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Structure of Binary Complexes of Mono- and Polynucleotides with Metal Ions of the First Transition Group

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Contents

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I. Introduction

It is well-known that divalent metal ions play an important role in molecular biology, as they participate in several functions of nucleic acids, including their replication processes. Moreover, metal ions are involved in the action of several nucleic acid derivatives. It is well-known, for instance, that adenosine triphosphate (ATP) acts as a mediator for the energy flux occurring in living organisms. ATP is formed in the living cells from adenosine diphosphate (ADP) and orthophosphate; through this synthesis, chemical energy is stored in the ATP molecule. The transfer of the ATP phosphoryl group to the acceptors is at the basis of the utilization of this stored energy. Most of the mechanisms responsible for the phosphate transfer are mediated by enzymes, which generally require the presence of metal ions. Similarly, the cleavage of pyrophosphate from the four deoxynucleotide triphosphates, dATP, dCTP, dGTP, and dTTP, involved in the DNA–polymerase reaction, together with the formation of phosphodiester linkages, requires the presence of divalent metal ions, mainly Mg^{2+}. However it has been shown that Mg^{2+} ions can be replaced by other divalent metal ions, this replacement modifying the selectivity of the reactions, possibly through an intermediate metal complexation.

The direct formation of binary complexes between nucleotide triphosphates and metal ions is able to promote hydrolysis of these phosphate derivatives only to a moderate extent.[1] This is taken as evidence that the configuration of a binary complex formed by a metal ion and the phosphate group of a mononucleotide is generally different from the configuration adopted by these interacting groups when they are a part of a ternary complex with a phosphate transfer enzyme. It seems therefore reasonable to assume that the binding of metal ions with the electron donor groups of nucleotides might be altered when the metal–nucleotide complex closely interacts with an enzymatic active site. Moreover, the enzymatic process occurs under the influence of so many factors (related either to the steric organization as well as to the dynamics of the various chemical groups involved) that a tentative explanation of the enzyme action in terms of a binary binding will immediately appear as an oversimplified approach. However, the participation of the phosphate group of monomeric nucleotides in a large number of metabolic processes already demonstrates the interest of studying even the structure of simple, binary metal–nucleotide complexes. Moreover, structural studies on binary complexes might help in identifying the preferential sites of binding for the various metal ions on mononucleotides involved in enzymatic mechanisms. These studies present a particular interest in the case of the metals of the first transition group, in view of their relevant inhibiting or activating effects on enzymatic reactions.

Metal ions are also particularly important in the biochemistry of polynucleotides. It is known in fact that, due to the nature of polyelectrolytes, polynucleotides and especially natural nucleic acids are profoundly affected by their ionic environment. In particular, it has been shown that the T_m transition exhibited by both DNA and polynucleotides is closely dependent on salt concentration. The various theoretical treatments used in the past to explain these effects have alternatively emphasized either the concept of "ionic atmospheric binding"[2] or the opposed principle of "binding site of counterions".[3] The treatments based exclusively upon the concept of "ionic atmospheric binding" are not able to account for the "specificity" of the effects that can be induced by part of different ionic species on conformational transitions of polynucleotides. This specificity, also found for simple monovalent cations, appears to play a predominant role in the case of divalent metal ions. In other words, divalent counterions can be considered as specific ligands that are either bound or released in the course of the conformational transition of polynucleotides, thus affecting their stability. In this sense, it is well-known, for instance, that the DNA double helix is generally stabilized by metal ions through the neutralization of the negative charges on the phosphoryl groups that would otherwise exert a repulsive driving force for unwinding.[4] On the other hand, some metal ions are able to express a different influence on the stability of the double helical structure of the DNA molecule. This is, for instance, the case of Cu^{2+} ions, which destabilize the normal conformation of DNA by decreasing its melting temperature. The destabilization induced by Cu^{2+} can be explained in terms of the tendency of this metal ion to bind to bases in addition to phosphate. There is a strong competition between the binding of this metal ion and the normal hydrogen bonds between paired bases, which helps to destroy the double helix. However, high electrolyte concentrations can stabilize the ordered structure of DNA so as to prevent copper from binding to the bases.

Some evidence that Cu^{2+} ions form cross-links between the strands of unwound DNA comes from the fact that these ions can also facilitate the regeneration of native DNA from each strand pair. In the denatured state, such cross-links would maintain the single strands in a favorable position for the regeneration of the double helix when temperature is lowered and the concentration of the electrolyte is increased. Thus, there is always an equilibrium between single- and double-stranded species, depending on concentration of Cu^{2+} ions, concentration of other electrolytes, and temperature. Similar equilibrium situations occur with Zn^{2+} ions, but in this case no other electrolyte is needed. This difference could only be interpreted on the basis of the relative affinity of these metal ions for the nucleotide bases. Cu^{2+} ions bind fairly strongly to the electron donor groups of the bases whereas other metal ions can be displaced more easily from the base binding sites when thermodynamic conditions favor the formation of hydrogen bonds between the strands.

It has not yet been clarified if metal ions, which generally seem to be necessary for the processes of replication of DNA and for the transcription of the genetic code, participate in the unwinding and rewinding of the DNA strands during these biological processes.

For RNA, an ordered conformation is less evident than for DNA, because hydrogen bonding takes place between bases of a single strand, but, nevertheless, such a structure seems also to be stabilized by metal ions.

Optical rotatory dispersion (ORD) studies[5] have shown that even coiled forms can be protected from denaturation by the presence of some metal ions. The conclusions of these experimental results suggest that the sensitivity of polyribonucleotides to metal ions is a quite general property, although each metal ion probably acts in a peculiar way.

An enumeration of striking effects which can be induced by the presence of various divalent metal ions, including some cations of the first transition group, on the activity of ribonucleic acids, the occurrence of mutations in cells, and the perturbation of enzymatic reactions involving nucleic acids, has already been done by M. Daune.[6] During the course of these biological processes, conformational changes of polyribonucleotides are brought about by complexation of the metal ions with electron-donor chemical groups.

At the present time, it is not possible to ascertain, especially in in vivo systems, to what extent and at which step of the reactions such complexations take place, but a study of the possible binding sites can be looked at as a contribution to the clarification of the problem, as it was already understood by many investigators.

At the end of the sixties different experimental methods had already afforded some evidence about the characteristics of the interaction of metal ions with DNA and RNA. Thus, it had been shown by several authors[7-11] that complexes formed by DNA and metal ions have a stability constant of an order of magnitude

ranging around 10^4. Base binding alone produces a much lower constant than the combination of base and phosphate binding, but metal ions bind to denatured DNA more strongly than to native DNA,[10] which reveals an additional binding capacity of the exposed bases.

In general, it is expected that the possible binding sites for divalent metal ions on polynucleotides have, at least, to be similar to some of those occurring in metal complexes of mononucleotides. In this sense, a detailed knowledge of the conformation adopted by mononucleotides in the presence of metal ions was generally sought by most investigators on the matter, with the aim of understanding local effects on nucleic acids. However, any result obtained in the study of metal complexes of mononucleotides cannot be directly extrapolated to nucleic acids because of the complexity brought about on them by further covalent bonds (which render some of the sites inaccessible to metal ions) as well as by hydrogen bonds and electrostatic interactions. Also, steric impediments are opposed by the regular structure of the nucleic acids to the approach of hydrated metal ions to the probable metal binding sites.

As a consequence of all that has been said above, the most accurate structural techniques are actually needed to ascertain which are the interaction sites of the different metal ions on polynucleotides and to detect the conformational changes induced on the latter by the formation of localized complexes. The recent developments of the magnetic resonance methods, both nuclear and electronic, as well as the increasing possibilities of the X-ray diffraction techniques because of the crystallization of new compounds, have favored remarkable advances in the field, which are expected to continue in the coming years.

In the present review we intend to cover the literature of the seventies through the years 1978–1979, although some necessary earlier references will also be included. Much information concerning earlier research on the subject can be found in the article by Izatt et al.[12] For the sake of clarity, the structure and numbering system for the main compounds are given in Figure 1. The abbreviations used throughout this article follow the 1970 recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature.[13]

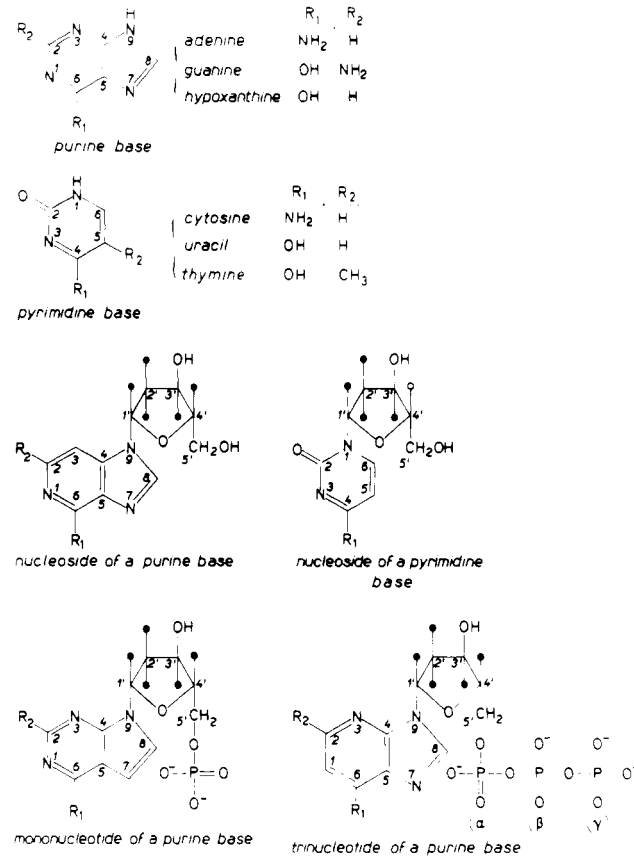


Figure 1. Chemical structures of the purine and pyrimidine bases and of the corresponding nucleosides and nucleotides.

II. Fundamentals of Some Physical Methods Used for the Determination of Metal-Nucleotide Interaction Sites

The important technical and theoretical advances experienced in the last few years by molecular physical methods have focused the general attention on the potential of these techniques for solving several problems of molecular structure relevant to the understanding of biochemical mechanisms. These expectations have often proved to rest on realistic bases. In particular, knowledge of the structure of complexes of mono- and polynucleotides with metal ions of the first transition group has been greatly extended by the application of a number of physical methods which include radiation and particle diffraction on crystals, nuclear and electron magnetic resonance, and X-ray spectroscopy, while optical methods continue to provide some interesting results. Potentiometry and conductometry, widely used in the sixties for the determination of the dissociation constants of various metal–nucleic acid derivative complexes, are still employed for complementary information. We will briefly summarize in the following some aspects of the physical methods which have become especially significant for the study of our subject.

A. Crystallographic Measurements

Among the various physical techniques suitable for studying substances in the crystalline state, X-ray diffraction has proved to be especially powerful to elucidate the conformational structure of complexes formed by transition metals with nucleic acid constituents. The data obtained from X-ray crystallographic measurements have made it possible to ascertain the precise locations of all atoms in many different metal–nucleic derivative complexes. Although elaboration of these data represents a laborious task, the new developments in the field of high-speed digital computers have encouraged efforts in the use of the X-ray instrumentation. The copper Kα X-radiation has been the one most widely used in studies on complex compounds, in view of its penetration power in organic crystals, as well as of the quality of photographic recordings.

Nickel and zirconium filters are commonly used for removing the accompanying Kβ radiation.

The technique aspects of X-ray diffraction methods have been extensively described elsewhere.[14,15] We will only briefly mention here the general analytical procedures which are usually applied on diverse patterns obtained from scattered X-rays. An X-ray diagram can be geometrically analyzed by determining the locations of the independent X-ray reflections in the diffraction pattern. From the knowledge of the corresponding coordinate parameters it is possible to compute the size and shape of the unit cell. Such geometrical analysis can even be performed on an assembly of small, randomly oriented crystals. A single crystal will provide a well-resolved diagram if the crystal position is adjusted in such a way that one of its crystallographic axes (generally the c axis) comes to be parallel to the rotation (or oscillation) axis. In this case a complete geometrical analysis can be performed, providing that the difficulties of identifying the individual reflections are overcome by some special procedure, such as oscillation of the crystal over a small angular range. The observed reflections can then be collected in an automatic diffractometer and the space group and unit cell dimensions computed by the Weissenberg (moving film) method or by precession photographs. Accurate unit cell dimensions can be obtained by measuring high-angle reflections and by

performing a least-squares calculation. However, to go farther, that is to get information on the atomic distribution within the unit cell, a study of the intensities of the independent reflections from single-crystal scattering is required. Such intensities depend on the scattering efficacy of the electrons within each atom, which is a function of sin θ/λ and can be theoretically evaluated.[16] The values of the intensities of the independent reflections correspond to the coefficients of a three-dimensional Fourier series, which represents a regular periodic function in space, as it is the electronic density in a crystal. Usually a microdensitometer is used for measuring the relative intensities of the reflections, and these are corrected by Lorentz and polarization factors. The structure is then solved by Fourier methods which, sometimes, may be reduced to those of a two-dimensional series. Also, some simplification is possible by the use of the Patterson method, which gives a physical interpretation to the summation of the series terms. In all cases, a least-squares refinement using thermal parameters can be applied.

The crystalline structures of the transition metal–nucleic derivative complexes contain heavy transition-metal atoms. Thus, the standard heavy-atom technique can be applied in these cases; the locations of the metal atoms (and of other heavy atoms such as S and Cl) are determined from the three-dimensional Patterson function. Moreover, this mathematical procedure facilitates the computation of the whole structure, since the parameters of the remaining (nonheavy) atoms can be determined from the three-dimensional electron density maps. Very often, the metallic complex and the original nucleic ligand are almost isomorphously structured; however, since the contribution of a massive atom to the intensities of the independent reflections is very high, it is sufficient to determine the signs of the structure factors in the Fourier summation. Furthermore, for facilitation of calculations in asymmetrically structured complexes, there are computing programs which have been especially developed for noncentrosymmetric crystal structures.[17]

B. NMR Measurements

Nuclear magnetic resonance techniques applied to poly- or mononucleotides can be directly used to detect the effects caused by the binding of a metal ion on these molecules in aqueous solutions. The magnetic resonance of protons has been most widely used.

Proton NMR spectra of nucleotide molecules are characterized by a great number of lines; however, the very high resolution attained with modern spectrometers makes an accurate study of the individual signals possible. On the other hand, the use of the resonance of other nuclei (e.g., ^{13}C, ^{31}P, ^{14}N, ^{15}N) offers the possibility of observing larger chemical shifts associated with variations of electronic distributions. The practical disadvantages brought about by the low concentrations of natural isotopes of some of these nuclei are compensated by using NMR spectrometers operating in the pulse-Fourier-transform mode, in which accumulations of pulse interferograms (free induction decay signals) are carried out with the aid of computers. Fourier-transformed NMR spectra can also be obtained from free induction decays accumulated under conditions of proton band decoupling.

Depending on the nature of the metal in the complex, it will be convenient to measure one or another NMR parameter. Selective line broadenings observed in the ligand's spectrum may provide useful indications about the proximity of a paramagnetic metal ion to the nuclear species whose characteristic line happens to be broadened. Also, measurements of chemical shift variations, induced on the NMR signals of the ligand by modifications of the chemical environment, represents a handy method for determining the proximity of a metal ion to a particular chemical group of the ligand.[18]

Relaxation processes in a nuclear spin system are responsible for the finite values of line width exhibited by the absorption signals. However, several different mechanisms can lead to spin relaxation. The prevalence of one mechanism over the others depends on the molecular properties and also on the physical conditions of the sample.

For complexes of transition-metal ions with organic molecules, the interaction of the odd-electron spin of the metal ion with the nuclear spins of the ligand is particularly important in the relaxation processes, since the electron spin relaxes at a high rate, thus inducing transitions between the nuclear spin levels. Information on the relaxation processes can be obtained by measuring the relaxation times of nuclear species possessing a magnetic moment.[19] Both the longitudinal and transverse nuclear magnetic relaxation times (T_1 and T_2) of a ligand molecule are reduced by the presence of a paramagnetic ion in solution when the interaction takes place under conditions of dynamic equilibrium. An effective correlation time, τ_e, is defined for the fluctuations of the contact energy. τ_e is related to the relaxation rate of the electron spin, τ_s, and to the correlation time of the chemical exchange, τ_c (the time spent by the electron spin while contacting the nuclear spin):

$$\frac{1}{\tau_e} = \frac{1}{\tau_c} + \frac{1}{\tau_s} \tag{1}$$

Since other processes depending on time, such as Brownian motions with an average correlation time τ_r, are also important in affecting the relaxation mechanisms, a correlation time τ_d is defined for the dipolar interactions between the electron and the nuclear spins such that

$$\frac{1}{\tau_d} = \frac{1}{\tau_c} + \frac{1}{\tau_s} + \frac{1}{\tau_r} \tag{2}$$

The relaxation time for the nuclear spin will be mostly influenced by the shortest correlation time corresponding to one of these processes.

Under various conditions, the dynamic nature of all relaxation mechanisms acting on the spin system will determine observable variations on the shape of the absorption line, and particularly on the line-width parameter. The latter is defined in a conventional way to permit it to be conveniently analyzed in mathematical terms. Most commonly, the shape of NMR absorption lines can be approximated, according to the case, either by Gaussian or by Lorentzian functions. This distinction is important for a proper definition of the line width which is experimentally evaluated at the half-height of the peak and expressed henceforth as $\Delta\nu_{1/2}$. Such a line width is related to the spin–spin relaxation time of the nuclear spins, T_2, by the expressions $\Delta\nu_{1/2} = 1.476/(\pi T_2)$ for a Gaussian line and $\Delta\nu_{1/2} = 1/(\pi T_2)$ for a Lorentzian line.

For the detection of the formation of metal–organic molecule complexes as well as for the determination of the number of binding sites available for the metal ion in the ligand molecule, observable broadenings of the spectral lines arising from the latter have been widely used. These broadenings indicate the proximity of paramagnetic ions to the atoms whose characteristic lines have been broadened[20] and are proportional to the concentrations of the interacting metal ions.

The formulation developed by Bloembergen and Morgan[21] for the transverse nuclear relaxation time of a nonquadrupolar nucleus affected by an electron spin–nuclear spin interaction has the general form

$$\frac{1}{T_{2m}} = \text{const}\,\frac{1}{r^6}\tau_d + \text{const}\,a^2\tau_e \tag{3}$$

where T_{2m} = transverse relaxation time of the nucleus considered in the ligand molecule; r = electron spin–nuclear spin distance; a = hyperfine nuclear spin coupling constant.

The T_{2m}^{-1} rate is related to the T_{2p}^{-1} rate, the corresponding magnitude attributable to equivalent protons, by

$$T_{2p}^{-1} = pqT_{2m}^{-1} \quad (4)$$

where p is the ratio of the molar concentrations of the metal ion to the ligand and q is the average number of ligands bound in an identical way.

In the case of relatively little incidence of the scalar electron–nucleus coupling, T_{2m}^{-1} can become practically proportional to the inverse of the sixth power of the distance between the nuclear and the electron spins, that is, of the distance between the nucleus and the metal atom. Under these conditions, the selective broadening of some of the ligand NMR lines can give structural information on the complex in solution, since the dipolar relaxation is very sensitive to variations of the electron–nucleus distances.

According to the procedure developed by Eaton and Phillips,[22] the distance r_i of the various atoms of the ligand from the metal ion can be estimated by plotting the values of T_{2m}^{-1} vs. N_s, the total number of paramagnetic species per volume unit. Measurements of T_{2m} can simply be accomplished by measuring the spectral NMR line widths.[23] In order to facilitate line-width measurements in the case of multiplet structures of the resonance lines, nuclear spins can be decoupled by applying double-irradiation techniques.

However, it should be emphasized that meaningful results can be obtained by these techniques provided that the following conditions are met by the system: (a) negligible scalar coupling vs. dipolar interaction; (b) rapid chemical exchange of the metal ion in solution with all binding sites.

Experiments on D_2O solutions of various ligand molecules (at a concentration of about 0.1 M) and a metal ion (in the range of 10^{-7}–10^{-8} M) have been carried out by Natusch in 1973.[24] The experimental values obtained for r_i agree well with those determined by the same author from molecular models of rigid complexes, such as those formed by transition metal ions with adenosine monophosphate, histidine, proline, and hydroxyproline. Similar experiments on D_2O solutions of diverse hydrogen-containing small and simple ligands have also been carried out recently by Espersen and others.[25] The ratio of metal ion (Cu(II)) to ligand concentrations was varied over the range 10^{-2}–10^{-4}. During each experiment, both proton spin–lattice (T_1) and spin–spin (T_2) relaxation times were measured on the ligand molecule, either in the absence or in the presence of the metal ion. It was supposed that the situation was not complicated by spin–spin coupling among the various hydrogen atoms, due to the simple structure of the ligand chosen for the investigation. The inverse proton relaxation times of the ligand in the complex, T_{1p}^{-1} and T_{2p}^{-1}, have been calculated (under conditions of fast exchange) on the basis of the difference between the values found for the free ligand and the ligand in a dynamic equilibrium with the complex.

Assuming the electron–nucleus scalar coupling to be negligible, both relaxation times depend upon r^{-6}, and simple equations can be written under "extreme narrowing" conditions[26] in the following way:

$$\frac{1}{T_{1m}} = 6k'\frac{1}{r^6}\tau_r \quad (5)$$

$$\frac{1}{T_{2m}} = 7k'\frac{1}{r^6}\tau_r \quad (6)$$

where τ_r is the rotational correlation time of the paramagnetic ion bound to the ligand molecule. Under such conditions,

$$\frac{T_{1p}}{T_{2p}} = \frac{T_{1m}}{T_{2m}} = \frac{7}{6} \quad (7)$$

In some cases, however, comparisons of experimental and theoretical values of the ratio T_{1p}/T_{2p} have indicated that line broadenings induced by transition-metal ions on ligand proton NMR spectra could not be ascribed uniquely to dipolar contributions. Consequently, conclusions derived from the assumption of a simple r^{-6} dependence are not justified in these cases. A deviation from a linear dependence upon r^{-6} is also found if the fast exchange limit is not attained. In this case, an additional correctional term must be added to the expression of T_{2p}^{-1}, which is formulated as $pq\tau_m\Delta\omega_m^2$, where τ_m is the lifetime of a ligand bound to the metal and $\Delta\omega_m$ is the chemical shift between bound and unbound ligand resonances.

Since the scalar coupling (a) and the chemical shift ($\Delta\omega_m$) are generally different for the various proton groups of a ligand molecule, only extensive studies on the effects of temperature and frequency variations will make possible the evaluation of both the relative contributions of an intermediate exchange and a scalar coupling.

C. Other Physical Methods

1. Magnetic Susceptibility

Measurements of the bulk magnetic susceptibility of a transition-metal–nucleic acid derivative complex give an evaluation of the effective magnetic moment per magnetic ion. When compared with the values normally found for magnetically diluted compounds, the experimental value of this magnetic parameter can provide evidence for the existence of an intramolecular coupling between two paramagnetic ions. The exchange coupling constant, J, measures the energy difference between the excited ($S = 1$) level and the ground state ($S = 0$) and gives an indication of the magnitude of the exchange. By using a quantitative approach[27–29] for coupled paramagnetic ions, the J value which fits the experimental results can be found.

A weak temperature dependence of the magnetic susceptibility for highly paramagnetic metal ion complexes also suggests the existence of an intramolecular spin–spin interaction.

2. Electron Paramagnetic Resonance

The electron paramagnetic resonance methods, as applied to the study of the transition-metal ions, have been theoretically analyzed by Abragam, Bleaney, and coll.[30,31] The most interesting information given by the EPR methods concerns the interactions of the paramagnetic ions with their molecular environments. Computer simulations of the experimental EPR spectra can be performed by giving variable values to the spectroscopic g factor, coupling constants, line widths (inversely related to the electron relaxation times), and the relative intensities of the component lines.

Three theories have been developed to account for the effect of the symmetry of the immediate environment of a paramagnetic center: crystal-field, ligand-field, and molecular orbital theories, respectively. According to crystal-field theory a decrease of the g values should be expected as the stability constant of the complex increases. This electrostatic approach can be, however, complemented by the assumption of the existence of some chemical bond between the central ion and each one of the ligand molecules. If second-order terms are neglected, the principal components of the g tensor in a d^1 tetragonal complex are

$$g_\parallel = 2\left(1 + \frac{4\zeta}{\Delta_1}\right)$$

$$g_\perp = 2\left(1 + \frac{\zeta}{\Delta_2}\right) \quad (8)$$

where ζ is the spin–orbit coupling constant, Δ_1 is the separation between the $d_{x^2-y^2}$ and d_{xy} orbital levels, and Δ_2 is the separation between $d_{x^2-y^2}$ and d_{xz}, d_{yz}. This approximation is generally

found to be in agreement with the predictions of molecular orbital theory, if only a small amount of covalency can be attributed to the binding around the paramagnetic ion. In this regard, $g_{\parallel}$ is more sensitive than $g_{\perp}$ to the degree of covalency of the bonds. On the other hand, the observation of a superhyperfine structure of the EPR lines, due to a spin–spin interaction with the ligand nuclei, indicates the delocalization of the spin density and thus is evidence of the highly covalent nature of the bonds between the central atom and the ligand atoms. In such a case, it will be necessary to treat the problem utilizing the principles of molecular orbital theories developed for metal complexes.[32,33] On the basis of the experimentally determined magnetic parameters, one can calculate α^2 and β^2, i.e., the spin densities on the orbitals $d_{x^2-y^2}$ and d_{xy} of the central ion, respectively, as well as the spin density, α'^2, of the unpaired electron on the ligand.

Some transition-metal complexes (most commonly copper complexes) present a dimeric character: two identical metal ions stand fairly close one to the other, while they are exchange coupled. Here, the EPR absorptions occur at higher and lower fields than the normal resonance field for $S = {}^1/_2$,[34,35] and the forbidden transition lines with $\Delta_{ms} = 2$ can be seen.

The spectra of the dimeric complexes can be interpreted with the assumption of a spin Hamiltonian for $S = 1$ and axial symmetry. The assignment of the absorption bands can be done by using the approach elaborated by Wasserman and coworkers.[36]

3. X-ray Photoelectron Spectrometry

The nature of molecular binding in transition-metal complexes can also be conveniently studied by the use of X-ray photoelectron spectrometry, based upon the determination of the kinetic energies of photoelectrons induced by X-ray photons interacting with target atoms in the molecules.[37] The knowledge of the energy difference between the exciting radiation and the emitted photoelectrons makes possible a direct computation of the binding energies of the inner-shell electrons. In this way, the orbital structures of the elements are directly related to the resulting X-ray photoelectron spectra, and information can be obtained from the chemical shifts of the photoelectron lines. Additional phenomena taking place during excitation processes in transition-metal complexes lead to satellite peaks for both the core and the valence X-ray photoelectron spectra.[38] Such satellite photoelectron lines, assigned to a multielectron excitation,[39] can be used to derive conclusions concerning the oxidation state and the chemical environment of the transition-metal atom, through the observation of their fine structure and position. Experimental details of the method have been described by Weser and collaborators.[40] Modern spectrometers perform a fully automatic operation for the recording of the spectra through the use of a computer system.

4. Other Methods

Other methods which have been used for the study of transition-metal–nucleic acid derivative complexes include circular dichroism (CD), magnetic optical rotatory dispersions (ORD), temperature-jump spectrometry, field-jump relaxation, potentiometry, polarography, conductometry, and visible, ultraviolet (UV), infrared (IR), Raman, and Mössbauer spectroscopy, together with molecular orbital (MO) calculations. Most of these methods have been concisely discussed by Phillips[41] and Weser.[42]

D. Potential of Various Physical Methods

Physical methods such as X-ray crystallography, nuclear and electron magnetic resonance, and X-ray photoelectron spectrometry are presently considered as the most reliable procedures for the elucidation of molecular structures. Physical parameters directly related to molecular dimensions, configurations, and binding energies between atoms are obtained through the use of the techniques mentioned above.

The highly precise results obtained by crystallographic (diffraction) methods have undoubtedly a cost of laborious evaluation and calculation. However, the growing sophistication of the experimental procedures can allow highly experienced research groups to obtain exciting results about the conformational structure of complex molecules. Furthermore, developing techniques for the crystallization of organic substances have extended the application of X-ray crystallographic methods to the study of large molecules. The chemistry of the metal–nucleotide and –polynucleotide complexes is a good example.

Knowledge of molecular conformations can also be gained, although in an indirect fashion, through the analysis of nuclear (mainly proton–proton) coupling constants in ^{1}H NMR spectra. In general, an interaction between a transition-metal ion and a nucleo base can be indicated by broadenings in the nucleo base spectrum. However, the resonance peaks arising from the base protons of a mononucleotide may also be broadened in a metal complex formed on the phosphate group if a pseudocontact interaction of the metal with the nucleo base takes place. Also, some chemical shifts produced in the NMR spectra of mononucleotides by the presence of metal ions may be caused by changes of the electronic configuration induced at sites far apart from the actual binding site. In both cases, the magnetic resonances of nuclei other than protons (^{13}C, ^{31}P, and ^{14}N) offer additional potentials to the study of the complex chemical structure. When a correct identification of the structure resists all NMR procedures, the precise positions of the atoms can still be determined by using X-ray diffraction methods.

The delocalization of the spin density on complexes of transition-metal ions with organic molecules can be directly determined on the basis of the hyperfine structure of the EPR lines. Thus, the EPR method is unique in demonstrating a covalent nature of the bond between the central atom and the ligands. A good approximation to the experimental values of the EPR spectral parameters can be obtained by varying the values of the coupling constants, line widths, and relative intensities of the theoretical lines and by performing a computer simulation of the spectrum. Different types of complexes with one or more base molecules involved in the metal binding can thus be evaluated by their EPR spectroscopic effects.

X-ray photoelectron spectroscopy will be particularly useful when a single-crystal X-ray structural analysis is either impossible or inappropriate. Metal ions interacting in low concentrations with large molecules can be detected by this method, which promises to be a very valuable analytical technique for studying the role of metals on nucleic acids and affording new possibilities for metal ions that cannot be detected either by EPR or by Mössbauer spectroscopy. The interpretation of X-ray photoelectron spectroscopy data may, however, be complicated by some crystal-field effects that cannot be quantitatively measured nor correctly defined at the present time.[43]

III. Complexation of Metal Ions of the First Transition Group with Mono- and Polynucleotides

A. Complexes with Nucleotide Bases

The investigation of metal complexes of nucleotide bases has been generally considered as a convenient step toward understanding the configurations of more complicated complexes, such as those arising from the interaction of metal ions with mono- and polynucleotides.

Among the various nucleotide bases with the capacity of interacting with metal ions, particular attention has been devoted to adenine and guanine (purine bases) and cytosine (pyrimidine

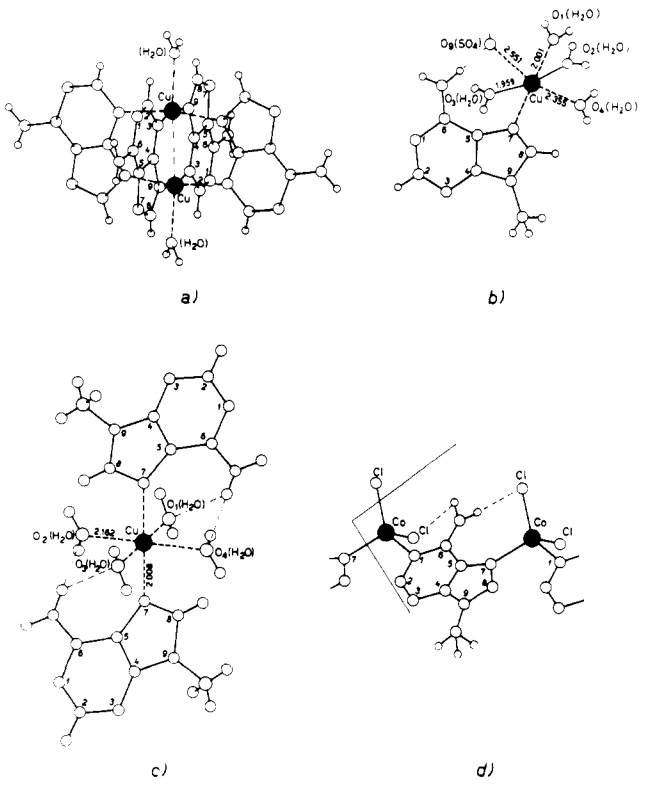


Figure 2. (a) Coordination around the copper atom in the dimer of $[(adenine)_2Cu^{II}H_2O]Cl_2 \cdot 3H_2O$, as viewed along the *b* axis (after Sletten[44]). (Reprinted with permission from ref 44. Copyright 1967, The Chemical Society). (b) Coordination around the copper atom in the complex compound $[(9\text{-methyladenine})Cu^{II}(H_2O)_4]SO_4 \cdot H_2O$ (after Sletten and Thorstensen[52]). (c) Coordination around the copper atom in the complex $[(9\text{-methyladenine})_2Cu^{II}(H_2O)_4]^{2+}$ (after Sletten and Rund[53]). (d) View of the fragment of the structure forming a chain of formula $[(9\text{-methyladenine})Co^{II}Cl_2]_n$ (after De Meester et al.[54] Reproduced with permission from ref 54. Copyright 1973, Elsevier Publishing Co.)

base) (Figure 1). A hydroxylated derivative, hypoxanthine, has also deserved some attention.

Metal complexes of the nucleic bases can be generally easily prepared in the crystalline state by mixing saturated solutions of metallic salts and acidic solutions of the heterocyclic bases. A variety of metal complexes, analytically differing in their stoichiometric composition and grade of hydration, can be obtained by changing the experimental conditions of preparation, in view of a possible identification of the preferential binding sites in each case.

1. *Complexes with Adenine*

In view of the great biochemical importance of the adenine nucleotides, the structures of complexes formed by various metal ions with the adenine base have been extensively studied, both in the crystalline state and in solution.

The formation of crystalline dimeric complexes formed by adenine and cupric ion has been first demonstrated by Sletten,[44,45] who succeeded in obtaining, from aqueous solutions, blue-violet prisms with the composition $[(adenine)_2Cu^{II}H_2O]Cl_2 \cdot 3H_2O$. X-ray crystallography has shown in fact that this complex has a dimeric configuration, in which each of the two adenine moieties is bound to two copper ions through the high electron density centers N(3) and N(9). The copper–copper distance was found to be 2.947 ± 0.002 Å. This value is appreciably higher than the distance generally found in other dimeric complexes formed by copper ions (Figure 2a). Compounds of this type generally possess a subnormal magnetic moment (as it is the case here). This result has been interpreted as due to spin pairing between the odd electrons of the two copper atoms. However, the large copper–copper distance in the copper–adenine complex makes a direct overlap of copper orbitals unlikely. A superexchange mechanism has been therefore postulated, which involves a migration of electrons through the π system of the ligands.[46]

Electron spin resonance studies[47] indicate that the hyperfine structure constant of this dimeric compound is one-half that exhibited by the monomer. On the other hand, when the cupric complex is prepared under various pH conditions of the adenine aqueous solution, large differences are found among the A_t values of the polycrystal precipitates. At high pH, adenine deprotonation occurs, this fact bringing about a change in the electronic delocalization via the bonding bridges across the Cu(II) ions. The change of electron density on the copper nuclei is the cause of the changes undergone by the A_t values and, consequently, by the character of the bonding bridges.

Also, a cupric complex of neutral adenine, expressed by the formula $[(adenine)_2Cu^{II}ClO_4]_2(ClO_4)_2 \cdot nH_2O$, can be prepared by adding a solution of copper(II) perchlorate and perchloric acid to a hot aqueous solution of adenine in a mole ratio of 1:2.[48] The X-band EPR spectrum of the blue-violet polycrystalline substance which is thus obtained shows a "spin of one" characteristic absorption. Such a spectral feature could arise from two exchange-coupled Cu(II) ions, each contributing a spin of one-half.

Another interesting dimeric structure, corresponding to the complex cation $[(adenine)_2Cu^{II}Cl]_2^{2+}$, has been studied by De Meester and others[49] on blue-green crystals of the substance, which also contain chloride ions and some molecules of solvation water. In this case, two pairs of coplanar adenine rings are arranged perpendicular to the other, while the chlorine and copper atoms lie axially in the 2/*m* crystallographic symmetry.

In order to get information on the structural changes induced on the adenine ligands by the binding with metal ions, Terzis et al.[50] studied, in 1973, a complex compound of the formula $[(adenine)_2Cu^{II}H_2O]_2(ClO_4)_4 \cdot 2H_2O$. Determination of the crystallographic parameters showed that the binding of the copper ion to N(3) has a real effect on the ligand geometry near N(3), since considerable differences are found between the C(2)–N(3)–C(4) and N(3)–C(4)–C(5) angles in the complex and the same angles in the nucleotide. The length of the bond N(7)–C(8), shorter than that of the bond C(8)–N(9) in the nucleotide, is modified in the metal–base complex so as to become longer. This variation is ascribed to a redistribution of the electron density over N(7)–C(8)–N(9), with the effect of increasing the double bond character of C(8)–N(9) at the expense of N(7)–C(8). Other small variations in the ring bonds are likely due to the formation of hydrogen bonds involving the ligands.

Cobalt(II) has less possibility of forming dimeric complexes than copper(II) does. De Meester and Skapski[51] have reported the X-ray crystal structure of an adenine–cobalt complex, which contains the centrosymmetric cation $[(Co^{II}(H_2O)_4(adenine)_2]^{2+}$. The two adenine moieties (in the trans position) are unidentate and bind to Co(II) via N(9). The four water molecules complete a nearly undistorted octahedral coordination. Diffraction studies on the crystalline structure of the compound have revealed the formation of hydrogen-bonded adenine–adeninium pairs; hydrogen bonds also hold together the bis(adeninium)-*trans*-bis(adenine)tetraaquocobalt(II) ion with sulfate ions and molecules of solvation water.

Crystallographic studies have also been carried out on two nondimeric copper–9-methyladenine complexes, prepared by addition of two different (saturated) copper salt solutions, $CuSO_4$[52] and $CuCl_2$,[53] respectively, to a solution of a nucleoside analogue $[(9\text{-methyladenine})Cu^{II}(H_2O)_4]SO_4 \cdot H_2O$ and $[(9\text{-methyladenine})_2Cu^{II}(H_2O)_4]Cl_2 \cdot 2H_2O$. In the first compound the copper ion has octahedral coordination (Figure 2b), with the equatorial positions occupied by three water molecules and by N(7) of the adenine ligand. The axial positions are occupied by a fourth

water molecule, O(4), and by one of the sulfate oxygens, O(9). Copper is displaced from the equatorial plane toward O(4), whereas its interaction with the sulfate ion, O(9), is very weak, as indicated by the value of the distance Cu–O(9), 2.551 Å. The preference for water molecules rather than for the sulfate ion in equatorial positions is also found in $CuSO_4$ hydrates. The sulfate ligand is accommodated far enough from the adenine binding site to permit an intramolecular hydrogen bond to be formed with the amino group, giving rise to an "indirect chelation" of the type N(6)–H(6)···O(9)–Cu–N(7). Similar hydrogen bonds are typically found in all complexes formed by copper ions and purine bases, whenever the N(9) position is not available for coordination. It is worth mentioning that uncomplexed 9-methyladenine molecules are packed in the crystal structure through an intermolecular hydrogen bond of the type N(6)–H-(6)···N(7), whereas in the crystal structure of the copper–9-methyladenine complex the intermolecular hydrogen bonds are formed with another nitrogen atom of the adenine ring, N(6)–H(6)···N(1). This is taken as additional evidence that N(7) is the preferential binding site for copper ions in the complex.

Some different particularities can be found in the copper–9-methyladenine complex obtained in $CuCl_2$ solution.[53] In this case the copper ion appears to be coordinated in a (2 + 4) inverted octahedral configuration, distorted by a Jahn–Teller effect (Figure 2c). The adenine base is axially coordinated to the metal ion at the N(7) position, whereas four water molecules are coordinated in the equatorial plane. The copper ion has here an unusual coordination geometry, with two short (2.008 Å) axial Cu–N(7) bonds and four long (2.162 Å) equatorial Cu–O bonds. All ligands are formally neutral, since the chloride ion does not enter the coordination sphere but is hydrogen bonded to adenine at the C(2) and C(8) positions. The coordinated water molecules are involved in intramolecular hydrogen bonds to the adenine amino group (the C(6) substituent). Therefore, an "indirect chelation" of the type discussed above is also found in this complex, where again the N(9) position is blocked by a covalently bound methyl group. Furthermore, coplanar hydrogen bonds are formed between N(3) and the chloride ions by the noncoordinated water molecules. The observation of the molecular packing of the crystalline structure formed by the copper chloride–adenine complex shows that coplanar adenine ligands, related by a center of symmetry, are hydrogen bonded through N(1) of one molecule and the amino group of another, as is also true for the copper sulfate–adenine complex. In both cases this feature of the complex molecular packing results in a DNA-like pattern. The N(1) site of adenine, which is directly involved in hydrogen bonds in both DNA and RNA structures (through the formation of a base pair with thymine or uracil), seems also to be a potential target for the interaction with metal ions. This conclusion derives from the work of De Meester et al. in 1973;[54] they had undertaken an X-ray structural study of a cobalt complex having the basic composition (9-methyladenine)$Co^{II}Cl_2$. This compound was obtained from an equimolar mixture of 9-methyladenine and hydrated cobalt chloride in ethanol and crystallized as dark blue prisms. Its crystal structure consists of infinite chains in which 9-methyladenine bridges are formed, by means of the N(1) and N(7) atoms, through the coordination sphere of the cobalt(II) ion. The metal atom is tetrahedrically coordinated, with the two chloride ions completing the coordination sphere (Figure 2d). The H atoms of the amino group in the adenine ring also form hydrogen bonds of the type N—H···Cl within each chain and of the type N—H···N between different chains. This was quoted as the first example ever found of metal N(1) bonding, but it is worth noting that X-ray powder analyses have already indicated that a zinc complex, $ZnCl_2$(9-methyladenine), has the same structure as the cobalt complex of equal basic formula.[55] More recently, additional crystallographic evidence for N(1) being exclusively preferred for metal coordination has been reported for the complex [(9-methyladenine)(protonated 9-methyladenine)$Zn^{II}Cl_2$]Cl·H_2O.[56] It is clear that such a binding scheme may be important in the metal-catalyzed unwinding and rewinding of DNA, since the N(1) site is directly involved in the pairing of adenine with thymine.

The complexation of iron with adenine seems to favor the N(3) and N(9) positions. A complex compound having the formula $[Fe^{II}(adenine)_2]SO_4 \cdot 2H_2O$ has been examined magnetochemically in 1974.[57] The magnetic moment of the compound has a weak dependence on temperature while the variation of its EPR line intensity with temperature confirms the monomeric character of the complex. Mössbauer spectra in the temperature range 77–300 K have also been presented for ^{57}Fe in this complex.[58] The temperature dependence of the spectral parameters suggests the high spin state for this compound.

Biological implications point, however, to the importance of knowing the characteristics of metal complexation in water solution. A potentiometric method has been used by Makar and Williams in 1974[59] in order to study the adenine–metal complexation in aqueous solution. The complexation constants determined for adenine in the presence of cobalt(II), nickel(II), copper(II), and zinc(II) have led to the conclusion that the interactions of these metal ions with adenine are rather weak. This fact explains the lack of spectroscopic studies on complexation of transition metals with nucleic bases in aqueous solution. Since the investigations in an aqueous medium appear then more difficult than in the crystalline state, the necessity of utilizing specifically sensitive techniques becomes unavoidable. In this sense, significant results for systems interacting in solution are to be expected from the use of magnetic resonance techniques.

To understand the complexation possibilities of the nucleic bases in aqueous solutions of metal ions, Karpel et al.[60] made an attempt to interpret the relaxation parameters and the NMR spectra obtained on an aqueous nickel(II)–purine system. Their choice of the nickelous ion for this investigation has been particularly convenient since this metal ion has a characteristic coordination number (six) as well as a typical rate constant for the release of the first bound water molecule, which will determine the penetration of any ligand into the Ni(II) inner-coordination shell. This rate constant is independent of the nature of the incoming ligand, so that a very consistent picture of the character of the complexation reactions for Ni(II)[61] can be attained. Furthermore, the application of proton NMR techniques to the study of these complexes is favored by the fact that, for purine, the resonances of H(2), H(6), and H(8) form a well-defined NMR spectrum[62] (Figure 3).

Two limiting cases can be analyzed for this system, slow exchange at low temperatures and fast exchange at elevated temperatures. Only the broadening of the H(8) line can be studied at low temperature since H(6) and H(2) show anomalies, possibly due to interactions between free base molecules in solution. At relatively high temperatures (300–400 K), the H(8) and H(6) line widths are comparable, with an excess of broadening for the H(6) proton, and both are significantly broader than H(2). The results thus point to N(7) as the preferential binding site. It is interesting to note here that the preferential location of the metal ion in the proximity of N(7) would not seem to be justified from a theoretical point of view, since molecular orbital calculations of charge distribution on purine bases[63] have shown that similar net charges are to be found on all four nitrogens of purine. The interpretation of the experimental results can be based on two facts: (a) for Ni(II) complexes the transverse relaxation time of a ligand proton has been found to be determined essentially by dipolar magnetic interactions[64,65] and (b) the mechanism for the formation of the complex is not changed as the ratio

$$f = [\text{bound ligand}]/[\text{free ligand}]$$

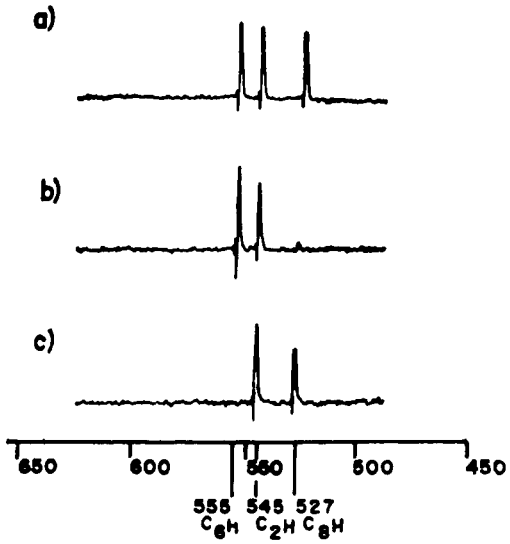


Figure 3. Proton magnetic resonance spectra at 60 MHz obtained on 0.1 M solutions of (a) purine, (b) 8-deuterated purine, (c) 6-deuterated purine (after Bullock and Jardetzky[62]).

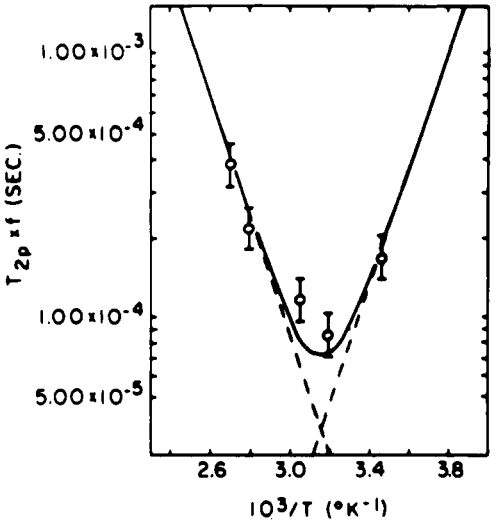


Figure 4. Temperature dependence of log (fT_{2p}) for the NMR peak of H(8) in a solution containing purine (0.476 M) and $Ni(NO_3)_2$ (3.0×10^{-4} M) at pH 5.7. The limiting slopes are drawn for $\Delta H^* = 9.8$ kcal mol^{-1} (after Karpel et al.[60]).

is varied within the experimental range ($f < 1$). This second property of the system has been asserted by temperature-jump studies and is also confirmed by NMR spectroscopy, since the proton line widths vary linearly with the total concentration of the Ni(II) ions.

At low temperature, the limiting process for the relaxation rate of a ligand proton is the chemical reaction

(proton in free purine) $\rightleftarrows$
(proton in coordination sphere of metal ion)

and then the relationship $1/T_{2p} = f/\tau_{Ni}$ can be used, where τ_{Ni} is the lifetime of the ligand proton in the coordination sphere of Ni(II). The dipolar term

$$1/T_{2p} = f\tau_{Ni}(\Delta\omega_{Ni})^2 \qquad (9)$$

(in which $\Delta\omega_{Ni}$ is the chemical shift produced by the nickelous ion) will be governing the line broadening at high temperatures, when the chemical exchange is sufficiently rapid to compensate for the low concentration of metal ions in equilibrium with the ligand molecule.

τ_{Ni} varies with temperature according to

$$\tau_{Ni} = \frac{kT^{-1}}{h} \exp\{(\Delta H^*/RT) - (\Delta S^*/R)\} \qquad (10)$$

where k is the Boltzmann constant, h is the Planck constant, T is temperature (K), R is the gas constant (cal·mol^{-1}·K^{-1}), and ΔH^* and ΔS^* are the enthalpy and entropy of activation, respectively. As a consequence of these facts, the study of the temperature behavior of the proton line widths appears to be essential for deriving any definite conclusion. This is generally done by means of plots of T_{2p} vs. T^{-1} (Figure 4).

The results obtained over a wide range of temperature, which indicate that the H(2) signal does not undergo significant line broadenings, suggest that N(7) is the preferred binding site. However, such preference does not mean that a single and exclusive mechanism is possible for the interaction of the nickelous ion with the purine base. The tautomerism of purine bases in solution implies the presence of various structural species in equilibrium, whose interconversion is catalyzed by H^+, OH^-, and the purine base anion.[66] The predominance of one or the other of these species in solution may have a direct influence on the reactivity of the potential sites for metal ion binding.

An example of the influence of ligand substituents on the strength of the interaction with metal ions is given by the potentiometric results obtained by Taqui Khan and Krishnamoorty[67] on the complexation of disubstituted purines, such as 2,6-diaminopurine. Here, the presence of two NH_2 groups on the purine ring increases considerably the basicity of the ligand and makes the groups NH_2 and $\equiv N$ better donors than in adenine. As a consequence, the complexation of diaminopurine with divalent metal ions (including those of the transition group) is generally expected to be accomplished through a chelate structure, with the participation of N(1).

The iron dinitrosyl group, $Fe^I(NO)_2$, with iron in a d^7 low-spin configuration and one unpaired electron, has been used by Tiezzi and co-workers[68] as a metal probe for investigating the coordination of the nucleotide bases with metal ions in solution. Various complexes in which the formal oxidation state of the coordinating ion is +1 can be prepared by adding nucleic bases to hydroalcoholic solutions of Fe(II) previously saturated with NO under a nitrogen stream.

The iron dinitrosyl complexes have an electron spin relaxation time which is similar to those of free radicals, so that the EPR nuclear hyperfine structures of the complexes are well resolved and can afford direct evidence of a Fermi contact interaction between the nuclei bonded to the metal ion and the unpaired electron. The EPR parameters can be determined by comparison with computer-simulated spectra, and the identification of the coordination sites can be effectively accomplished.

Adenine gives rise to a nine-line spectrum when using ^{14}NO for the metal probe and to a seven-line spectrum when using ^{15}NO, both in the pH range 7.0–9.5. The comparison of the nuclear hyperfine structures of the experimental EPR spectra with those of the simulated spectra indicates that the iron atom is coordinated with the two NO groups of the metal probe and with two equivalent nitrogens of the purine base. No spectral differences are found when 2,6-diaminopurines, 9-methyladenine, or pure imidazole are used. Thus, the experimental facts rule out the participation of pyrimidine nitrogens, namely N(1) and N(3), in the metal binding and point to N(7) as the ligand atom involved in the coordination. Also, the possibility of chelation through $-NH_2$ groups is excluded (Figure 5).

The same type of EPR patterns, indicating the imidazole site as the preferred one for the iron dinitrosyl group, is found in the spectra of the $Fe(NO)_2$ complexes of adenosine and of the natural polynucleotides (i.e., low molecular DNA and yeast RNA[69]), which is also confirmed by the selective broadening of

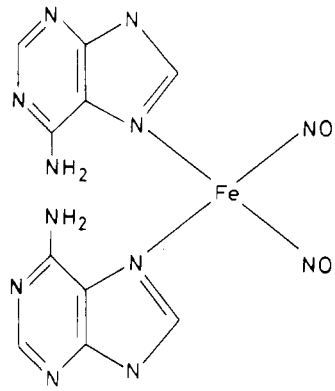


Figure 5. Proposed structure for the complex compound formed by $Fe(NO)_2$ and adenine. The substitution of adenine by imidazole does not modify the EPR spectrum of the compound (after Basosi, Tiezzi, and Valensin[68]).

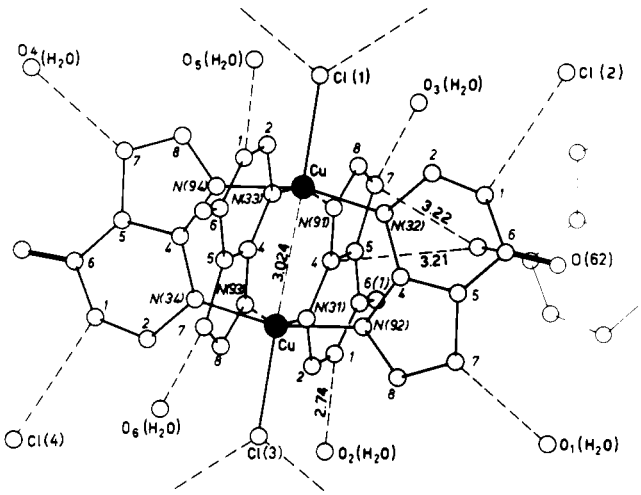


Figure 6. Spatial configuration of the dimeric complex $[(hypoxanthine)_2Cu^{II}]_2Cl_4 \cdot 6H_2O$ (after Sletten[70]).

the peaks in their Fourier transform NMR spectra. This so well-defined site of interaction of the iron dinitrosyl probe with the imidazole ring in a variety of compounds points to the suitability of this probe for the investigation of biological macromolecular structures.

2. Complexes with Hypoxanthine and Its Derivatives

The free base hypoxanthine presents, like adenine, a net tendency to coordinate metal ions on the N(3) and N(9) atoms. An example of this situation is given by a bis(hypoxanthine)-copper(II) chloride trihydrate which was first synthesized and obtained as deep turquoise crystals by Sletten.[70] The structure of this compound, as studied by X-ray diffraction, presents a close similarity with the structure of the bis(6-aminopurine)-copper(II) tetrahydrate complex (see Figure 2a). The copper–hypoxanthine complex (Figure 6) is a centrosymmetric dimer in which each copper atom has square-pyramidal coordination. Two of the ligand atoms are the N(9) nitrogen atoms in the imidazole rings and the other two are the N(3) nitrogen atoms in the pyrimidine rings. The apical position is occupied by a chlorine atom, which is hydrogen bonded to two water molecules. The bond length of Cu–Cl (2.431 Å) is intermediate between those of ionic and covalent bonds, and comparable with that of Cu–O (2.195 Å) in the bis(adenine)copper(II) tetrahydrate complex. The internuclear Cu–Cu distance is 3.024 Å long; here the presence of the chlorine atom favors a strong axial coordination and induces a large separation of the copper atoms. It is interesting to make here some consideration about the situation presented, in the crystalline state, by the electron donors still available for coordination, namely N(1) and N(7), as well as by the potential electron acceptor, O(6). The structural analysis indicates that the donor atoms N(11), N(71), and N(72) (the second number indicating one or the other of two purine ligands) participate in hydrogen bonds with three water oxygen atoms, O(2), O(3), and O(1), respectively, while the fourth donor atom, N(12), is hydrogen bonded to a chlorine atom, Cl(2). Carbonyl oxygen atoms do not participate in hydrogen bonding, but both O(61) and O(62) approach the imidazole rings in the neighboring dimers, with the C→O vector pointing nearly perpendicular to the plane of the imidazole ring.

The metal complexes of the nonsubstituted bases cannot, however, be directly correlated with those formed in nucleic acids, where ribosyl residues are attached to the base N(9) nitrogens.

As it is the case for 9-substituted adenine, it can be expected that the ligand properties of 9-methylhypoxanthine will be equivalent to those of the corresponding nucleoside, inosine. With N(9) engaged in a covalent bond, the N(7), N(1), and N(3) nitrogen atoms remain here as the high electron density centers still available for coordination. Also, a metal–hypoxanthine complex is very suitable to clarify the role of the electron donor substituent in the 6-position on the stabilization of the complex.

The study of the crystal structure of a copper–hypoxanthine complex with a blocked N(9) position was undertaken by Sletten[71] on a synthesized complex compound with the formula $[(9\text{-methylhypoxanthine})_2Cu^{II}(H_2O)_2]Cl_2 \cdot 3H_2O$. This cupric compound crystallizes as blue prisms when adding an excess of copper chloride to an aqueous solution of 9-methylhypoxanthine. The first results of the crystallographic determinations carried out on these crystals have immediately excluded the formation of any chelate structure, since the cupric complex appears to be monodentate on the imidazole N(7) nitrogen. This fact is compatible with the normal direction of the coordinating orbital on N(7), which prevents the involvement of the O(6) oxygen in a chelate structure. Similar impossibility for chelation has been encountered on metal complexes of nucleotide bases where the substituent on C(6) is an amino group.[72,73]

Careful X-ray diffractometric data obtained also by Sletten[74] on crystals of the complex mentioned above have demonstrated the location of the copper ion at the center of molecular symmetry with a (4 + 2) coordination geometry. This situation resembles very much the one encountered in the complex structure already discussed for the 9-methyladenine–copper coordination[53] (see Figure 2c). However, the relative positions of the ligand atoms in the coordination are different from those of the 9-methyladenine–copper complex of similar composition. Thus, N(7) nitrogens from two 9-methylhypoxanthine molecules and water oxygens from a pair of centrosymmetrically located water molecules lie in the equatorial plane, while the axial positions are occupied by chloride ions 2.787 Å apart from the copper ion (weak axial coordination). The planar purine rings are tilted 25° with respect to the equatorial plane of the octahedral geometry; this position makes possible the formation of intramolecular hydrogen bonds between the water molecules in the coordination sphere and the carbonyl oxygens, O(6). Then, the oxygen atom of the 6-substituent only contributes to consolidate the spatial configuration of the complex (as it is also the case for the cupric complex of 9-methyladenine).

The interest of elucidating the role of anions in these complex structures has been recently pointed out by Sletten,[75] who has undertaken an X-ray diffraction study on monoclinic single crystals of the complex compound $[(9\text{-methylhypoxanthine})_2Cu^{II}(H_2O)_4]SO_4$. Here the sulfate anion links crystallographically independent hypoxanthine ligands by forming hydrogen bonds with the N(1) positions of the purine rings (Figure 7). This is in contrast with the situation found in the corresponding adenine sulfate complex,[52] where the sulfate anion has

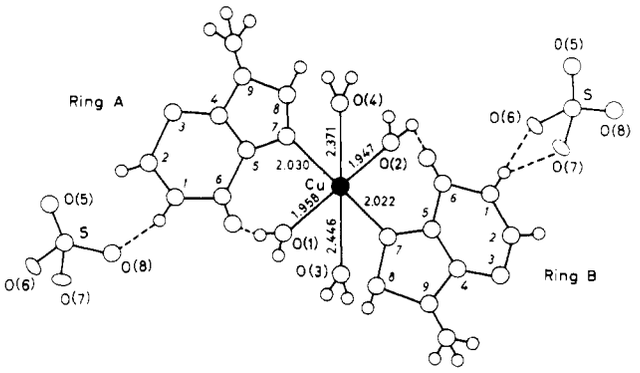


Figure 7. Spatial configuration of the complex compound $[(9\text{-methylhypoxanthine})_2Cu^{II}(H_2O)_4]SO_4$ (after Sletten and Kaale[75]).

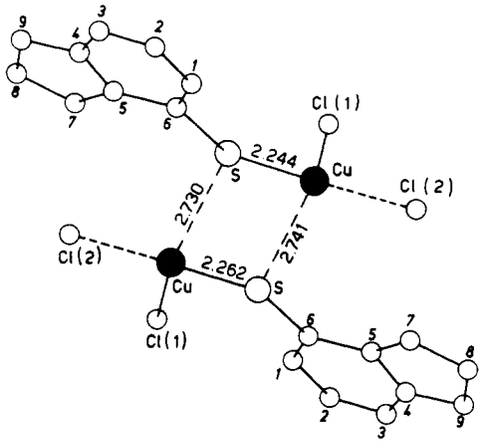


Figure 8. Molecular configuration of the dimer of copper-6-mercaptopurine, formula $[(\text{protonated 6-mercaptopurine})Cu^{I}Cl]Cl\cdot H_2O$ (after Caira and Nassimbeni[76]).

entered the coordination sphere and is hydrogen bonded to the amino substituent at C(6) (see Figure 2b). However, the connection between one of the sulfate oxygens (namely, O(5) in Figure 7) and the group C(2)-H of ring A can be regarded as a pseudo hydrogen bond. The two other sulfate oxygens (namely, O(6) and O(7)) interact, through a bifurcated hydrogen bond, with N(1) of the ring B, arranged in an anti configuration. The pronounced thermal motion of three of the oxygen atoms in the sulfate has been interpreted as a rotation around S–O(5), which tends to shift the sulfate position between slightly different symmetry arrangements. Thus, an axial metal–anion bond is not established in this complex because of the accommodation of hydrogen bonds between purine rings and sulfate anions.

The interaction of metals with mercaptopurines (especially when the sulfur atom is substituted to the hydroxyl oxygen of hypoxanthine) has an actual biological interest because of the significance of 6-mercaptopurine as an anticarcinogenic agent. On this research line, Caira and Nassimbeni[76] have studied a 6-mercaptopurine–cuprous chloride complex, which has the formula $[(\text{protonated 6-mercaptopurine})Cu^{I}Cl]Cl\cdot H_2O$ and has been obtained as deep red crystals from an acid solution (20% HCl) containing 6-mercaptopurine and copper(II) chloride in a 1:1 ratio. The measurement of the magnetic moment of the complex has yielded a value of $\mu = 0.21\ \mu_B$, clearly indicating that Cu(II) has been reduced to Cu(I) by the formation of the complex. Crystallographic determinations have indicated that the protonated 6-mercaptopurine ligand is complexed with copper(I) chloride through a Cu–S bond of normal length. Two crystallographic units of this sort are associated into a S-bridged dimer (Figure 8). The tetrahedral geometry around the copper atom is thus considerably distorted; however, the structure possesses a pseudosymmetry because the midpoint of the line joining the copper atoms is very nearly the center of molecular

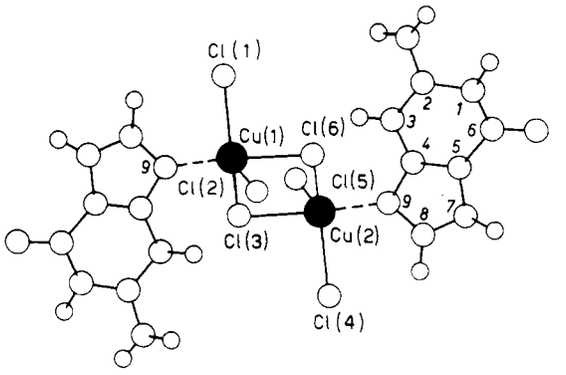


Figure 9. Spatial configuration of the dimeric complex $[(Cu^{II}(\text{protonated guanine})Cl_2]_2Cl_2\cdot 2H_2O$ (after Carrabine and Sundaralingam[72]). Reprinted with permission from ref 72. Copyright 1970, The Chemical Society.

geometry.

From the results on the crystalline state it is then concluded that the mercapto group in mercaptopurines is a strong binding site for copper atoms. In solution, the situation is somewhat dependent on the pH value since one of the two forms in the thiol–thione tautomerism of the mercaptopurine can predominate over the other. Thus in the thione form the base strength of the neighbor nitrogen is sensibly lowered as compared to the thiol form. It also appears, from the K_a values of various mercaptonucleo bases,[77] that the acidic properties of the mercapto group can be enhanced by the presence of some substituents on the ring; by this way, such group can be enabled to bind any metal ion in solution.

3. Complexes with Guanine

As is also the case for adenine and hypoxanthine, the free base guanine shows a tendency to form dimeric complexes with metal ions. However, the only preferred position for coordinated guanine seems to be N(9), as was demonstrated by Carrabine and Sundaralingam.[72] By means of diffractometric methods applied to single crystals, these authors have analyzed the dimeric structure of a guanine–copper chloride complex having the basic formula $[(\text{protonated guanine})Cu^{II}Cl_2]_2Cl_2\cdot 2H_2O$, which is probably protonated on N(3). This dimeric complex is seen to consist of chlorine-bridged copper atoms (Figure 9) possessing a somewhat distorted trigonal-bipyramidal environment. The alteration of normal bond lengths and angles is here brought about in the imidazole portion of guanine because of the perturbation of the π-electron system by the metal ion, which axially coordinates the N(9) position of guanine and one of the chlorine atoms, while the trigonal plane of each pyramid is formed by three Cu–Cl bonds. The N(9) of guanine is thus the only potential binding site which is favored by copper ions in this type of coordination. The N(7) and O(6) atoms are excluded from participating in any chelation and the (Cu(1),Cl(1),Cl(2),Cl(3)) or (Cu(2),Cl(4),Cl(5),Cl(6)) groups of atoms appear to be coplanar. The whole structure seems to be stabilized by hydrogen bonds between Cl(3), Cl(6) and the protonated N(3).

A trigonal-bipyramidal geometry, like that described above, is fairly unusual for a copper(II) complex, as compared to the more common arrangement of a distorted square pyramid.[78] However, a quite similar structure has been recently reported by Villa and co-workers[79] after having interpreted electronic, EPR, and IR spectra, as well as low magnetic susceptibility values, for a family of Cu(II) saltlike compounds having a complex, guaninium-type cation, $[(\text{protonated Gua})Cu^{II}X_2]_2X_2\cdot xH_2O$, where X is a halogen atom and x may be 2 or 0.

The possibility of synthesizing copper compounds which contain halogen-bridged coordination geometries shows that the copper ion exhibits a great ability to adapt itself to different stereochemical situations imposed by the interacting molecules.

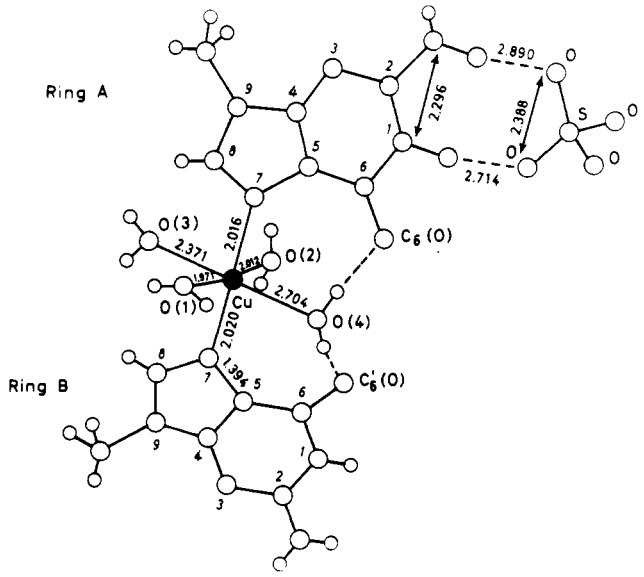


Figure 10. The octahedral coordination geometry in the cupric complex $[Cu^{II}(9\text{-methylguanine})_2(H_2O)_3]SO_4 \cdot 3H_2O$ (after Sletten and Fløgstad[81]).

Another copper(II)–guanine complex, having the composition $Cu(II)(guanine)(ClO_4)_2 \cdot (H_2O)_{3.5}$, has been synthesized by Bonnet and others,[80] but no complete structural studies seem to have been performed on this compound.

Also, an Fe(II)–guanine complex, of the basic formula $Fe^{II}(guanine)(OH)SO_4 \cdot 2H_2O$ has been synthesized and studied by magnetic methods.[57] Here, the magnetic measurements, as well as the EPR spectra, suggest a strong intramolecular spin–spin interaction, for which the formula $[Fe^{II}(guanine)(OH)]_2(SO_4)_2 \cdot 4H_2O$ has been assigned to the complex. The Fe(II) ions are here bridged by the two hydroxyl groups; they coordinate N(9) and N(3) of the two guanine molecules while the four water molecules occupy the axial positions.

By contrast, the dimeric character seems to be absent in the complexes formed by transition metal ions with 9-substituted guanine. A copper sulfate complex of 9-methylguanine, studied by Sletten and Fløgstad,[81] is also an example of an anion which does not enter the coordination sphere of the metal but interacts with the purine ring via hydrogen bonds. This complex compound has the formula $[(9\text{-methylguanine})_2Cu^{II}(H_2O)_3]SO_4 \cdot 3H_2O$ and has an octahedral coordination geometry (Figure 10), with two guanine-N(7) atoms and two water oxygens surrounding Cu in the equatorial plane, while two other water oxygens occupy the axial positions. One of these axial water oxygens is located far enough from the copper ion (2.704 Å) to keep it only weakly bound to the metal; however, the water molecule at this position contributes to the stabilization of the whole structure by extending hydrogen bridges to the carbonyl groups of two syn-arranged guanine ligands. This configuration is opposing that of the chloro complex of 9-methylhypoxanthine,[74] where the purine ligands are anti and the chlorine ions occupy axial positions. A similar situation to that of the chloro is found in the nitrato complex of 9-methylguanine[82] corresponding to the formula $[Cu^{II}(9\text{-methylguanine})_2(H_2O)_2(NO_3)_2]$, where an optimal π interaction between the p_z orbital of N(7) and the d_{xy} orbital of Cu(II) has to be expected, because of the close to 90° angle between the guanine ring and the basal plane of the complex.

In the sulfato complex of 9-methylguanine the noncoordinated anion participates in a strong bis hydrogen bond with both the amino group on the C(2) position and the N(1) nitrogen. It must be pointed out that such type of strong hydrogen bond involving N(1) has also been found by Sletten in a sulfato complex of 9-methylhypoxanthine.[75] The evidence of such a possible arrangement for the sulfato anion points to the importance of the anionic effect on the metal–nucleic acid interactions since the donor properties of the purines may favor a contribution of the anion to the disruption of the base-pairing scheme in a natural polynucleotide.

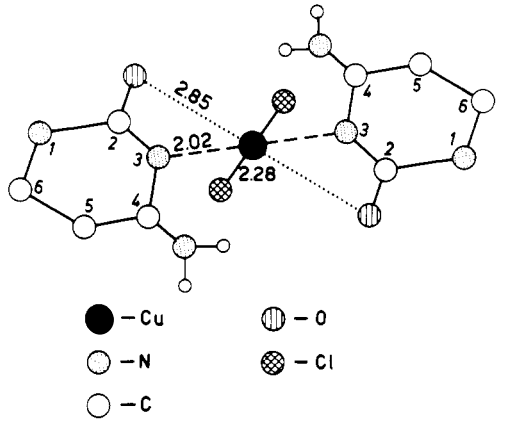


Figure 11. The distorted octahedral arrangement in the cupric complex $[Cu^{II}(cytosine)_2Cl_2]$ (after Carrabine and Sundaralingam.[83] Reprinted with permission from ref 83. Copyright 1968, The Chemical Society.)

4. Complexes with Cytosine

The N(3) position of cytosine is normally involved in hydrogen bonding of nucleic acids (GC pairs). Copper, a metal which disrupts the nucleic acids structures, preferentially binds to GC pairs rather than to AT pairs. This preference seems to be related to the Cu(II) binding to N(3) of cytosine, a site where the metal ion will also interact, in some way, with the carbonyl group of the base. The magnitude of such preference affords an explanation for the selective disruption of the base-pairing scheme in the presence of copper ions.

A demonstration of what has been said above is given by an already classical study of copper–pyrimidine bonding, accomplished in 1968 by Carrabine and Sundaralingham.[83] These authors have described a complex compound which, after being analyzed by X-ray diffractometric methods, was shown to have two chlorine ions and the N(3) nitrogens of two cytosine moieties involved in coordination around a copper atom. Thus, it corresponds to the formula $[Cu^{II}(cytosine)_2Cl_2]$. Here the octahedral arrangement is distorted to such an extent that it appears to be practically square planar (Figure 11). The O(2) atoms of the cytosine bases are found at an average distance of 2.85 Å from the copper ion and only dubiously could it be thought of them as forming a bond. However, some contribution is to be expected from the carbonyl group of cytosine for stabilizing the complexation of the metal ion on its preferred binding site in so highly distorted a configuration. This fact throws some light upon the possibilities to stabilize, by means of complementary donor groups, unusual coordinations in more complicated structures.

A confirmation of the role that the carbonyl group may play in complexation is afforded by the results obtained by Shirotake and Sakaguchi[84] on a (4:1) cytosine–copper complex having the probable formula $[Cu^{II}(protonated\ cytosine)_4Cl_2]Cl_4$. This complex compound has been synthesized by the following operations: (a) dissolution of cytosine and copper chloride in 0.1 N hydrochloric acid; (b) application of a long high-temperature treatment; (c) crystallization through a very slow concentration process. The yellow crystals of the compound give infrared absorption bands at 840, 1572, and 3140 cm^{-1} which, from a comparison with the IR spectrum of cytosine hydrochloride, are assignable to the N(3)–H out-of-plane deformation, the N(3)–H in-plane deformation, and the N(3)–H stretching vibrations, respectively, in a cytosinium copper chloride complex. These observations clearly indicate that the N(3) site is protonated in the complex and thus excluded from a direct interaction with copper ions.

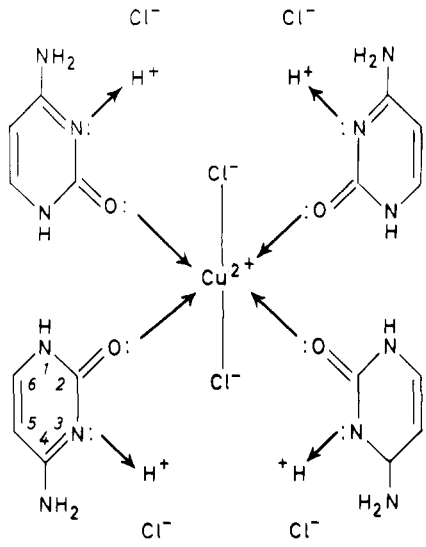


Figure 12. Presumed octahedral configuration of the cupric complex $[Cu^{II}(protonated\ cytosine)_4Cl_2]Cl_4$ (after Shirotake and Sakaguchi[84]).

Also, the cytosinium copper chloride complex presents a band at 1730 cm^{-1}, which has to be ascribed to the C(2)=O stretching vibration; however, this band is higher by 10 cm^{-1} than the corresponding one in cytosine hydrochloride, which suggests that copper ion coordinates with the carbonyl group (Figure 12).

On the other hand, the proton NMR spectrum of cytosine dissolved in CF_3COOH in the presence of copper chloride shows a broadening of the H(6) resonance that would indicate the binding of the copper ion to the N(1) nitrogen or to the C(2)=O group of the cytosine; however, N(1) has to be excluded as a binding site for copper ions when, under a strong acid condition, it also becomes protonated. In fact, the IR spectrum of the cytosinium copper chloride complex confirms the protonation of N(1) under highly acid condition because the bands assigned to the N(1)–H out-of-plane and the N(1)–H in-plane deformation vibrations are observed. These results favor the interpretation that, in the copper complex of protonated cytosine, the C(2)=O site remains as the only possible binding site for copper ions. As a contrast, the proton NMR results obtained from cytosine dissolved in Me_2SO-d_6 in the presence of copper chloride support the interpretation that copper ion coordinates with both the N(3) and the C(2)=O sites in a (2:1) cytosine–copper chloride complex of the type reported by Carrabine and Sundaralingam.[83]

It is interesting to point out here that the unusual polymeric silver(I) complex of 1-methylcytosine, where the metal binds to the exocyclic oxygen at C(2) of the base, has been reported by Marzilli and co-workers.[85] In fact, the binding at the C(2)=O site is strong for only one Ag(I) but it is still able to bind a second Ag(I). Also, it has been found that manganese forms a strong bond (2.08 Å) with the C(2)=O oxygen of the 5′-CMP ring in an octahedral complex of Mn(II) and cytosine 5′-monophosphate.[86]

All these observations point to the role that O(2) of the cytosine base may play in the formation of biopolymeric complexes.

5. Complexes with Uracil and Thymine

Uracil and thymine are weak acids in which two positions, namely C(2)=O and C(4)=O, have approximately equal tendencies for enolization and ionization. However, the enolization on one of these positions inhibits that of the other; uracil (or thymine) has then only one dissociation constant. Theoretical calculations[87] have indicated that the above-mentioned positions are the most probable binding sites for Mg(II), whereas the interaction of this metal ion with the rest of the base is essentially repulsive. However, the character of the uracil interaction with transition-metal ions is not expected to be the same as with Mg(II).

The uracil binding sites for transition-metal ions in aqueous solution have been investigated by potentiometric methods.[88] However, the possibilities of these measurements have to be limited to a selected pH region, because of the low solubility of the uracil base. The formation of very low concentrations of uracil complexes of Cu(II) and Ni(II) have been detected by using specific metal ion electrodes. They are saltlike (1:1) complexes, which are formed through the release of a proton. Their only possible complexing site appears to be N(3), since deoxyuridine (where N(1) is bonded to a sugar molecule) also forms (1:1) metal complexes, whose stabilities ($K \simeq 10^3$ for Cu(II) and $K \simeq 10^2$ for Ni(II)) are comparable to those of the complexes formed by uracil and the same metal ions. Also, the possibility of a chelation has to be excluded in uracil as well as in deoxyuridine because negative 5-substituents do not increase at all the stability constants of the complexes.

In solvents other than water, an indirect interaction of copper ions with the N(1) nitrogen of uracil has been detected in the proton NMR spectrum of the nucleobase.[89] In particular, the addition of Cu(II) to a Me_2SO-d_6 solution of uracil causes a splitting of the H(3)–H(1) originally broad line, together with the broadening of the H(5) and H(6) doublets. These variations in the NMR spectrum of uracil, induced by the presence of Cu(II), have been interpreted as effects of the interactions of the metal ions with the solvent molecules which are hydrogen bonded to the uracil N(1)–H and N(3)–H positions. Splitting of the H(3)–H(1) peak by the addition of Cu(II) is caused by an upfield shift of the H(1) peak (which can be identified in a double-resonance experiment) that means a weakening of the hydrogen bond at N(1).

It has to be expected that electron-releasing substituents (such as OH, CH_3, CH_2OH) at the uracil C(6) position would also modify the strength of the hydrogen bond between the proton on N(1) and the S=O group of the solvent molecule. In such a case, a direct interaction between Cu(II) and N(1) could occur because of the higher electron density at N(1) and the weaker hydrogen bond with the solvent. Optical studies[90] show the appearance of a new absorption band when Cu(II) is added to a solution of 6-methyluracil in Me_2SO, which indicates the formation of a charge-transfer complex between the copper ion and the methylated uracil. The electron charge transfer within the complex results in the reduction of Cu(II) to Cu(I); simultaneously, the proton NMR spectrum of the solution indicates that a further shift of the H(1) peak by the addition of Cu(II) does not take place in this system. It is possible, also, to induce an opposite (electron attracting) effect by means of a sulfur substituent on C(2). In the latter case, the sulfur atom competes with N(1) for the interaction with Cu(II), as evidenced by the appearance of a new peak in the EPR spectrum[90] of Cu(II) ions in Me_2SO when 2-thiouracil is added to the solution.

Thiouracils are minor components of transfer RNA, and their interactions with metal ions can be of special biological interest because of the possible role played by metal complexes of thio bases in protein synthesis. Great efforts have recently been devoted to the study of these interactions.

By following the thio base line of work, Hunt and co-workers[91] succeeded in crystallizing, by using Me_2SO as solvent, a thiouracil–copper(I) complex which can be precipitated from a warm aqueous solution of $CuCl_2$ and 2-thiouracil. The formula of the complex is $[(2\text{-thiouracil})_2Cu^{I}Cl]\cdot Me_2SO$, and its structure, according to X-ray crystallographic measurements, consists of nearly planar neutral molecules having corresponding molecules of Me_2SO as solvate (Figure 13). A contribution to the stabilization of the planar molecular geometry is made, in the crystal, by the base stacking between molecules as well as by some strong hydrogen bond between the C(2)–OH group of one

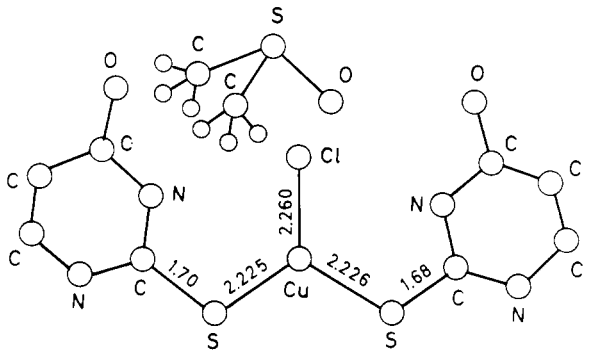


Figure 13. A nearly planar neutral molecule of the cuprous complex $[(2\text{-thiouracil})_2Cu^{I}Cl]$, having a Me_2SO molecule as solvate (after Hunt, Griffith, and Amma[91]).

molecule and a nitrogen site of another. The planar coordination around Cu(I) is thus formed by three atoms: two sulfurs and one chlorine. The positions of the sulfur atoms indicate that sp^2 orbitals and electron pairs are involved in the binding to the metal whereas the bond distances within the 2-thiouracil moieties are evidence for the keto (dilactam) form of the ligand. 2-Thiouracil acts then as a neutral ligand donating through the sulfur atom, which confirms the general observation that nitrogen atoms of uracil (and thymine) are normal donors only when the molecule is under strong basic conditions.

On the other hand, X-ray photoelectron spectrometry has shown[92] that the correct oxidation number of copper in the complex with 2-thiouracil (and also with 6-amino-2-thiouracil and 6-methyl-2-thiouracil) is always 1, regardless of the mode of preparation.

A number of complexes of Mn(II), Co(II), Ni(II), and Cu(II), containing uracil and thymine as neutral ligands, have also been synthesized by Goodgame and Johns,[93] using a variety of anions for the metal salts and ethyl acetate as solvent. Examples of typical complexes of this series are $[Mn(ClO_4)_2(ura)_2]\cdot H_2O$ (pale pink), $[CoCl_2(ura)(H_2O)_2]$ (pink), $[Ni(NO_3)_2(thy)_2]\cdot 4H_2O$ (pale green), and $[CuCl_2(thy)]\cdot 2.5H_2O$ (yellow-green). All complexes are obtained as microcrystalline powders that are insoluble in nonpolar solvents and become decomposed by polar ones.

Electronic spectra suggest that the complexes have essentially octahedral structures; however, for $[CoBr_2(thy)_2]$, the intensities and band profiles in the visible region are in agreement with a tetrahedral structure.

The EPR spectra are consistent with six-coordination in most cases. The single, rather broad EPR signals of the manganese halogeno complexes are indicative of polymeric octahedral structure, as it is also the case for $[CuCl_2(ura)]\cdot H_2O$.

Neutral ligands, where the donor atom is probably a carbonyl oxygen, generate a necessarily very weak field; however, both oxygen atoms in uracil and thymine are expected to act as moderately strong π donors for which it can be presumed that metal–ligand bonds may be fairly strong. The coordination by means of the C=O group seems to be confirmed, in the case of uracil, by the IR spectra of the ligands in the carbonyl stretching region, since a considerable variation is seen in the spectra after complexation.[93] The most striking evidence for the existence of a strong copper–O(4) bond, however, comes from the recent X-ray crystal determination on the bis(1,3-dimethyluracil)dichlorocopper(II) complex, reported by Cartwright et al. in 1978.[94]

Recently, some uracil and thymine analogues, having more than two ring nitrogens, have been studied as possible ligands for transition-metal ions. Mosset and co-workers[95] have reported the molecular and crystalline structure of a complex formed by copper(II) with 6-azauracil, an analogue of uracil having a nonprotonated nitrogen in the 6-position of the ring. Such metallic complex is of the 1:2 type and has the formula

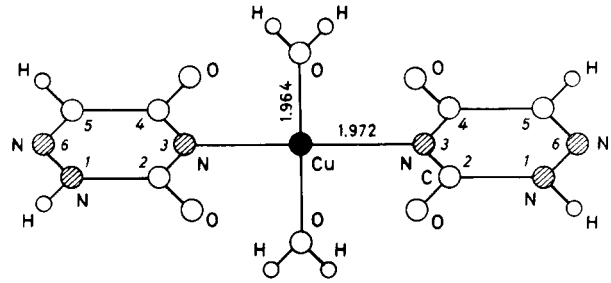


Figure 14. Square-planar geometry around the copper atom in the cupric complex $[Cu^{II}(6\text{-azauracilato})_2(H_2O)_2]$ (after Mosset, Bonnet, and Galy[95]).

$[Cu^{II}(6\text{-azauracilate})_2(H_2O)_2]$. The structure of this complex compound was crystallographically analyzed by application of the heavy atom method, which has revealed a square-planar environment for the copper atom (Figure 14). Here, two azauracil ligands are monodentate by way of their N(3) nitrogens, whereas two coordinated water molecules are arranged in trans positions. The distances Cu–O and Cu–N(3) have similar magnitudes and the regular square-planar geometry is thus practically not distorted by the 6-azauracil ligand. However, the heterocycle of the ligand is longer in the N(6)–N(3) direction than it is in the free 6-azauracil. It is known[96] that protonated and nonprotonated nitrogens of hexagonal heterocycles form internal angles of 125 and 115°, respectively. The reduced angle on N(3) observed when 6-azauracil is bonded to the metal strongly indicates that protonated N(3) in the free base has passed to the electronic situation of a nonprotonated nitrogen in the complex. Thus, the potential acceptor N(6) seems not to be coordinated by the metal to form any complex structure, but replaced by N(3). The third ring nitrogen, namely N(1), is the only one to have some participation in the stabilization of the crystalline structure by means of the N(1)—H···O(2) hydrogen bond.

Investigations on structures and functions of metallic complexes formed by a variety of biologically active analogues of nucleic acid constituents appear not to be completed.

B. Complexes with Nucleosides

Role of the Ribose Moiety in the Formation of the Complex. Nucleosides, having a ribose or deoxyribose moiety attached to a purine or pyrimidine base, are expected to form loosely associated complexes with first transition series metal ions.

The current methods for the study of the solid state would thus seem to be appropriate to detect the weak interactions involved in the metal–nucleoside complexation. However, the complexes formed by transition-metal ions with ribo- and deoxyribonucleosides have appeared little suitable for X-ray diffraction experiments because of the difficulties in crystallization.

As an alternative to the diffraction methods, various other experimental techniques have been applied to the study of lyophilized samples obtained from (generally 1:1) metal ion–nucleoside solutions. Thus, in an attempt to interpret the IR spectra of freeze-dried Cu(II)–ribonucleoside complexes, Tu and Friederich[97] have applied the freeze-drying treatment for adenosine and inosine in the presence of Cu(II) ions and have pointed out a considerable decrease in the intensity of the stretching vibration band in the region of 1680–1700 cm^{-1}, generally attributed to keto groups in aromatic rings, for both ribonucleosides. Here the simultaneous appearance of an intense band within the region of the aromatic C=C and C=N stretching bands in purine rings, precisely at 1610 cm^{-1}, also indicates that some sort of complexation is taking place between each purine ribonucleoside and the copper(II) ion.

Solid-state IR spectra have also allowed the detection of very weak interactions of Mn(II) ions with inosine and guanosine[98]

which closely resemble those already described for the Cu(II) ions.[97] Also, the results of IR spectroscopy in the solid state suggest the enolization of Guo and Ino upon formation of complexes with Cu(II) and Mn(II) ions, since, according to Miles,[99,100] the enolic bands of both Guo and Ino are expected to be found at or near 1615 cm^{-1}.

Metal–nucleoside complexation in solution offers a wide field for the application of advanced techniques. Here, dimethyl sulfoxide has been used as the main nonaqueous solvent, since it provides adequate solubilities for nucleosides as well as for metal salts; it also excludes any proton exchange, thus making the solutions particularly suitable for the detection of any possible engagement of base amino groups in complexation. Furthermore, the interaction of metal ions with the hydroxyl groups of nucleoside ribose or deoxyribose would be favored by the nonaqueous solvent. On the other hand, the fairly rapid exchange of molecules interacting with the metal ions in a Me_2SO solution would make them almost equally affected by the chemical interaction.

The results obtained by Eichhorn and co-workers[20] in 1966, in their NMR studies in Me_2SO solution, had already shown that the broadening effect of Cu(II) ions on the base H(8) line of deoxyguanosine is lacking the NH_2 proton lines, thus ruling out the possibility of a chelation with the amino group. Although some shifts of the NH_2 proton lines of adenosine in the presence of Zn(II) ions have been reported by Wang and Li[101] in 1968, this fact could be interpreted as an electronic perturbation at the level of the amino groups because of the binding of the metal ion on some other site of the molecule, without involving directly the NH_2 group attached to C(6). Such a point of view is supported by the fact that a similar shift is observed for the ^{15}N NMR line of the N(9) nitrogen of adenosine, to which the ribose moiety is attached, and by theoretical speculations considering the requirement of a mobilized electron from the amino nitrogen for the stabilization of an electron-deficient ring system.[102]

On the other hand Chang et al.[103] have reported an effect of the chloride counterions of divalent metal ions on guanosine molecules in Me_2SO solution, consisting of ^1H NMR spectral shifts of the base amino protons. By continuing these studies, Yokono and co-workers[104] have been able to demonstrate that both metal ions and anions can influence the chemical shifts in ^1H and ^{13}C NMR spectra of nucleosides in Me_2SO solution. It has also been observed by Shimokawa and co-workers[105] that addition of a divalent metal salt to a Me_2SO solution of cytidine reduces the relaxation time T_1 of the ^{13}C atom at the C(4) position, while the T_1 values of the other base carbon atoms remain practically unchanged. Further studies are obviously needed to clarify the metal interactions, especially those of transition metals, in the vicinity of the cytidine C(4)–NH_2 group.

Some important investigations on specific interactions of Cu(II) ions with non-amino-group-containing pyrimidine nucleosides in Me_2SO solution were undertaken by Berger and Eichhorn[106] in 1971. For uridine, which has an hydroxyl group at the C(4) position, they found a broadening of the base H(3) proton line which they have interpreted in terms of the binding of the metal ion with the oxygen atom at C(4). No interaction could instead be ascertained by the same authors between Cu(II) ions and deoxythymidine, which also has an hydroxyl group at C(4).

Fourier-transform (FT) natural-abundant ^{13}C NMR, which is being currently used as a nuclear probe to test previous results obtained by ^1H NMR, has been particularly useful to clarify the early results of Berger and Eichhorn.[106] Thus, by using the ^{13}C NMR approach, Fritzsche and others[107] have carried out a study on the effects of the presence of both Cu(II) and Mn(II) ions on nucleosides in Me_2SO solutions. Their experiments were based on the assignments made by Jones et al.[108,109] of the ^{13}C NMR peaks in the spectra of the base moieties; also, a precise order of peak broadening induced on the spectral lines by metal

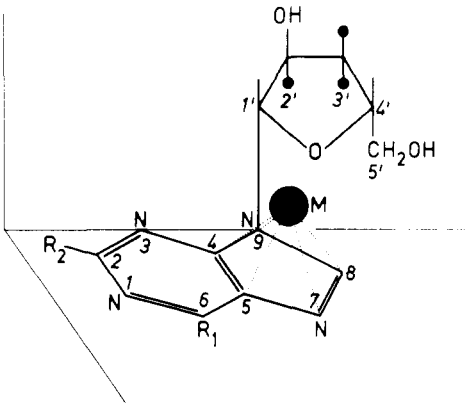


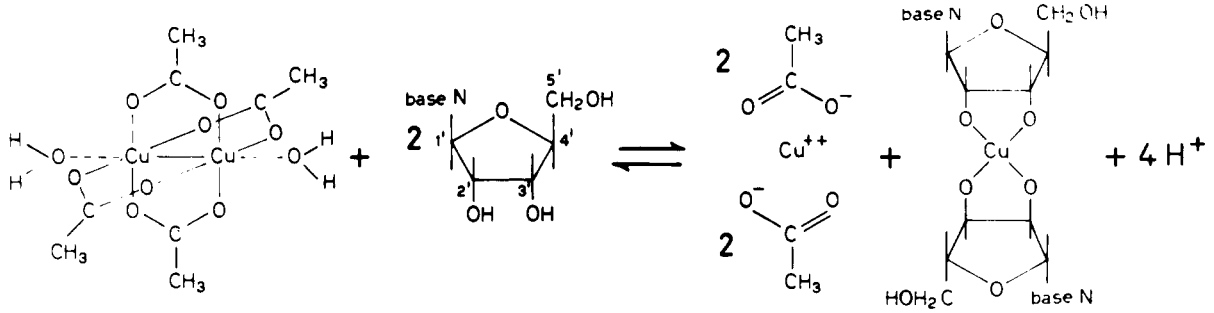
Figure 15. Probable location of a transition metal ion complexed with a purine nucleoside (in the figure a purine deoxyribonucleoside) in Me_2SO; a similar situation would be expected in neutral aqueous solution. The scheme is mainly based on data for the Cu(II) ion (after Berger and Eichhorn[106] and Fritzsche, Arnold, and Krusche[107]).

ions was determined from plots of measured line widths, $\Delta\nu_{1/2}$, vs. the concentration [M]/[L] = [metal ion]/[ligand]. Possibly, a quantitative revision of the data would be needed in the light of a nonnegligible scalar interaction effect. Fritzsche's investigations have indicated that the C(4) and C(2) peaks of the pyrimidine nucleosides cytidine and deoxycytidine are selectively broadened by the presence of Cu(II), thus suggesting an interaction of this metal ion with N(3). By contrast, a rather wide action was found to be exerted by the Cu(II) ion on deoxypurine nucleosides, as shown by the fact that this metal ion preferentially broadens the C(8), C(4), and C(5) peaks in the ^{13}C NMR spectra of both deoxyguanosine and deoxyadenosine in Me_2SO solution. Instead, the broadening effect of Cu(II) ions appears to be highly selective for the C(4) and C(8) peaks of inosine, the ribonucleoside of hypoxanthine. Mn(II) ions also induce selective broadening effects on the ^{13}C NMR spectra of purine nucleosides in Me_2SO solution, as has been observed by Fritzsche and co-workers;[107] such effects take place on the C(8) and C(6) peaks of the base residue of guanosine as well as on the C(6) peak of inosine. The results point then to N(7) of the purine nucleosides as the most favorable site for the interaction with both Cu(II) and Mn(II) ions. No broadening effects, on the other hand, have been observed on the ^{13}C peaks of the ribose moieties during Fritzsche's experiments.[107] However, a weak interaction of all the hydroxyl groups of the uridine ribose moiety had already been detected by Berger and Eichhorn in Me_2SO solution.[106]

The conclusion derived from the experimental results obtained in Me_2SO solution is that the interaction of a transition-metal ion with either a purine or a pyrimidine nucleoside probably takes place by the metal approaching to the plane of the base five-membered ring. The metal ion would locate itself on a plane parallel to this ring, and under the ring of the ribose moiety (Figure 15). The nature of both the metal ion and any substituent in the base ring may alter this scheme slightly; however, the whole situation always suggests a possible interaction with the ribose hydroxyl groups.

A distinction has to be made, however, when considering metal ions as a part of a dimeric species, since the interaction of a metal ion pair with the 2′- and 3′-hydroxyl groups of the ribose ring would appear to be particularly favored by stereoselective considerations. Such an hypothesis has appeared to be supported by the variations of the optical spectra of copper(II) acetate in Me_2SO by the effect of the presence of ribonucleosides.[110] The result of the interaction would be the formation of a dimeric 1:1 copper(II) acetate–ribonucleoside complex and the release of two acetate ligands; thus, the sugar 2′- and 3′-hydroxyl groups would lead to the formation of a chelate ring. By contrast, deoxyribonucleosides would not be

SCHEME I. Disruption of the Dimeric Structure of Cu(II) Acetate by a Ribonucleoside in Me_2SO Solution, with the Formation of a Monomeric Cu(II) Species[a]



[a] Protonation of the base N(3) nitrogen keeps it out from being a major donor site (after Brun, Goodgame and Skapski[111]).

able to form such stabilized complex structures. However, EPR studies carried out by Brun and co-workers[111] have shown that addition of a ribonucleoside to a Me_2SO solution of copper(II) acetate results in the change from $S = 1$ EPR bands (characteristically distributed in a wide spectral range, as for any dimeric copper(II) compound) to a typical $S = {}^1/_2$ EPR band, thus indicating the cleavage of the dimer to give a monomeric copper(II) species.

It is already known[112] that polyalcohols, especially *cis*-glycols, can form strong complexes with transition-metal ions and that, in the case of the Cu(II) ion, such complexes are monomers having a square-planar coordination and containing two chelate rings.[113] Hence, ribose as well as deoxyribose and ribonucleosides will disrupt the dimeric structure of copper(II) acetate through the formation of chelate rings with the donor hydroxyl groups.[114]

The formation of chelate rings in metal–nucleoside complexes has been demonstrated in some particular cases;[115,116] moreover, a nonaqueous solvent such as Me_2SO may favor the interactions of metal ions with the hydroxyl groups.

Thus, the overall reaction of a ribonucleoside with the copper(II) dimer will be that shown below. (See Scheme I.)

$$[Cu^{II}(OAc)_2]_2{\cdot}2H_2O + 2\text{ribonucleoside} \rightleftarrows Cu^{II}(OAc)_2 + Cu^{II}(\text{ribonucleoside})_2 + 2AcO^-$$

Monodentate ligands, such as water and monoalcohols, would also interact with the copper(II) acetate dimer, but their driving force in the reaction

$$Cu^{II}\ \text{dimer} \rightleftarrows Cu^{II}\ \text{monomer}$$

will depend on their capacity to form a strongly bound monomeric complex.

^1H NMR experiments[106,114] support that point of view, since it was shown that all the hydroxyl proton peaks in the ribonucleosides as well as in the deoxyribonucleosides in Me_2SO solution are broadened by the effect of Cu(II) ions. Such results strongly indicate that all the donor hydroxyls are competing for coordination with Cu(II) ions in Me_2SO solution. It is suggested that even the base N(3) and N(7) nitrogens could operate, through the favorable formation of a chelate ring, to disrupt a copper(II) dimeric structure. The various extents to which such interactions occur, according to the nature of the molecules competing with the acetate ligand, have been demonstrated by performing X-band EPR spectra of the frozen Me_2SO solutions.[111] Ribo- and deoxyribonucleosides could then be differentiated by way of the relative ease by which they cleave the copper(II) dimer.

The nature of the interactions which, under physiological conditions, take place between metal ions and either ribo- or deoxyribonucleosides must obviously be studied in aqueous solution.

Since the base ring imino group, when in contact with polar solvent molecules, would undergo a keto–enol tautomerism, it is expected that transition-metal ions would associate weakly with the basic sites of nucleosides in aqueous solution.

Early attempts to measure the low formation constants of metal–nucleoside complexes in aqueous solution were made by using potentiometric and EPR methods.[7,20,97,117] The ability of deoxyribonucleosides to form Cu(II) complexes, as determined from the measured EPR g values, was found to decrease in the order deoxyguanosine > deoxycytidine > deoxyadenosine,[7] and no interaction was detected for deoxythymidine. The same method was used by Bemsky and co-workers[118] to establish the corresponding sequence for ribonucleosides: cytidine > guanosine > adenosine > thymidine > uridine = deoxyribose.

On the other hand, the results of IR spectroscopy for D_2O solutions of dGuo, dCyd, and Ino have shown that additions of Cu(II) ions result in the appearance of a new band at 1562 cm^{-1}, due to complex formation. However, neither a decrease of the keto stretching band at 1680–1700 cm^{-1} nor an increase of the enolic band at 1615 cm^{-1} have been detected in the IR spectra.[119] These observations are strong evidence for the existence of the keto form in the copper(II)–nucleoside complexes. However, no new band can be detected in the IR spectra of 6-aza-Cyd in D_2O solution upon the addition of Cu(II) ions,[119] which suggests that the nitrogen atom occupying the 6-position of the cytosine pyrimidine ring has an important influence on the whole electronic distribution. The determination of the EPR spectral g values has confirmed these results[119] and further suggests a stronger association of copper(II) ions with purine nucleosides than with pyrimidine nucleosides.

The pH of an aqueous solution can obviously be an important factor in determining the nature of the interaction sites in metal–nucleoside complexes. Actually, it has been found that adenine binds copper(II) ions in acid as well as in neutral solutions, without releasing protons;[117] however, the corresponding nucleoside, Ado, can form a variety of complexes which can be differentiated by their kinetics of formation and dissociation in thermal relaxation and stop-flow experiments.[120] As an example, adenosine in basic aqueous solution can form two types of complex with Cu(II) ions, by release of one or two protons from the hydroxyl groups of ribose.[121] However, Anderson and co-workers[122] have indicated the preferential interaction of Mn(II) ions in D_2O solution with the guanine moiety of both Guo and dGuo, as seen from the broadening effects on ^1H NMR spectra. Also, Kotowycz and Suzuki[123,124] have studied the effects of the Mn(II) ion on the proton-decoupled ^{13}C NMR spectra of the nucleosides Cyd and Urd in D_2O solution adjusted to pH 7.0 (pD = 7.4) and have found that the metal ion broadens the C(2) carbon resonance preferentially to the C(4), C(5), and C(6) carbon resonances and to the ribose carbon resonances. Thus, the results of Kotowycz and Suzuki essentially agree with those of Fritzsche and co-workers[107] for the interactions of Cu(II) and Mn(II) ions with pyrimidine nucleosides in Me_2SO solution.

The EPR spectra of copper(II) ions in aqueous solution and in the presence of various nucleosides, at pH 5.5–5.6, were analyzed by Maskos[125] in 1974. The delocalization of spin

TABLE I. Spin Hamiltonian Constants Indicating Covalency of Bonds between Cu(II) and Nucleoside Bases (after Maskos[125])

nucleoside	$g_\parallel$ = −2.0023	$g_\perp$ = −2.0023	α^2	α'^2	β_1^2
cytidine	0.230	0.020	0.697	0.401	0.804
deoxycytidine	0.236	0.020	0.697	0.401	0.816
guanosine	0.273	0.048	0.707	0.391	0.919
deoxyguanosine	0.272	0.046	0.709	0.389	0.914
adenosine	0.276	0.052	0.704	0.394	0.932
deoxyadenosine	0.275	0.054	0.701	0.397	0.942

TABLE II. Properties of the Complexes Formed by Copper(II) Ions and Adenosine at Different pH Values (after Chao and Kearns[126])

complex species	pH[a]	probable ligand	magnetic property
I	3.5	water	paramagnetic
II	6.5	a ring nitrogen	paramagnetic
III	9.5	ribose hydroxyls	diamagnetic
IV	11.6	ribose hydroxyls	paramagnetic

[a] Each pH corresponds to a maximum of formation for each complex.

density which is brought about by complexation is shown here by the detected superhyperfine structure of the spectra and is an indication of the covalency of the bonds between the central atom and the ligands. The corresponding EPR data can be interpreted on the basis of the theory of molecular orbitals[32,33] from the values of the following EPR parameters for the unpaired electron: α^2, spin density on the d_{x-y} orbital of the central ion (σ bond); β_1^2, spin density on the d_{xy} orbital of the central ion (π bond); α'^2, spin density of the unpaired electron on the ligand.

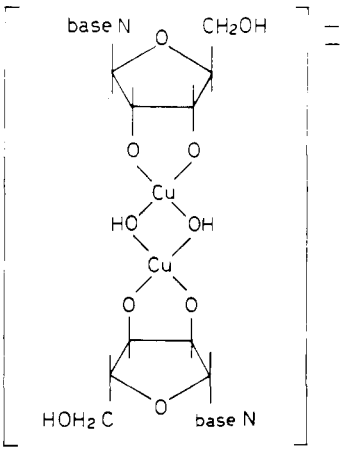
The values obtained for the EPR parameters, including the g values, indicate a narrow range of variation in the covalent nature of the copper–ligand bonds for different ribo- and deoxyribonucleoside complexes.[125] Furthermore, no selective differences are found in the binding with Cu(II) between ribo- and deoxyribonucleosides. The sequence of decreasing covalency for Cu(II)–nucleoside complexes is the same as that of the decreasing basicity of the donor nitrogen sites: N(3) of cytosine > N(7) of guanine > N(7) of adenine (Table I). It is interesting to note the high degree of covalency of the copper(II)–cytidine complex as derived from the values of the parameters mentioned above.

The relationship between covalency of the bond and basicity of the donor atom in the ligand points to the effect of pH on the mode of interaction of the nucleoside bases with the copper(II) ion. Following this line of research, Chao and Kearns[126] have examined the complexation of copper(II) ions with various nucleosides over the pH 3.5–12 range, using the EPR technique. Instead of water as a solvent for the system, these investigators have used a H_2O–Me_2SO glass (at 77 K), which effectively prevents the rotational motion of the complexes, thus avoiding some spectral complications. Their results for copper(II)–adenosine complexes are summarized in Table II.

NMR experiments performed by Chao and Kearns[126] in Me_2SO at "pH" 9.5 have confirmed the formation of a soluble diamagnetic copper(II) complex with adenosine, because no broadening of the ribose proton peaks is seen, but a well-resolved splitting in the H(1′) resonance is observed. Deoxyadenosine is unable to form a similar soluble diamagnetic complex at pH 9.5, thus indicating the requirement of the vicinal hydroxyl groups of ribose for the formation of the diamagnetic Cu(II)–adenosine complex. The proposed structure for the latter can be seen in Scheme II. A further increase of the pH leads to a paramagnetic monomeric structure.

The nature of the copper(II) complexes of guanosine in aqueous solution also depends on the pH, but in the case of this nucleoside no diamagnetic complex is formed at high pH. A

SCHEME II. Proposed Structure for the Diamagnetic Cu(II)–Adenosine and Cu(II)–Uridine Complexes, Formed in Aqueous Solution at pH Values between 8 and 10 (after Chao and Kearns[126])



SCHEME III. Proposed Structure for the Paramagnetic Cu(II)–Guanosine Complex, Formed in Aqueous Solution at about pH 10 (after Chao and Kearns[126])

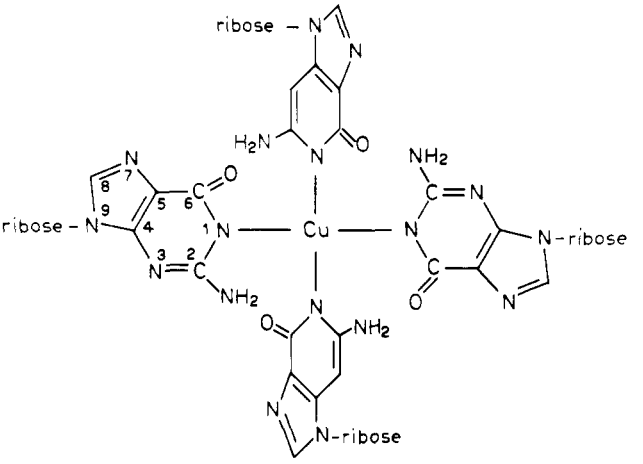


TABLE III. Properties of the Complexes Formed by Copper(II) Ions and Guanosine at Different pH Values (after Chao and Kearns[126])

complex species	pH	probable ligand	magnetic property
I (partial)	2.8	water	paramagnetic
II (partial)	2.8	a ring nitrogen	paramagnetic
II	6.6	a ring nitrogen	paramagnetic
III	9.9	ring nitrogens (at least two)	paramagnetic
IV	11.5	ribose hydroxyls	paramagnetic

description of the situation is given in Table III.

Possibly, the diamagnetic copper(II)–ribose species is prevented from being formed in the case of guanosine because of the ionization, at high pH, of the proton associated with the keto–enol tautomerism between ring positions 1 and 6. Such ionization favors a strong binding of copper(II) to the N(1) nitrogen, thus resulting in the formation of a paramagnetic complex. The EPR spectrum of the latter shows a superhyperfine structure which can be fitted by two sets of five lines each, each set corresponding to a pair of equivalent nitrogens. From the EPR data it appears that each copper(II) ion could be bound to the four N(1) nitrogens of two guanosine pairs, each pair being contained in one molecular plane (Scheme III). A further increase in the pH (up to 11.5) leads to the formation of a monomeric complex presenting EPR parameter values identical with those already observed for the adenosine complex at the same pH; thus the ligand groups for Cu(II)–guanosine would also

TABLE IV. Properties of the Cu(II)–Cytidine Complexes at Different pH and Cyd/Cu(II) Values (after Chao and Kearns[126])

complex species	pH	Cyd/Cu(II)	probable ligand	magnetic property
I	7.0	1:1	water	paramagnetic
II	7.0	1:1	a ring nitrogen	paramagnetic
III	7.0	26:1	ring nitrogens (4 equiv nitrogen)	paramagnetic
III	5.9	8:1	ring nitrogens (4 equiv)	paramagnetic
IV	5.9	8:1	a ring nitrogen (and probably H_2O)	paramagnetic
V	10.2	8:1	ribose hydroxyls	paramagnetic

TABLE V. Properties of the Complexes Formed by Copper(II) Ions and Uridine at Different pH Values (after Chao and Kearns[126])

complex species	pH	probable ligand	magnetic property
I	4.0	water	paramagnetic
II	6.0	a ring nitrogen	paramagnetic
III	8.2–10	ribose hydroxyls	diamagnetic
IV	11.4	ribose hydroxyls	paramagnetic

be the ribose hydroxyls of two nucleoside molecules.

In the case of copper(II)–cytidine complexes, whose nature in aqueous solution is dependent on both the pH and the cytidine-to-copper ratio, the EPR studies[126] were aimed at analyzing these dependencies. Table IV shows the results of the experiments.

At neutral pH and with equimolar concentrations of cytidine and Cu(II) ions, the relative proportions of Cu(II) free and bound to the base N(3) nitrogen can be estimated from the EPR pattern, where the characteristics of a Cu(II)(Cyd) complex seem to be present. However, when the Cyd/Cu(II) ratio is very high at neutral pH, the superhyperfine pattern of the EPR spectrum indicates the formation of a $Cu(II)(Cyd)_4$ complex. The latter is also formed at pH 5.9 with a Cyd/Cu(II) ratio of 8:1, together with a minor component with the probable formula $Cu^{II}(Cyd)(H_2O)_3$.

For copper(II)–uridine complexes in H_2O–Me_2SO solution, the EPR spectra suggest N(3) as the base binding site. The results of the analysis of the EPR data are summarized in Table V.

It can be presumed that, at neutral pH, copper(II) binds to the N(3) nitrogen of uridine in the tautomeric state, which results in a weak association. The ionization process for uridine at pH 8.2–10 appears instead to be unfavorable for the binding of two or more uridine molecules, through their N(3) nitrogens, to a single copper(II) ion. Since no EPR spectrum is detected under the latter conditions, the most probable structure to be formed would be that of a dimeric diamagnetic copper(II)–ribose complex, which is disrupted into a monomeric structure at higher pH.

As a result of these studies, it can be concluded that pH is an important parameter in determining the ratio of the metal–nucleoside complexes in aqueous solution. Also, the concentration ratio [metal]/[nucleoside] can be a determining factor. The ability of copper(II) to distinguish among different ligands, at a convenient pH, also enables this metal ion to be used for reaction controls.

C. Complexes with Mononucleotides

1. Interaction with the Base Portion of the Nucleotide

The first series transition-metal ions have not been seen to cause any appreciable change in the IR absorption peaks attributable to the base portions of 5′-AMP and 5′-GMP in D_2O solutions, at least under conditions which make the C=O, C=C, and C=N bands sensitive to interactions of heavy metal ions with the π-electron system of the bases.[127] However, it is expected that Cu(II) ions would interact more strongly with the electron donor groups of the purine and pyrimidine bases of mononucleotides than they do with the phosphate groups.

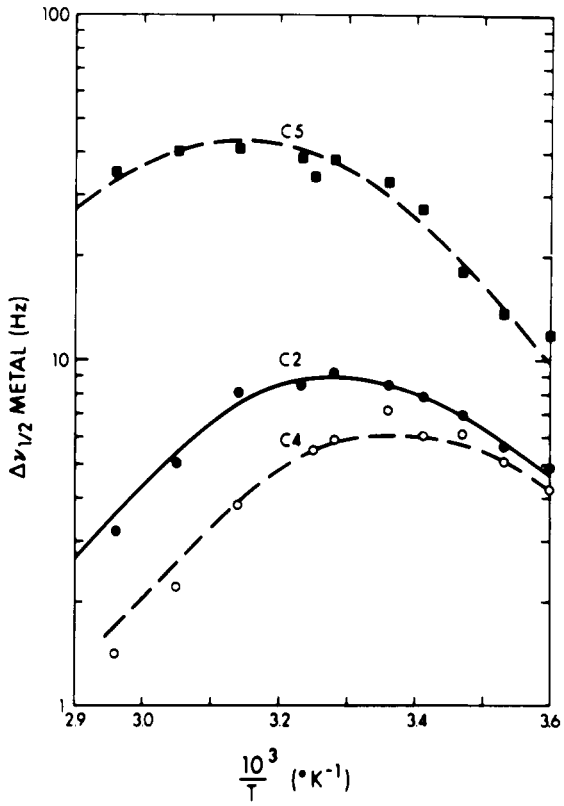


Figure 16. Temperature dependence of $\Delta\nu_{1/2}$ for the C(2), C(4), and C(5) carbon nuclei of 5′-CMP (0.87 M) in D_2O (pD 7.4). The Cu(II) ion concentration is 2.5×10^{-4} M for a [nucleotide]/[metal] ratio of 3.5×10^3 (after Kotowycz[124]).

The early results obtained by Eichhorn and collaborators[20] for the effects of the Cu(II) ion on the proton NMR spectra of 5′-CMP and 5′-dCMP had indicated that the metal induces a strong broadening of the base H(5) resonance. This observation suggests a direct binding to the N(3) nitrogen. No substantial broadening of the H(5) peak could be observed, however, in the ^{1}H NMR spectra of 5′-TMP, under similar conditions. Obviously, the validity of these conclusions is limited by the fact that only three proton resonances can be studied in D_2O solutions of pyrimidine mononucleotides, namely, H(5), H(6), and H(1′).

The proton-decoupled, natural abundance, Fourier-transformed ^{13}C NMR spectroscopy of mononucleotides has afforded broader possibilities for studying these metal interactions. The ^{13}C NMR line assignments, ^{13}C chemical shifts, and ^{31}P–^{13}C coupling constants have been derived from the spectra of mononucleotides and reported in the literature.[128-131]

The study of the effects of Cu(II) ions on the ^{13}C NMR spectra of 5′-CMP, 5′-UMP, and 5′-TMP was undertaken by Kotowycz[124] in 1974 by using the Fourier transform approach. A strong broadening of the C(5) carbon resonance was observed in all cases, followed by weaker broadenings of the C(2) and C(4) carbon resonances. The quantitative analysis of the dependence of these line broadenings on temperature was based upon the observation that such dependence is determined by (a) the temperature dependence of the metal ion average lifetime, at the binding site, τ_m, and (b) the relaxation time of the carbon nucleus, $1/T_{2m}$.[132] The plots of the experimental line widths, $\Delta\nu_{1/2}$, for the C(2), C(4), and C(5) resonances in the spectra of the interacting system Cu^{II}–5′-CMP vs. the reciprocal absolute temperature, $1/T$, have shown[124] that at high temperatures the nuclear relaxation rate, $1/T_{2p}$, is controlled only by $1/T_{2m}$, but at low temperatures $1/T_{2p}$ is predominantly controlled by the exchange, characterized by τ_m (Figure 16). Thus, the values

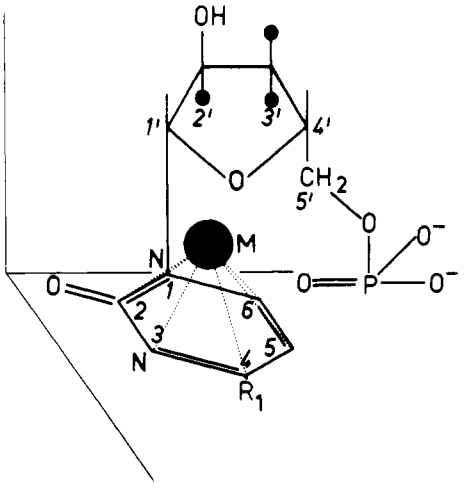


Figure 17. Probable location of the metal ion in Mn(II)–pyrimidine mononucleotide complexes.

of $1/T_{2p}$ for C(2) and C(4) at 35 °C fall in the region controlled by $1/T_{2m}$, whereas at the same temperature $1/T_{2p}$ for C(5) is determined by contributions of both $1/T_{2m}$ and τ_m. It could then be said that at 35 °C the equation $1/T_{1p} = 1/T_{1m}$ is valid for the nuclei C(2) and C(4).[133]

The spin–lattice relaxation time measurements for the system Cu^{II}–5′-CMP have permitted the evaluation of the ratio T_{1p}/T_{2p}; this ratio is 3.3 for C(2) and 2.0 for C(4). These values are small enough to conclude that the main mechanism for relaxation is of dipolar nature. Also, the inverse proportionality of the broadening with the sixth power of the distance indicates that the copper atom is located at equal distances from the C(2) and C(4) atoms, which implies a binding to N(3) (see also Figure 17). On the other hand, since the metal atom is located farther apart from C(5) than it is from C(2) and C(4), the strong broadening of the C(5) resonance in the presence of copper ions would indicate that the transverse relaxation time of the C(5) nucleus is controlled by scalar effects. Such line-broadening behavior is common to all three mononucleotides 5′-UMP, 5′-TMP, and 5′-CMP.

On the other hand, the proton NMR spectra of purine nucleotides have shown a strong preferential broadening of the base H(8) line when they are in the presence of Cu(II) ions, especially in the case of inosine 5′-monophosphate.[98,134,135] Under the same experimental conditions, Mn(II) and Co(II) are less specific than Cu(II) for the broadening effect. Also, specificity decreases in cyclic nucleotides, such as 2′,3′-cyclic AMP and 3′,5′-cyclic AMP, for which Berger and Eichhorn[136] have detected multiple complexation sites.

The specificity of the binding of Cu(II) ions to both 5′-AMP and 5′-GMP has also been demonstrated by the strong preferential broadening of the C(8) carbon resonance on the ^{13}C FT NMR spectra of these purine nucleotides.[107] However, the C(4) and C(5) resonances of 5′-AMP are also visibly affected by the Cu(II) ions as well as the C(4) resonance of 5′-GMP, whereas the C(5) resonance of 5′-GMP is only weakly broadened and the C(2) and C(6) resonances of both 5′-AMP and 5′-GMP are practically unaffected. These results point to the N(7) nitrogen as the principal binding site for metals on the purine ring of mononucleotides. On the other hand, X-ray photoelectron spectroscopy studies have shown that the binding energy of the copper core electrons in the Cu^{II}–AMP complex is remarkably lower than in other low molecular weight copper complexes.[137]

Differences in the nature of binding sites for Cu(II) ions on purine and pyrimidine nucleotides have also been characterized by different g values and hyperfine splittings, as measured by the EPR spectra of the complexes formed under conditions close to physiological ones.[118]

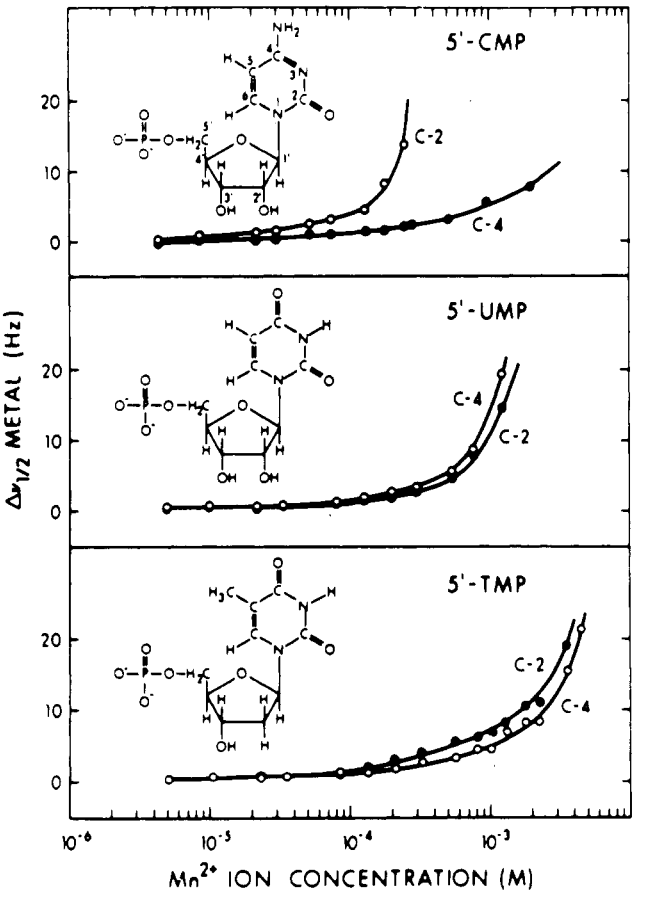


Figure 18. Dependence of $\Delta\nu_{1/2}$ on the Mn(II) ion concentration for the C(2) and C(4) carbon nuclei of 5′-CMP, 5′-UMP, and 5′-TMP (after Kotowycz and Suzuki[123]).

The interaction of Mn(II) ions with the base portion of purine nucleotides in aqueous solution has also been detected by proton NMR,[138] while water proton relaxation time (T_2) studies[139] seemed to show the existence of only one binding site for manganese ions on the purine and pyrimidine bases of mononucleotides. However, although several investigators[98,136,140] have reported a preferential broadening of the H(8) resonance of purine nucleotides by the Mn(II) ions, no clear preferential effect was found for any proton resonance in the case of pyrimidine nucleotides.

The results obtained by Fritzsche and collaborators[107] on the natural-abundance ^{13}C FT NMR of 5′-AMP and 5′-GMP in aqueous solution and in the presence of various concentrations of Mn(II) ions are in essential agreement with the conclusions drawn from ^1H NMR studies about the existence of a preferential base binding site; thus, only the ring C(8) carbon resonance is broadened in the spectra of both 5′-AMP and 5′-GMP at low Mn(II) ion concentrations. Nevertheless, broadenings of the C(5) and C(6) resonances can be seen at high Mn(II) concentrations, indicating that competitive actions from other than N(7) potential purine binding sites are to be expected.

Also, the ^{13}C FT NMR studies carried out by Kotowycz and Suzuki[123] on pyrimidine nucleotides suggest the existence of multiple binding sites for the manganese ion on the pyrimidine ring. The effect of the Mn(II) ion on the spectra of aqueous 5′-CMP, for instance, is somehow preferential for C(2), but also the C(4), C(5), and C(6) carbon resonances are broadened. The C(2) and C(4) resonances are equally affected by Mn(II) ions in the NMR spectra of both 5′-UMP and 5′-TMP, but their broadenings are clearly preferential as compared with the very small effects on the other base carbon resonances and the ribose carbon resonances (Figure 18). These results have led to the interpretation that the manganese ions can be held near

the carbonyl oxygen at C(2) in 5′-CMP and near the carbonyl oxygens at C(2) and C(4) in both 5′-UMP and 5′-TMP (Figure 17).

2. Role of the Phosphate Group in the Formation of the Complex

The early results of Shulman and co-workers[141] on the relaxation time of the ^{31}P nucleus in an aqueous solution of 5′-AMP containing either Mn(II) or Co(II) ions had given support to the idea that the phosphate group of the nucleotide is the primary binding site for these metal ions. Such results have been confirmed in 1972 by Missen and collaborators,[142] when studying the effect of the Mn(II) ion on the ^{31}P NMR spectrum of 5′-AMP. Differential spectrophotometry studies have also shown[143] that both Co(II) and Ni(II) ions in aqueous solution bind preferentially to the phosphate group of mononucleotides.

However, the decrease in signal intensities and the increase in line widths observed in EPR spectra of the Mn(II) ion in the presence of various concentrations of 5′-GMP have been interpreted in terms of a stepwise formation of successive outer- and inner-sphere Mn–GMP complexes.[144] The stability constants for these complexes, as calculated from the EPR data, seem to indicate that both the phosphate and the guanine base are involved in the binding. The reaction kinetics of the complexation of the Ni(II) ion with AMP, as studied by temperature-jump techniques, has revealed[145] that the formation of both the Ni^{II}–5′-AMP and Ni^{II}–3′-AMP complexes involves more than one substitution step and that metal binding to both phosphate and adenine is relevant.

The EPR and temperature-jump experiments have confirmed the conclusion derived from ^{13}C NMR observations of the similarity of the pyrimidine base spectrum in nucleotides and nucleosides[123] that the interaction of the metal ion with the pyrimidine ring is independent of the interaction with the phosphate group. The detection of both ^{13}C and ^{31}P relaxation effects, induced by Cu(II) ions on pyrimidine nucleotides[124] and by Mn(II) ions on purine nucleotides,[107] as well as the absence of any influence of the position of the phosphate group on the preferential base binding site for the transition-metal ions,[146] is evidence of the simultaneous and independent interactions of these ions with the two main binding sites on nucleoside monophosphates. However, since the [ligand]/[metal] ratio in aqueous solution is always very large under the experimental conditions for ^{13}C NMR, the possibility cannot be excluded that the metal ion is simultaneously bonded to the phosphate group of one ligand molecule and to the base ring of a second ligand molecule.

Studies on general conformations of transition-metal–nucleotide complexes in the solid state have been somewhat delayed because of the difficulties encountered in obtaining the crystallized compounds. The first successful attempts in this direction were those of Ogawa and Sakaguchi,[147] who obtained crystallized Ni(II) and Co(II) complexes, having chemical compositions of outstanding reproducibility. The nickel(II) inosine 5′-monophosphate, for instance, crystallizes from aqueous solution as blue needles having the chemical composition $Ni(C_{10}H_{11}N_4O_8P)(H_2O)_7$. The results of X-ray diffraction studies on this compound have clearly demonstrated[148] that here the nickel ion is directly coordinated to the N(7) nitrogen of the hypoxanthine ring but no bond occurs with the phosphate group (Figure 19). The geometry of the purine ring system is practically not perturbed by the Ni–N(7) bond, since the octahedral coordination of the complex is completed by five water molecules. Then, the proper molecular formula for the complex is $[(IMP)Ni^{II}(H_2O)_5]\cdot 2H_2O$. The whole structure of this compound is stabilized by intramolecular hydrogen bonds linking coordinated water molecules with oxygen atoms from both the phosphate group and the hypoxanthine moiety. The metal–phosphate interaction is thus accomplished only in an indirect way, via intramolecular

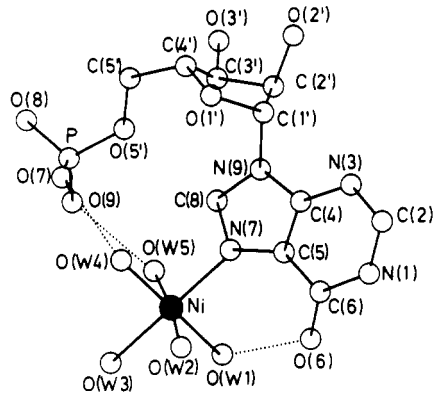


Figure 19. Spatial configuration of the nickel(II)–inosine monophosphate complex (after Clark and Orbell[148]). Reprinted with permission from ref 148. Copyright 1974, The Chemical Society.)

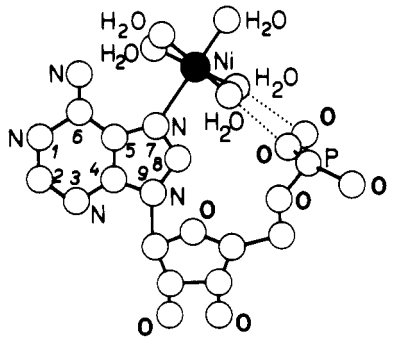


Figure 20. A schematic view of the structure of the $[Ni^{II}(5'\text{-AMP})(H_2O)_5]$ complex (after Collins et al.[153] Reproduced with permission from ref 153. Copyright 1975, Elsevier Publishing Co.)

water bridges. Single-crystal studies on a similar hydrated Co^{II}–IMP complex have confirmed[149] a high concentration of water molecules around the metal ion and an approximately octahedral geometry completed by the N(7) nitrogen. In both nickel and cobalt complexes of IMP, no interbase hydrogen bonds are formed, but an intermolecular hydrogen bond involves the O(3′) oxygen of a ribose moiety and the O(9) oxygen of a phosphate. However, the base, sugar, and phosphate moieties are all involved, although to different extents, in hydrogen bonds with water molecules.

The crystallized Ni(II) and Co(II) complexes of guanosine 5′-monophosphate have been shown[150] to have structures which do not differ essentially from those of the complexes of inosine 5′-monophosphate with the same metal ions, which implies a lack of interaction of the latter with the base amino group. Such complexes show a slightly distorted octahedral geometry[151] and have the molecular formula $[(5'\text{-GMP})M^{II}(H_2O)_5]\cdot 3H_2O$. Here, three intramolecular hydrogen bridges between coordinated water molecules and three oxygen atoms, namely O(6) and two phosphate oxygens, make a contribution for the stabilization of the complex. A similar structure has been found for the cadmium(II) complex of guanosine 5′-monophosphate.[152]

Adenosine monophosphate differs from inosine and guanosine monophosphates because of the absence of any keto–enol tautomerism of the amino substituent on C(6), which prevents a proton transfer to N(1). Consequently, the N(1) site in adenosine monophosphates is expected to be available for metal binding. However, the single-crystal X-ray studies carried out by Collins et al.[153] have shown that even in that case a direct metal binding on N(1) does not occur. This is the case with the nickel complex $[(5'\text{-AMP})Ni^{II}(H_2O)_5]\cdot H_2O$, which crystallizes from aqueous solutions as small green plates. Here the nickel atom binds to the nucleotide only through the adenine N(7) site and the phosphate group is connected to the octahedral geometry via intramolecular hydrogen bonds with two coordinated water

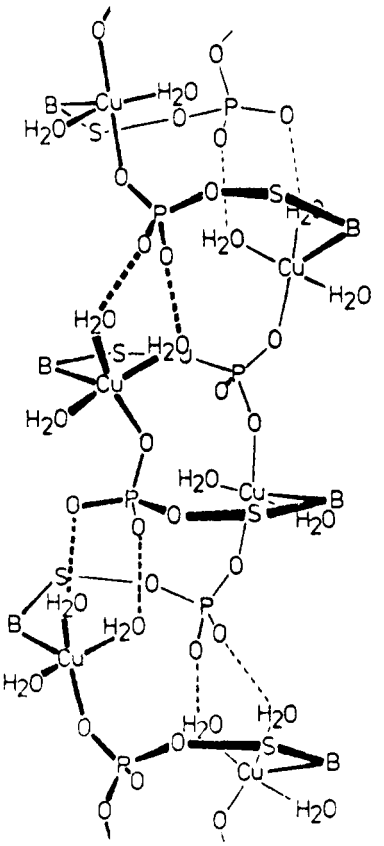


Figure 21. Schematic representation of a segment of the polymeric chain structure of $[Cu_3(5'\text{-GMP})_3(8H_2O)\cdot 4H_2O]_n$. The sugar and base groups are depicted as S and B, respectively. Broken lines represent hydrogen bonds. Noncoordinated water molecules have been omitted. (After Aoki et al.[157]. Reproduced with permission of the author.)

molecules (Figure 20). This complex has, however, a great tendency for formation of polymeric species in which a potential binding site, such as N(1), is likely to be involved.

A direct metal–phosphate binding in the solid state could take place when the conditions for crystallization do not favor the capture of water molecules. Such types of binding have been found[154] in a Zn(II) complex of inosine monophosphate having a polymeric structure. The formula of this compound is $[Zn(5'\text{-IMP})\cdot H_2O]_n$, and it can be prepared from concentrated solutions of the reactants at pH 4. Here, the distorted tetrahedral coordination around the zinc atom involves both the N(7) donor nitrogen of a hypoxanthine moiety and three neighboring phosphates. The water molecules do not coordinate but link different chains, via hydrogen bonds between base sites and oxygen atoms. A recent X-ray analysis by Clark et al.[155] of $[Cu^{II}(5'\text{-IMP})\cdot H_2O]$ has shown structural features similar to those of the Zn(II) analogue, but the distorted tetrahedral geometry of the Zn(II) complex is flattened in the Cu(II) complex, toward a square plane.

Transition-metal ions have also shown the capacity of forming nucleotide polymeric complexes in which both direct and indirect binding to phosphate is involved. Thus, the polymeric copper(II) complex of guanosine 5′-monophosphate, which can be prepared by the method of Ogawa and Sakaguchi[156] and has the molecular unit composition $[Cu_3(C_{10}H_{12}N_5O_8P)_3(H_2O)_8]\cdot 4H_2O$, is an example of polymeric structure which has been studied by X-ray diffraction.[157,158] The three independent copper atoms have here a slightly irregular square-pyramidal (4 + 1) coordination with the base N(7) nitrogen in the axial position and four oxygen atoms in the equatorial plane (Figure 21). The oxygen donors come from two water molecules and two different phosphate groups for one of the copper atoms and from three water molecules and one phosphate for the other two copper

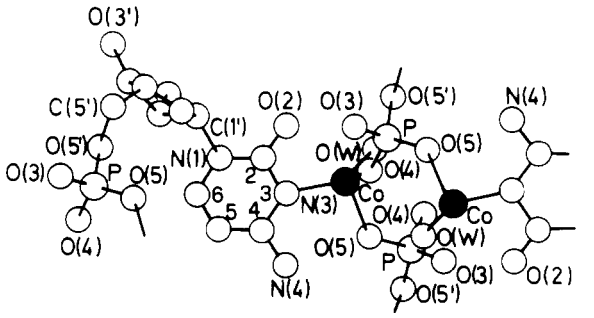


Figure 22. A schematic view of a segment of the polymeric structure of $[Co^{II}(5'\text{-CMP})(H_2O)]_n$ (after Clark and Orbell.[160] Reproduced with permission from ref 160. Copyright 1975, The Chemical Society.)

atoms. An irregular helix is the result of such bonding arrangements. Crystallized cobalt complexes of pyrimidine nucleotides, as prepared by the method of Ogawa and Sakaguchi,[159] have also been shown to be polymeric. Thus, the complex $[(5'\text{-CMP})Co^{II}(H_2O)]_n$, which forms layers connected by H bonds, has been studied by X-ray diffraction methods.[160,161] The experimental results have indicated here that the cobalt ion is at the center of a tetrahedral geometry, being directly involved in the coordination the N(3) nitrogen of the pyrimidine ring as well as two oxygen atoms of different phosphate groups and the oxygen atom of a water molecule (Figure 22). In this way, a spirally organized sequence of cobalt, oxygen, and phosphorus atoms is formed on going from one unit cell to the other. The planes of the pyrimidine rings are here partially overlapped, but this situation is also partially stabilized by interactions between the amino groups and the π-electron systems. The cobalt(II) complex exhibits close similarities with both the complexes formed by 5′-CMP with cadmium(II)[161,162] and zinc(II)[163] in which the base N(3) nitrogen and the phosphate oxygen atoms are the most relevant binding sites for metals on the pyrimidine nucleotide molecule in the solid state. The overall structural configuration can, however, result in being strongly dependent on the nature of the metal ion, as shown by Clark and Orbell[161] for Cd–CMP and Co–CMP, respectively. A weak intramolecular interaction with the O(2) oxygen of the pyrimidine ring has also been detected in the Zn–5′-CMP complex.[163]

A direct binding of a transition metal with the base O(2) oxygen, together with a great participation of phosphate groups in the coordination, is found in the polymeric three-dimensional Mn(II)–CMP complex,[164] which has been prepared by the method of Ogawa and Sakaguchi.[159] Here, the X-ray structure analysis has shown[164] a square-bipyramidal coordination involving a base O(2) oxygen, a water molecule, and four oxygen atoms of two different phosphate groups. The N(3) position of the pyrimidine ring is not involved in the coordination, but two manganese ions are bridged together by two phosphate oxygens. The molecular formula of this complex can be written $[(5'\text{-CMP})_4Mn_2(H_2O)_2\cdot 3H_2O]_n$.

The preparation of a cobalt(II) uridine monophosphate complex has been reported by Mosset et al.,[165] who obtained monoclinic crystals of a compound with the global formula $Co(5'\text{-UMP})(H_2O)_7$. The existence of only metal–phosphate bonds in this sort of complex has been demonstrated by Cartwright and collaborators,[166] from X-ray diffraction evidence, on a polymeric complex having the composition $[(5'\text{-UMP})_2Co_2(H_2O)_4]_n$. The sugar and uracil moieties do not play any part in the metal coordination sphere of this complex, which is octahedrally occupied by four oxygen atoms from four different phosphate groups and two water molecules. The central double chain of the polymer is formed by pairs of cobalt atoms linked by phosphate groups; it appears thus surrounded by the ribose and uracil moieties. Such a polymeric structure seems to be repeated in a Mn–5′-UMP complex, as the data from the X-ray powder analysis have indicated.[166]

From all these studies it can be concluded that the metal–phosphate bonding can become strongly preferential in transition-metal complexes of nucleoside monophosphates when the ligand molecules have been forced to arrange themselves in orderly arrays within a crystal lattice, what seems to be the case for uracil nucleotides having no nonprotonated base nitrogen atom. Some interaction between a metal ion and a base carbonyl oxygen of a uracil nucleotide may take place, however, in aqueous solution. On the other hand, metal complexes in polymeric structures may appear feasible in local situations of macromolecules.

3. Role of the Ribose Moiety in the Formation of the Complex

Small copper-induced broadenings of the ribose H(2′) and H(3′) proton resonances have been detected in the ^{1}H NMR spectra of both 5′-AMP and 3′-AMP.[136] This observation could be ascribed to the fact that the metal ion approaches the H(2′) and H(3′) hydrogens, when linking together the phosphate group and the base N(7) nitrogen. The metal location would then be on the side of the furanose ring which opposes the H(1′) hydrogen.

Relevant broadenings of the ribose carbon resonances have also been observed[137] on the ^{13}C NMR spectra of 5′-AMP when in the presence of a high concentration of Cu(II) ions. A direct binding of Cu(II) to the hydroxyl groups attached to C(2′) and C(3′) in the sugar moiety appears then to be possible. This interaction could be favored by the flexibility of the ribose ring system in aqueous solution.[167] The situation seems to be different, however, in the solid state, since no direct binding of the metal with the furanose hydroxyl groups has been observed in the crystalline structures of the Co^{II}–5′-IMP and Ni^{II}–5′-IMP complexes.[149] This observation is in contrast with the finding of a direct coordination of the 2′- and 3′-ribose oxygen atoms with cadmium in a polymeric Cd–5′-IMP complex.[168]

On the other hand, no broadening effects on the ribose carbon resonances have been observed on the ^{13}C NMR spectra of transition-metal–pyrimidine nucleotide interacting systems.[123]

D. Complexes with Mononucleotides Possessing a Phosphate Chain

1. Role of the Phosphate Group in the Formation of the Complex

The interactions of the paramagnetic ions of the first transition series with nucleotides possessing a phosphate chain have been systematically investigated, for the first time, by using NMR techniques, by Cohn and Hughes,[169,170] who have found that the resonance peaks assigned to the α-, β-, and γ-phosphorus atoms are broadened upon the addition of paramagnetic metal ions. Sternlicht and others[64,171] have extended these studies to the measurements of the relaxation times, T_1 and T_2, for the phosphorus nuclei of adenosine triphosphate interacting with manganous ions in aqueous solution. Although all three phosphorus resonances appear to be equally broadened by the addition of paramagnetic manganous ions and show an equal saturation rate when the system is above room temperature, different behavior is observed at room temperature in the dependence of T_1 on the Mn(II) concentration: the α-phosphorus resonance saturates more rapidly than the β- or γ-phosphorus resonances.

The spin–spin relaxation time (T_2) for water protons in a Mn(II)–nucleotide system has been determined quantitatively by means of ^{1}H NMR techniques,[139] and a calculation of the number of the ligand binding sites can be made from these data, according to Eisinger et al.[172] By using a simplified form of the equation for T_2,[64] Heller and collaborators[139] have obtained a value of between two and three binding sites for the manganese ions on the adenosine 5′-triphosphate.

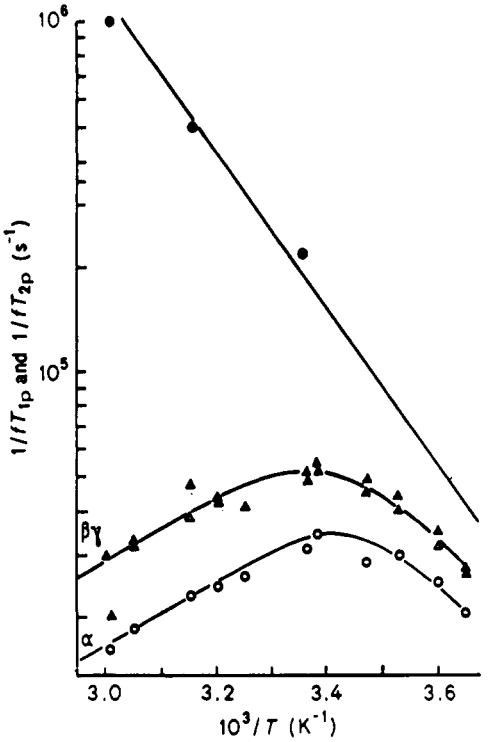


Figure 23. Temperature dependence of the normalized paramagnetic contribution to the spin–lattice and spin–spin relaxation rates of the phosphorus nuclei in aqueous solutions containing 0.1 M ATP and 0.1 mM manganese(II) perchlorate at pH 8.5 (resonance frequency 86 MHz): (●) T_2^{-1} for the α-, β-, and γ-phosphorus resonances; (○) T_1^{-1} for the α-phosphorus resonance; (△) T_1^{-1} for the β-phosphorus resonance; (▲) T_1^{-1} for the γ-phosphorus resonance (after Brown et al.[173]).

Brown and co-workers[173] have carried out a more systematic study of the situation in the Mn(II)–ATP system by measuring the spin–lattice and spin–spin relaxation rates of phosphorus nuclei as a function of temperature. The study was based upon the modified Bloch equations for the NMR effects produced when a ligand molecule exchanges between a free solution and the first coordination sphere of a paramagnetic ion.[132,174] Then, the paramagnetic contributions to the relaxation times, T_{1p} and T_{2p}, are

$$1/T_{1p} = f/T_{1m} + \tau_m \tag{11}$$

$$1/T_{2p} = \frac{f}{\tau_m}\frac{T_{2m}^{-1}(T_{2m}^{-1} + \tau_m^{-1}) + \Delta\omega_m^2}{(T_{2m}^{-1} + \tau_m^{-1})^2 + \Delta\omega_m^2} \tag{12}$$

where f = fraction of the ligand bound to the cation, τ_m = lifetime of the ligand in the bound state, T_{2m}, T_{1m} = relaxation times in the bound state, and $\Delta\omega_m$ = shift between the resonances of the free and bound ligand in the limit of long τ_m.

The plots representing the experimental data show that both the curves for $(fT_{1p})^{-1}$ and $(fT_{2p})^{-1}$ exhibit a negative activation energy at low temperatures, but as the temperature increases, $(fT_{1p})^{-1}$ passes through a maximum at about 30 °C. Above 30 °C, the curve for $(fT_{1p})^{-1}$ of the α-phosphorus resonance can be more easily distinguished from the curves of the β and γ resonances (Figure 23).

From the analysis of the data obtained by Brown and coworkers,[173] it is concluded that both $(fT_{1p})^{-1}$ and $(fT_{2p})^{-1}$ are dominated by τ_m at low temperatures. However, it is expected that T_{1m} would exceed τ_m as the temperature is raised. Then

$$(fT_{1p})^{-1} = T_{1m}^{-1} \tag{13}$$

Since at high temperatures the relaxation rates of the α-^{31}P resonance are smaller than those of the β- and γ-^{31}P resonances, assuming that all three phosphorus atoms have the same correlation time, it can be concluded that the Mn(II) ion

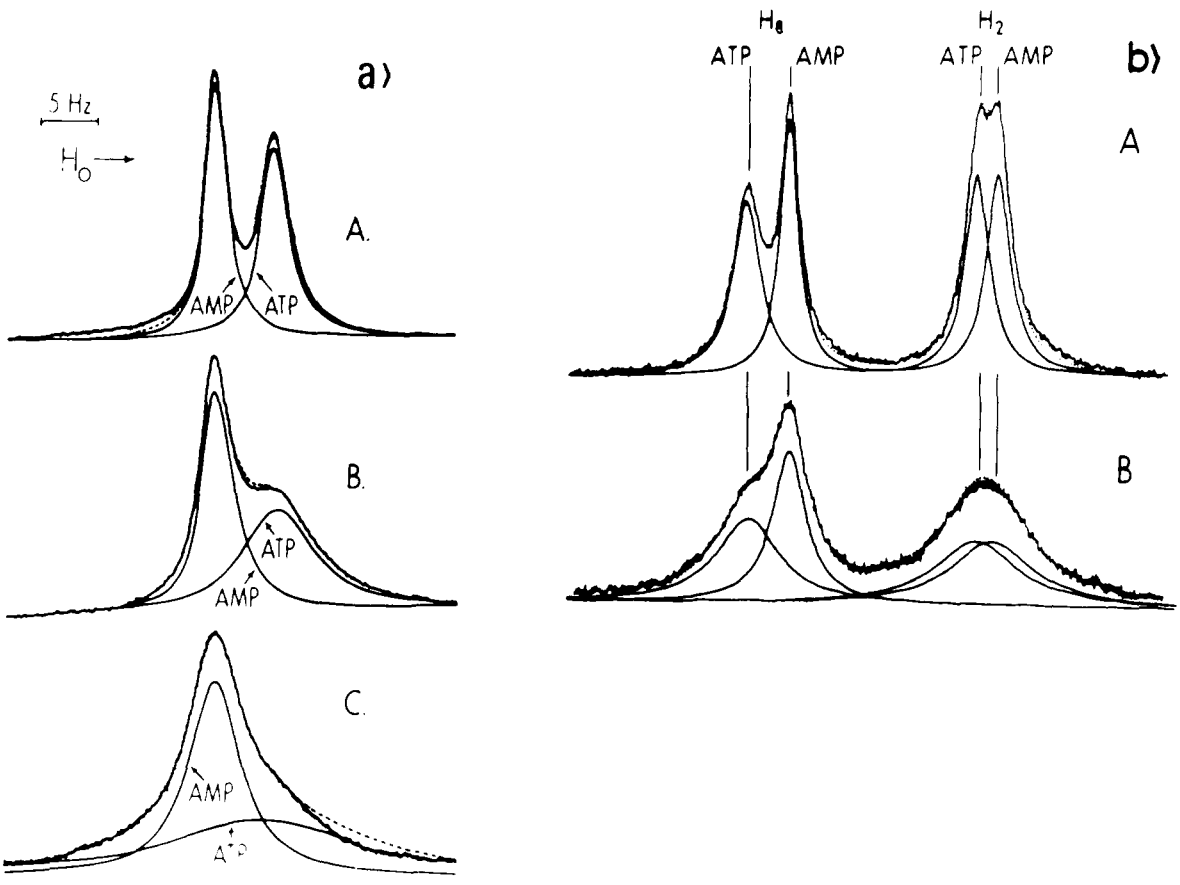


Figure 24. Competition study of Mn(II)-induced proton resonance broadening on an equimolar mixture of AMP and ATP at 27 °C: (a) the H(8) resonance line at pD 8; (b) the H(8) and H(2) resonance lines at pD 5.4. The solution was 0.25 M in both AMP and ATP: (A) no Mn(II) present; (B) 5×10^{-5} M Mn(II) present; (C) 1.0×10^{-4} M Mn(II) present. Outer curves are observed spectra. Resolved ATP and AMP components are indicated in the figure. Dotted segments indicate deviation of the sum of the components from the observed spectrum (after Wee et al.[179]).

is more distant from the α-phosphate group than it is from the β- and γ-phosphate groups. The calculation of the actual distance, r, requires the knowledge of the dipolar correlation time, τ_d, which can be estimated from the determination of the proton relaxation rates of the water molecules in the presence of the ligand.[133] A rough estimation of the distance r, as provided by this method, would be around the value of 0.7 nm. This value is higher than the value of 0.33 nm which is obtained from the considerations of the X-ray data for Na_3HATP,[175] the Pauling ionic radii, and an assumed octahedral coordination geometry with two oxygen atoms from the β- and γ-phosphate groups occupying two adjacent positions.

On the other hand, by studying the pH-dependent frequency of a phosphate indicator Raman line, Rimai and Heyde[176] have found that in the Mn–ATP complex this line is shifting at two pK_a values, which are those for the dissociation of phosphate and of adenine, thus indicating a Mn(II)-mediated interaction between the phosphate chain and the base of ATP. The interaction of metal ions with the base portion of nucleotides has been directly observed by Glassman et al.,[177] through the predominant broadening effect which is induced by Mn(II) and Ni(II) ions on the base H(8) proton resonance of ATP in aqueous solution. In these experiments the water relaxation times of solutions of ATP containing either Mn(II) or Co(II) ions have been measured at various temperatures and compared with those obtained for the corresponding solutions of CTP, in which the metal ions mentioned above have a known number of coordinated water molecules. The identity of the values for the solvent relaxation seems to show that Mn(II)–ATP and Co(II)–ATP are outer-sphere complexes with no metal–nitrogen bond. However, the ratio 2:3 of rapidly exchanging water molecules in Ni(II)–ATP vs. Ni(II)–CTP is consistent with the hypothesis of an inner-sphere metal–nucleotide complex, formed with the elimination of a coordinated water molecule. The latter observation could account for an outer-sphere complex only if a water molecule, bridged between the metal ion and the adenine moiety, would be exchanging so slowly that it would not contribute to the overall water relaxation.

An interesting dynamical model which takes into account a distribution of correlation times resulting from the jumps of the solvent molecules between the various configurational states has been proposed for the Mn(II)–ATP system by Basosi et al.[178] In the combined EPR–NMR experiments carried out by these investigators, the temperature dependence of both the EPR line widths and the T_{1p}^{-1} and T_{2p}^{-1} relaxation rates have suggested the close relation of the variation of these parameters with the formation of the complex, through the displacement of water molecules from the coordination sphere of the metal ion. The results obtained from this EPR–NMR analysis have been interpreted on the basis of the model mentioned above as supporting the formation of a bond between the metal and the base N(7) nitrogen.

However, the phosphate chain seems to bring about some differences in the nature of the binding metal ion–phosphate, when compared with the single phosphate group, since the same metal ions do not produce an equal broadening of the H(8) line in ATP and AMP. The experiments to test the effects of the Mn(II) ions[179] on D_2O solutions of equimolar ATP–AMP at 27 °C and pD 8 have shown that the H(8) resonance of ATP is being broadened about four times as much as the H(8) resonance of AMP. At pD 5.4, a broadening of the H(2) resonance of both ATP and AMP has also been observed (Figure 24a,b). Wee and co-workers[179] have also studied the dependence of the ATP proton line broadenings upon varying temperature, pD, and concentration of the metal ion. Their results have indicated that the H(8) resonance broadening predominates at low concen-

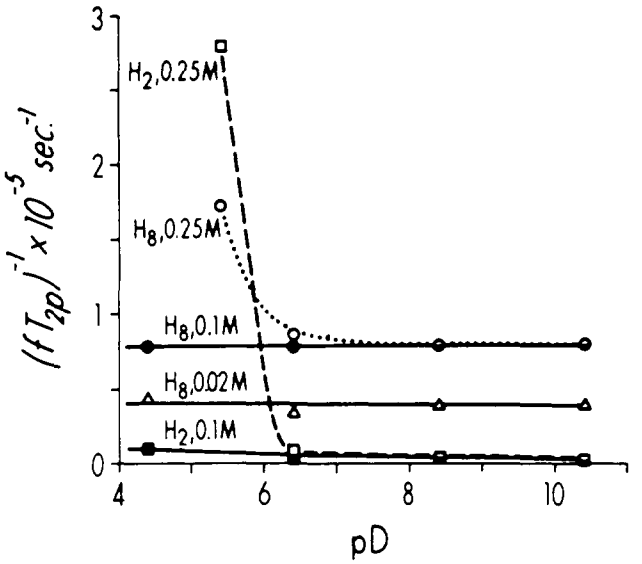


Figure 25. pD dependence of $(fT_{2p})^{-1}$ for the ATP–H(8) and ATP–H(2) proton magnetic resonances at various concentrations of ATP and in the presence of Mn(II) ions (t = 27 °C). The identity of the proton and the ATP concentration corresponding to each curve are indicated in the figure (after Wee et al.[179]).

trations of ATP and that the H(2) resonance broadening can instead be observed only at concentrations of ATP above 0.1 M. However, very small concentrations of Mn(II) ions are able to bring about the broadening of both the H(8) and H(2) resonances when the nucleotide/metal ion ratio is about 10^5. The broadening depends strongly on the concentration of ATP as well as on the pD of the solution (Figure 25). The results are consistent with the hypothesis of a chemical equilibrium in aqueous solution between the forms $[Mn^{II}\text{–ATP}]^{2-}$ and $[Mn^{II}(ATP)_2]^{6-}$ as well as with the assumption of two equal Mn–H(8) distances in the latter complex, where the interaction of the metal ion probably proceeds by way of a water molecule bridged to the N(7) atom. Furthermore, the considerable changes observed in the values of $(fT_{2p})^{-1}$ for the H(8) and H(2) lines of ATP when the total concentration of ATP is above 0.1 M and the pD is varied between 5.4 and 6.4 suggest that drastic structural alterations of the complexes take place with the variation of the pD. According to Wee and co-workers[179] these changes are accompanied by variations in the ligand exchange mechanism and could be related to a transition from an α,β,γ-triphosphate chelation to a β,γ one, resulting from a phosphate protonation with decreasing pD. In this way, the Mn(II)–ATP interaction could result in the formation of complex polymers (Figure 26).

As also emphasized by Wee,[180] the results on Mn(II)–ATP are consistent with a 1:1 $\rightleftarrows$ 1:2 equilibrium, governed by a constant, $([1:2]/[1:1])[ATP]^{4-}$, equal to <29.

Chang et al.[181] have attempted to determine the equilibrium constant for the binding of ATP to Mn(II) by using the EPR method for detection of free Mn(II) ions. By assuming the formation of a 1:1 complex (the total concentration of ATP was around 0.001 M), they found an average value of K, 4.7 × 10^4, which is in fair agreement with values obtained from pH titrations.[182-184] It has also been found[185] that the K values for Mn(II)–adenine nucleotide complexes increase with the length of the phosphate chain and with temperature. Furthermore, the divergences between calorimetric and potentiometric values of the enthalpic variation in the formation of Mn–AXP complexes (X = 1, 2, 3), which can be observed at low temperatures, as well as the hypochromic effect induced by Mn(II) ions on the UV spectra of the nucleotides have led to postulations that polymolecular complexes are formed.[185]

It is interesting to note that UV and CD studies of ATP in aqueous solution[186,187] as well as measurements of the proton

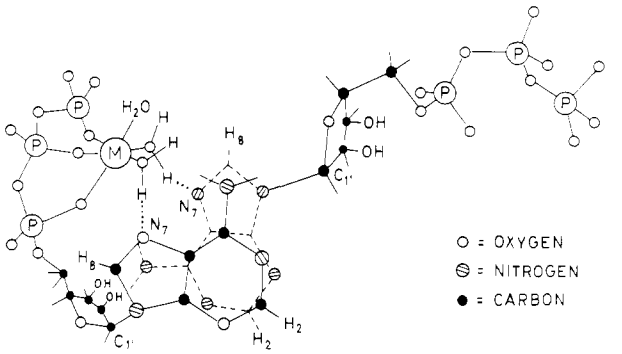


Figure 26. Structure proposed by Wee et al. for the 1:2 Mn(II)–ATP complex in aqueous solution (after Wee et al.[179]).

chemical shifts of ATP[188] have indicated that the ATP molecules can form various oligomers in aqueous solution, by way of a linear self-association. However, the degree of self-association is practically negligible when the concentration of ATP in the solution is less than 20 mM. This fact explains results obtained from solvent relaxation measurements in aqueous solutions of ATP containing Mn(II) ions since the relaxation times are independent on the ATP concentration, provided it is below that limit.[173]

The high sensitivity of the circular dichroism spectra to structural changes has also confirmed the existence of various Mn(II)–ATP as well as Mn(II)–ADP complex species in solution.[189] The UV CD spectra of these interacting systems exhibit drastic variations which are dependent on the ligand concentration as well as on the pH of the solution. A low amount of the polymeric complex $[Mn(ADP)_2]_n^{4-}$ can be detected in the Mn(II)–ADP system, while the $[Mn(ATPH)_2]_n^{4-}$ complex can be easily detected in the Mn(II)–ATP system at a 0.1 M ATP concentration, as well as a self-associated species with ATP^{4-}. With regard to the behavior of these complexes with the variation of ATP concentration, the CD results confirm the NMR observations.[179] Also, a CD study of the spectral dependence on pH, temperature, and ligand concentration has shown[190] the existence of a self-association in the complex Cu(II)–ADP, which is destabilized if ADP is replaced by dADP or by IDP. The dichroic spectra have been interpreted here by the formation of two oligomeric species, depending on the pH: a homooligomer of $Cu(ADP)^-$ and a heterooligomer of $Cu(ADP)^-$ and $Cu(ADP)(OH)^{2-}$.

Recently Fazakerley and Reid[191] have determined, by using ^1H and ^{31}P FT NMR, the conformations in aqueous solution of the 1:1 ADP– and dADP–lanthanum(III) complexes, obtained by titration of the nucleotides with diamagnetic lanthanum nitrate. The dipolar shifts and the variations of spin–lattice relaxation rates induced by the lanthanide probe have then been examined to draw conclusions about (a) the position of the ribose diphosphate and (b) the orientation of the base relative to the ribose. It was shown in this study that the complex exhibits an extended diphosphate group and that its adenine base displays a small syn contribution. On the other hand the relaxation rate studies carried out on Mn(II) titrations led to results that are consistent with an α,β bidentate coordination of the diphosphate to the manganese ion, if it is assumed at the same time that ADP and dADP retain the same configuration as in the lanthanum complex. Under such conditions, the distance between the metal ion and the base N(7) would be long enough to rule out a mediated water bridge interaction. Also, the T_{1m} values for water protons in solutions of coenzyme A (which contains an adenosine moiety within its structure) with and without Mn(II) ions have been used to calculate the correlation time, τ_c, of the water molecules bound to Mn(II) in the weak 1:1 CoA–Mn interacting system.[192] A correlation time which had been previously determined for propionyl-CoA was then used for the calculation of the distances between the Mn(II) ion and the three phosphorus nuclei (two

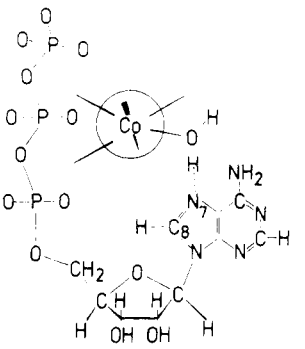


Figure 27. The direct and equally distributed interaction between the cobalt ion and the triphosphate chain and the probably indirect interaction between the cobalt ion and the adenine ring in the Co(II)–ATP complex (after Torreilles and Crastes de Paulet[195]).

of which belong to a 5′-diphosphate and one to a 3′-phosphate), whose paramagnetic spin–lattice relaxation rates have been determined by proton-decoupled FT ^{31}P NMR. The results have been interpreted in the light of an equal coordination of the Mn(II) ion with the oxygens of the three phosphate groups in the CoA–Mn(II) complex.

A NMR relaxation time study of the ^{13}C, ^{1}H, and ^{31}P nuclei of the Mn(II)–inosine 5′-triphosphate complex in aqueous solution was undertaken by Kuntz and Kotowycz in 1975.[193] Here, the spin–lattice relaxation data for the H(8) and H(1′) protons have indicated that this is a 1:1 Mn–ITP complex. On the other hand, the dependence on temperature of the spin–spin relaxation time of the hypoxanthine ring carbon nuclei suggests that here the Mn(II) ion interacts at two distinct base ring sites, namely C-(6)–OH and N(7). Since the Mn(II) ion cannot bind simultaneously to these two sites, a theory for a four-site exchange system has been postulated and the equations for four species in equilibrium (free ITP, Mn–phosphate bound ITP, phosphate–Mn–ring C=O bound ITP, and phosphate–Mn–ring N(7) bound ITP) has been developed.[193] On the other hand, the chemical shifts of the ^{17}O and ^{1}H NMR water signals in the interacting systems Co(II)–ATP and Co(II)–CTP, in aqueous solution, has shown the shifting effectiveness (dependent on the conditions of observation) of the Co(II) ion in the Co–ATP complex as compared to that in Co–CTP.[194] The experimental results obtained on these systems can be explained if a slowly exchanging water molecule is coordinated to the phosphate-bound metal and simultaneously H-bonded to the adenine ring. Also, Torreilles and Crastes de Paulet[195] have observed, by studying the chemical shifts of the ^{1}H NMR lines induced by the Co(II) ion on AMP, ADP, and ATP, that the H(8) and H(2) resonances are affected in all three cases in a similar way. These investigators have then concluded that the same type of binding is formed in the three cobalt(II) nucleotide complexes. Furthermore, the low values of the observed chemical shifts exclude in all cases a direct binding of the metal to the adenine ring (Figure 27). However, the nature of the interaction between the cobaltous ion and a nucleotide is influenced by the physicochemical conditions of the solution. Thus, the visible and UV CD spectra of either the Co(II)–ADP or Co(II)–ATP system in aqueous solution show great changes with the variation of the pH when the nucleotide concentration amounts to 0.1 M.[196] The changes, which take effect on the d–d transitions of Co(II) as well as on the adenine transitions, are related to the pH-dependent modifications in the intermolecular interactions and follow the secondary hydrogen ionization of the terminal phosphate group and the deprotonation of the adenine N(1). The spectral effects are thus closely related to the formation of higher nucleotide polymers, as is the case for the Mn–ATP complex.[179] Also, the effects of both the ring deprotonation and the secondary phosphate ionization on the metal–ATP interactions have been investigated by ^{1}H NMR, at different pD values.[197] In the case of the Co(II)– and Ni(II)–ATP complexes, the values of the broadening and shifting of the proton lines have indicated that a protonated ring acts in a way that brings the adenine moiety nearer to the negative phosphate chain, thus enhancing the metal–ring interaction. The latter, however, does not appear to be affected by the secondary phosphate ionization. A method for calculating the formation constants for the 1:1 and 1:2 metal–ligand complexes from the NMR shifts has been presented by Granot and Fiat.[197]

On the other hand, it has been observed by Glassman and co-workers[198] that the interaction of Ni(II) ions with the adenine ring of adenosine is facilitated by the presence in solution of inorganic polytriphosphate, as indicated by the measurements of the chemical shifts of H(8) and H(2). The extrapolated values of the proton line shifts at a metal–triphosphate ratio 1:1 were consistent with those found for the Ni(II)–ATP complex. It should be noted here that, in contrast with the interacting effects of paramagnetic ions, some ^{31}P NMR studies have shown that the diamagnetic Mg(II) ion binds only to the β-phosphate group of nucleoside triphosphates[199] and to the α-phosphate of nucleoside diphosphates.[200] On the other hand, no chemical shift variations are observed for the proton lines of the Mg(II)–ATP complex in aqueous solution with respect to the ^{1}H NMR spectrum of ATP alone.[197]

Kinetic studies on the formation of the metal–nucleotide complexes have mostly been performed on the systems Ni-(II)–AMP, –ADP, –ATP and Ni(II)–CMP, –CDP, –CTP. Several techniques have been used for the elucidation of the reaction mechanisms UV spectrometry,[201] polarography,[202] and temperature-jump relaxation spectrometry.[203–206] The experiments have clearly demonstrated that the rate-determining step for the formation of the complexes is the loss of water molecules from the Ni(II) ion, thus indicating an inner-sphere coordination.

The interaction of the Cu(II) ion with nucleotides has presented a special interest because of the known destabilizing effect of this metal ion on nucleic acids. The effects of the interaction Cu(II)–ATP on the IR spectra of ATP in D_2O solution have been observed early in 1964.[207] These spectra show a pD dependence, but when Cu(II) ions are also present in solution, a shift is observed for the pD values at which the ionizations of the ATP protons occur. It has then been proposed[207] that the complex is formed at the triphosphate group, and before that any deprotonation could take place on it. Also, by means of UV difference spectrophotometry, Sigel[208] has determined the difference in the acidity constant of the N(1) proton which is brought about by Cu(II) ions in several nucleoside triphosphates. The values of ΔpK for the Cu(II)–nucleotide systems are of the same order of magnitude as the ΔpK value for Cu(II)–nucleoside, which suggests the formation of Cu(II)–nucleotide macrochelates. However, for elucidation of the role of the triphosphate chain in the copper complexes, NMR analyses were undertaken in 1974 by Feldman and Wee[209] on equimolar ATP–AMP mixtures. Very low concentrations of Cu(II) ions were then used to induce the broadening of the proton lines in the D_2O solution. In particular, the Cu(II)-induced broadenings of the H(8) and H(2) lines have been correlated with the pD-dependent distribution of the various species in solution. The experimental results have shown here that the normalized value of $(fT_{2p})^{-1}$ for H(8) decreases rapidly between pD 5.4 and 8.4 but remains practically constant when going to higher pD. However, the line width of the ATP–H(8) resonance appears to be larger than that of the AMP–H(8) resonance, at any concentration and pD, whereas the H(2) resonance of both ATP and AMP are equally broadened by Cu(II) at any pD. According to the studies of Cohn and Hughes,[170] the Cu(II) ion has four coplanar coordination sites and two of them are occupied by the β- and γ-oxygen atoms of the triphosphate chain of ATP. As the pD increases, the metal ion binds to OD^- groups in the metal–nucleotide complex. Further hydroxylation of a 1:1 copper–nucleotide complex above

TABLE VI. Rate Constants for the Phosphate Hydrolysis of Copper-Purine NTP and Copper-purine NDP at $I = 0.1$ (in $NaClO_4$) and 50 °C (after Sigel, Buisson and Prijs[216])

species	pH range	$k' \times 10^{-11}$, M s^{-1}
$Cu(ATP)^{2-}$	5.3-6.6	5.47 ± 0.58
$Cu(GTP)^{2-}$	4.7-6.0	2.27 ± 0.60
$Cu(ITP)^{2-}$	5.8-6.5	0.274 ± 0.040
$Cu(ADP)^{-}$	5.2-6.2	7.67 ± 0.89
$Cu(GDP)^{-}$	4.8-6.1	11.12 ± 3.93
$Cu(IDP)^{-}$	5.0-6.0	0.840 ± 0.244

pD 8.4 does not lead to a decreased width of the adenine H(8) resonance because the Cu(II) ion still keeps its binding site on the N(7) atom. The influence of a pD increase is seen, however, in the case of a 1:2 copper–nucleotide complex, since the binding to OD^- groups competes here with the binding to the nitrogen donor. The relative values of the stability constants, 1.5×10^3 M^{-1} for Cu(II)–AMP^- and 1.4×10^6 M^{-1} for Cu(II)–ATP^{2-},[210,211] indicate that the concentration of the Cu(II)–AMP complex would be negligible in an equimolar mixture of AMP and ATP if only 1:1 complexes were to be formed. A broadening of the AMP–H(8) resonance occurring at pD 6.4 and lower thus indicates that 1:2 complexations are also present in the solution.

By using the formula given by Glassman and co-workers[177] for calculating the distance between a nucleus and an odd electron, Naumann, Sigel, and Prijs[212,213] computed a value of 0.27 nm for the distance Cu–H(8) in $Cu(ATP)^{2-}$. The corresponding calculations for $Cu(GTP)^{2-}$ have revealed that the distance Cu–H(8) in this complex is also between 0.25 and 0.28 nm. This fact indicates that both chemical species have probably the same macrochelate structure involving the β- and γ-phosphate groups and the N(7) of the purine ring. This macrochelate structure is crucial for a Cu(II)-accelerated dephosphorylation of ATP.[214,215] On the other hand, the similarity of the Cu–H(8) distances in the $Cu(ATP)^{2-}$ and $Cu(GTP)^{2-}$ complexes is in close correspondence with the similarity of the dephosphorylation rates of these complexes, much higher than that of the copper–inosine triphosphate complex, $Cu(ITP)^{2-}$, where the coordination tendency of the hypoxanthine N(7) donor is also lower.[215,216] Calculations of the rate constants, k', have been based on eq 14 and 15, where NTP = nucleoside triphosphate and NDP = nucleoside diphosphate. Quantitative results have been obtained for CTP, GTP, ITP, ATP, CDP, GDP, IDP, and ADP; some of them are summarized in Table VI.

$$v_0 = d[PO_4^{3-}]/dt = k'[Cu(NTP)^{2-}]/[H^+] \quad (14)$$

$$v_0 = d[PO_4^{3-}]/dt = k'[Cu(NDP)^{-}]/[H^+] \quad (15)$$

Also, there are pH optima for the dephosphorylation rates which depend on the concentration distribution of the various species present in the interacting systems. By studying this pH dependence, it was concluded that the most reactive species are those where N(1) is protonated. A reaction mechanism has been proposed for dephosphorylation,[216] where an intermolecular attack of a terminal phosphate OH^- occurs. It has also been postulated that the dephosphorylation reaction proceeds via a dimer complex species, probably being $[Cu(ATP)]_2^{4-}$,[217] which would play a role to lower the energy barrier.

In contrast to the Cu(II) complexes, no pH optimum is observed in the dephosphorylation reactions of the Mn(II)– and Ni(II)–purine nucleotide complexes.[218] The latter also appears to be less effective for the phosphate hydrolysis than do the corresponding Cu(II)–purine nucleotide complexes. This observation has to be related to the fact that the Ni(II) and Mn(II) ions coordinate to all three phosphate groups of NTP (in contrast with the interactions of Cu(II) occurring only at the level of the β- and γ-phosphates), which implies a weaker interaction with the purine moiety.

With regard to the complexation of transition-metal ions, it is worth noting the use of stable Cr(III)–nucleotide complexes for studies of enzymatic inhibitions. In Cr(III)–NTP as well as in Cr(III)–NDP, as prepared by several investigators,[219,220] all phosphates in the phosphate chain appear to be coordinated to the chromium ion and the remaining coordination positions are occupied by water molecules. Cr(III)–nucleotide complexes can be used as probes for biological processes, in replacement of the corresponding Mg(II)–nucleotide complexes.[221]

2. Role of the Ribose Moiety in the Formation of the Complex

Several studies have shown[121,222,223] that the interactions between a metal ion and the ribose hydroxyl groups are promoted by a basic medium. On the other hand, Buisson and Sigel[215] have shown that the catalytic effect of the Cu(II) ions on the phosphate hydrolysis is quenched on the complex $[Cu^{II}(ATP)(OH)]^{3-}$, whose concentration increases slowly at pH higher than 6. The dichroic absorption for this complex seems to indicate an unfolded structure without a N(7) binding site.

At pH higher than 10.5, two ribose hydroxyls are ionized and, as Reinert and Weiss[222] have pointed out, they will be strongly bound to the copper ion, without any participation of the phosphate groups in the coordination. On the other hand, the CD studies carried out by Gabriel and co-workers[224] have shown the similarity of the CD spectra of AMP, ADP, and ATP in the presence of Cu(II) ions when all three systems are at a pH value higher than 9. Such observation suggests that the dichroic absorptions at a high pH are only due to the ribose–metal interactions. Two different kinds of CD spectra have also been detected between pH 10 and 12,[224] thus confirming earlier results by Reinert and Weiss,[222] for any Cu(II)–ribonucleotide system. These two characteristic CD spectra, which have not instead been observed for Cu(II)–deoxyribonucleotide systems in the basic range of the pH, would correspond to the successive ionization of the 2′- and 3′-hydroxyl groups on the same side of the sugar plane. Similar observations have been made by Voelter[225] on the CD difference spectra between sugar–metal complexes in Cu(II)–nucleotide systems and the nucleotide alone. These results have been confirmed by a ^{31}P NMR study carried out by Gabriel and co-workers[226] in 1978. The behavior of the P_β and P_γ resonances of a Cu(II)–ATP system was then observed with variation of pH: at pH 8 they become wider with increasing the Cu(II) ion concentration and finally disappear in the noise for a Cu(II):ATP ratio of 4×10^{-3}, but they again progressively reappear when the pH is increased; finally, at pH 11.5, the spectrum is similar to that of ATP alone, which demonstrates that the interaction with the ribose hydroxyls excludes the binding with the phosphate groups. Also, the ^1H NMR study of the same system has shown[226] that at pD 7.5 the ribose H(1′) resonance is only weakly affected by Cu(II) ions (in contrast with the H(8) and H(2) resonances); however, when the pD of the solution is increased to 12.0 the H(8) and H(2) signals are narrowed, indicating a weakening of the Cu(II)–N(7) binding. For the quantitative determination of the corresponding binding distances in the high pD complexes, on the basis of NMR line-broadening studies, a number of theoretical considerations would have to be taken into account.[227,228]

In any event, the interaction with the two ribose hydroxyl groups in 2′ and 3′ is likely to occur, even at pH around neutral, as was also noted during a ^{13}C NMR study,[137] although in that case relatively high concentrations of Cu(II) ions were required. However, the catalytic effect of Cu(II) on dephosphorylation was decreased at high pH, thus clearly indicating that this loss of effectiveness must arise from a modification of the binding sites.

E. Complexes with Synthetic Polynucleotides

The first observations on the effects induced on polynucleotides by transition-metal ions were intended to ascertain if the metal binding sites in the polymer are the same as those

TABLE VII. EPR Parameters of Various Cu(II)-Mono- and Polynucleotide Complexes at $T = 77$ K and pH 5.5[a] (after Bemski, Rieber, and Wust[118])

species	g_m	$g_{\parallel_1}$	$g_{\parallel_2}$	A_1, G	A_2, G
CMP	2.080	2.38		135	
poly(C)	2.067	2.34	2.27	179	154
GMP	2.122				
poly(G)	2.082	2.33		154	
AMP	2.080	2.36		135	
poly(A)	2.086	2.34		156	
UMP	2.083	2.37		150	
poly(U)	2.085	2.41		125	

[a] The subindexes 1 and 2 refer to two binding sites.

in the monomer. In this sense, the results obtained by Berger and Eichhorn in 1971[106,136] by ^{1}H NMR have indicated that the Cu(II) ions bind to N(7) in polyriboadenylate (poly(A)) and in polyriboinosilate (poly(I)) as they do in 5′-AMP and in 5′-IMP, respectively. However, the Cu(II)-induced broadening of the H(8) resonance is preferential over that of the H(2) resonance in poly(I), thus contrasting with a parallel broadening of the H(8) and H(2) resonances in IMP. This observation suggests that the binding of the metal to N(7) is restricted because of the involvement of the O(6) atom in hydrogen bonding. On the other hand, ^{1}H NMR spectra have shown[106] that binding of copper to pyrimidine homopolymers occurs preferentially at the N(3) position, just as in the monomer.

The EPR studies conducted by Bemski and co-workers[118] have indicated that polynucleotides can bind Cu(II) ions up to a 1:5 Cu:polymer ratio and that the observed EPR parameters for the various Cu(II)–polynucleotide complexes differ from those for the corresponding Cu(II)–mononucleotide complexes (Table VII). Here, the EPR parameter, g_m, which indicates the g value at maximum absorption, has been considered to be very close to $g_\perp$, following the assumption made in a classic EPR study by Malmstrom and Vanngard.[229] According to the EPR observations, poly(C) exhibits two binding sites for copper, as shown by the appearance of two sets of hyperfine lines. These would correspond to a site on the base and to another on the phosphate, the former being of a more covalent nature, as indicated by the values of A and g.[33] By contrast, the mononucleotide CMP exhibits a single binding site which would correspond to the N(1) nitrogen of cytosine, as suggested by earlier NMR evidence.[20]

Natural nucleotides already offer a number of possible conformations; the most evident among them are defined by the angle of torsion about the glycosyl bond (Figure 28). Consequently, differences in the interaction of metal ions with polynucleotides may also be related to the influence that the nature and orientation of the base have on the polynucleotide backbone conformation. In particular, the effects exerted by the phosphate groups on the backbone conformations of different polyribopurine and polyribopyrimidine nucleotides have been studied theoretically by Yathindra and Sundaralingam.[230] Also, the studies carried out by Sundaralingam[231] on the structures and conformations of nucleoside and nucleotide analogues, capable of incorporation in the synthesis of polynucleotides, offer new possibilities for structural studies and throw new light on the diversity of binding sites for transition-metal ions.

One of the best clear-cut effects induced by metal ions on nucleotide polymers is that brought about by the Cu(II) ions, which produce random coil forms from either single- or double-stranded ordered structures.[5,232] On this line of research, the experimental observations on the ORD spectra of purine as well as of pyrimidine homopolynucleotides[233] have confirmed the direct action of the Cu(II) ion in the unstacking of the bases, which results in the random-coil form; experimentally, the transition is revealed by a decrease of the optical rotation which is paralleled by an increase of the UV absorption. The effect

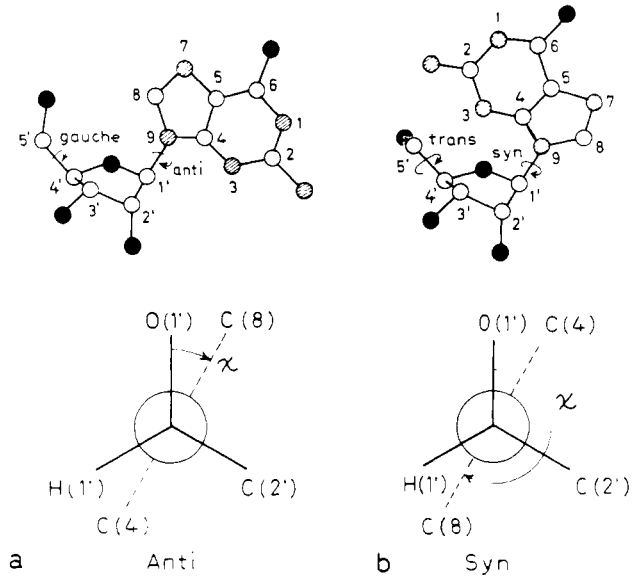


Figure 28. Two familiar conformations about the glycosyl bond, (a) anti and (b) syn, illustrated by the guanine base. The projections down to the glycosyl bond are also shown. The torsion angle about the glycosyl bond is defined by the bond sequence: O(1′)–C(1′)–N(9)–C(8) (after Sundaralingam[231]).

can only be attributed to the binding of the copper ions to the nucleoside bases, which disrupts the stacking and consequently the helical structure of the polynucleotide. The binding is at least of a partly covalent nature, as shown by the EPR observations.[118]

In order to set differences between thermal and Cu(II)-induced transitions, Rifkind et al.[234] have analyzed the UV and ORD spectral changes induced by the activity of the Cu(II) ion on the single-stranded helical structures of poly(A) and poly(C). Here, the comparison of the spectral variations with the results of simultaneous equilibrium dialysis binding studies have indicated that initially the Cu(II) ion binds to phosphate groups of the ordered polymer in a noncooperative fashion; however, subsequent cooperative spectral changes are being caused by the phosphate-bound Cu(II) ions, by forming additional bonds with nonadjacent donor sites on the bases of the same or another polymer strand. The cooperative disordering of the helical structure induced by the Cu(II) ion is thus brought about by the intramolecular and intermolecular copper cross-linkings to the bases; it opposes the noncooperative thermal transitions of polynucleotides. The structural destabilization produced by the Cu(II) ion on ordered polynucleotides is contrasted by the stabilizing effects of the Mg(II) and Ca(II) ions, which suggests a different type of binding for these metal ions. In this regard, thermodynamic and kinetic parameters measured by ion titration and field-jump relaxation for the Mg(II) and Ca(II) binding to poly(A) and poly(C) demonstrate that these metal ions do not form inner-sphere complexes with the polynucleotides.[235,236] Moreover, the results of these experiments favor the hypothesis that the Mg(II) and Ca(II) ions form mobile clouds surrounding the polymers without any definite site of binding. However, these metal ions may also stabilize the helical polymer conformation through some more specific phosphate interaction.

Differently from the two extreme cases described above, the Mn(II), Ni(II), and Co(II) ions may have either a stabilizing or destabilizing effect on a polynucleotide helix, as some ORD studies have shown.[233] The actual situation will depend mainly on the nature of the nucleoside bases. The affinities of these metal ions for the base sites and for the phosphates are comparable; only the relative strength of the two types of binding will determine, in fact, the conformational change experienced by the polynucleotide.

Through UV spectral measurements, Murray and Flessel[237] have followed the effect of the Mn(II) ion on the base pairing

of synthetic polyribonucleotides. The experiments have been carried out on complexes formed by the metal ion with a mixture of two polymers which are capable of forming base pairs: poly(I) and poly(C,U), or poly(I) and poly(C,A). The relative absorbance of the mixture, A_r, could be calculated by using eq 16, where

$$A_r = A_{obsd}/\{X_{HO}A_{HO} + (1 - X_{HO})A_{CO}\} \quad (16)$$

A_{obsd} = observed absorbance, A_{HO} = mole fraction of the homopolymer (poly(I)); A_{CO} = absorbance of the copolymer (either poly(C,U) or poly(C,A)), and $1 - X_{HO}$ = mole fraction of the copolymer. The plots of A_r as a function of the mole fraction of poly(I) have shown that the addition of Mn(II) ions to the system causes a remarkable hypochromicity in the spectra of the mixed polyribonucleotides. This contrasts with the minimal effects produced by the Mg(II) and Zn(II) ions and the less pronounced effect caused by the Cd(II) ion. The comparison of all these spectral observations suggests that important conformational changes take place in a polymer pair as a consequence of the base mispairing ability of the Mn(II) ion.

The ^1H and ^{31}P nuclear spin–lattice and spin–spin relaxation times, T_1 and T_2, of a single-stranded poly(A)–Mn(II) system in neutral D_2O solution have been measured by means of the FT NMR method.[238] This has permitted a fairly good estimation of the H(2)–Mn, H(8)–Mn, H(1′)–Mn, and P–Mn distances, which have come out to be 4.7, 4.1, 5.2 and 3.0 Å, respectively. On the other hand, the values of the hyperfine coupling constants for the H(8) and H(2) protons of poly(A) in the presence of the Mn(II) ions, which are around 10^5 Hz, are evidence for the penetration of the odd electron spin into those hydrogen nuclei and suggest a direct coordination of the metal ion with the N(7) nitrogen of adenine; that is, there is no formation of a water bridge between the metal and the adenine ring. However, it must be taken into account that the manganese–phosphorus and manganese–proton distances, as calculated by NMR relaxation, are probably averages of actual distances in structurally different Mn–poly(A) complexes. In any event, results obtained from water proton relaxation experiments in the same poly(A)–Mn(II) system have led to the conclusion[238] that every Mn(II) ion binds to two phosphate groups and, besides, a good probability exists for every Mn(II) of being simultaneously bonded to the adenine ring. Also, by estimation of the rotational correlation times, it has been found that the local motion of the polyriboadenylate in the vicinity of the manganese binding site is much slower than the rotational motion of the free polymer.

The problem of the metal binding to polynucleotides is expected to be further clarified by way of the results obtained from the studies on metallic complexes of small oligonucleotides. In this direction, a good help for the study of the problem is afforded by the known conformations of several dinucleoside phosphates[239-241] and dinucleotides.[242,243] Theoretical studies have also been related to the problem of the interaction of metal ions with phosphate groups belonging to a polymeric structure. Nanda and Govil,[244] for instance, have concluded that the metal ions may affect the conformation of the dimethyl phosphate anion around its O–P bonds by favoring an extended form. Detailed computations on similar effects have been carried out by Pullman and collaborators.[245]

On the other hand, Guschlbauer et al.[246] have shown that a change in the degree of stacking occurs with the variation of the pH in di- and oligonucleotides having guanine as the nucleoside base, the G residues being in a syn conformation (Figure 29). This observation is obviously related to the finding by Zimmer and co-workers[247] that the CD spectra of the complexes of Zn(II), Mn(II), and Cu(II) with GpG and ApGpG are closely similar in shape to those reported for these oligonucleotides at low pH values and in the absence of any of those metal ions;[248] in the second case, one guanine residue has become protonated. Thus, it appears that the effect of the interaction of Zn(II), Mn(II),

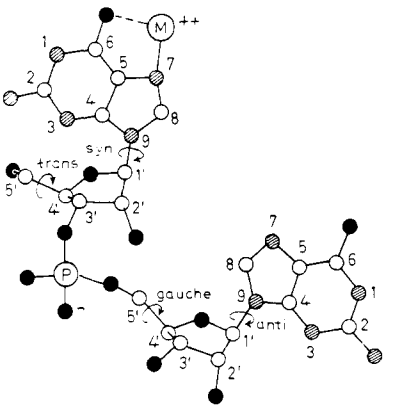
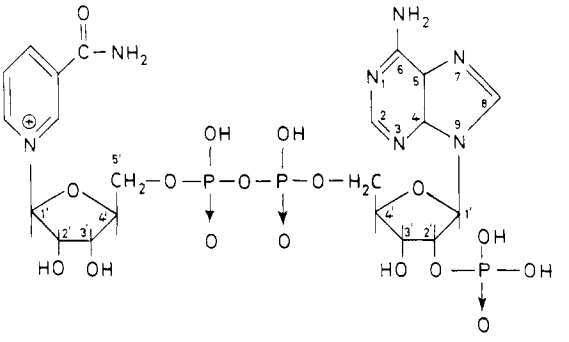


Figure 29. Schematic representation of the structure M^{2+}(syn)Guo-3′-P-(anti)-5′-Guo (after Zimmer, Luck, and Holy[247]).

SCHEME IV. Structure of Nicotinamide Adenine Dinucleotide Phosphate (Deprotonation of N(1) Occurs with $pK = 4$)



or Cu(II) on oligonucleotides containing at least two guanine residues is the electrostatic repulsion of the guanine rings.

The interaction of metal ions with dinucleoside monophosphates is particularly amenable for studies by ^1H NMR because of the simple and well-resolved spectra of these molecules, whose proton peak assignments have been made by Ts'o et al.[249] It would be expected here that the metal ion undergoes a primary binding to the phosphate group and that the two bases compete for an additional binding with the metal ion. The ^1H NMR data obtained in 1971 by Anderson and collaborators[122] on the interaction of the Mn(II) ion with GpG, dGpG, and other dinucleoside monophosphates had already shown that the magnetic influence of this metal ion is stronger on the H(8) ring proton of deoxyguanosine than on any other. However, these authors have suggested that such an effect could be brought about by a simple vicinity of the metal ion to the guanine ring, favored by a conformational situation and not by a real binding with a ring donor atom.

The application of the generalized perturbation theory has led to the conclusion that, although the phosphates are preferential fixation sites for metal ions, an extra binding may occur either with N(7) or N(1) or N(3), depending on the energy levels of the metal orbitals.[201,250] On the other hand, the calculation of the metal–proton distances, based on line width measurements in dinucleotide ^1H NMR spectra, have indicated that both the Mn(II)[251] and the Co(II) ion[252] may be bonded indirectly to the ring N(7), via a water molecule.

Visible CD, which appears to be particularly suitable to get an insight into the geometry of the ligands around a metal ion, has been used by Bolard and Chottard[196] to study the transitions of Co(II) in the presence of nicotinamide dinucleotide (either NAD or NADP) (Scheme IV). The visible CD spectral changes of these systems with the value of the pH and of the [Co(II)]/[dinucleotide] ratio are related to modifications in the self-association of the dinucleotide molecules but not to any gross change in the molecular shape. ^{13}C NMR studies have also been

carried out on the Co(II)–NAD^+ system,[253] and the results have suggested that both types of interaction of the Co(II) ion with the dinucleotide molecule, viz., Co(II) linked to phosphate groups and Co(II) linked to the adenine N(7), are allowed to exist in both the folded and unfolded conformations of NAD. However, the Co(II) ion may also interact with the adenine amino group in the NAD^+ folded conformation, as the observed paramagnetic effects on the C(6) carbon atom seem to indicate.

On the other hand, the shapes of the visible CD spectra of the N(1)-deprotonated (pH 6) Co(II)–NAD^+ and Co(II)–$NADP^+$ systems can only be explained by the presence of a 1:2 Co(II)–dinucleotide complex.[254] This would mean that an intermolecular association of two dinucleotide molecules could be maintained in the presence of Co(II) ions. The two adenine bases in the complex would be situated in two parallel planes and the two nicotinamides on each side of the interacting adenines, as is the case for the free dinucleotide.[251] In the complex the Co(II) ion may be linked to the pyrophosphate and to the N(7) atom of one dinucleotide molecule as well as to the N(1) atom of the other molecule. Here, the vanishing of the visible CD spectra of both Co(II)–NAD^+ and Co(II)–$NADP^+$ with the protonation of the N(1) atom could be explained by the repulsion of the adenine bases and not only by the prevention of a Co(II)–N(1) bond. However, the Co(II) ion may also bind to the 2′-PO_4 group of $NADP^+$, as is suggested by the UV CD spectral changes in the range of deprotonation of 2′-PO_4. Also, the temperature variations of the UV CD spectra of Co(II) and Mn(II) complexes of nicotinamide adenine dinucleotides are the same as those found for the free dinucleotides and lead to the conclusion that neither the manganese ion nor the cobalt ion bridges the nicotinamide and the purine base together.[254] The question arises whether the binding of a second metal ion in the 1:2 metal–dinucleotide complex would be reflected in the CD spectra.

F. Complexes with Natural Polynucleotides

As evidenced by a number of experimental observations, the stability of metal ion–nucleic acid complexes varies as a consequence of the action of structural factors which include the neutralization of negative charges, the change of conformation brought about by the metal complexation, and the competition between metal and hydrogen bonding. The consideration of such situations may afford an explanation for the fact that the overall stability constant of Mg(II)–DNA has been found to be higher than that of Cu(II)–DNA,[255] in spite of the fact that the copper ions are expected to bind tightly to a mononucleotide by way of both base and phosphate sites. Concerning the stability of metal complexes with DNA, the rest of the divalent transition-metal ions are situated between the two extreme cases of magnesium(II) and copper(II), depending on their relative affinities for the two types of binding sites.[55] However, the binding of metal ions to the bases can be prevented by high electrolyte concentrations which stabilize the ordered structure of DNA in aqueous solution.

1. *Interaction of Cu(II) Ion with Nucleic Acids*

By observing the EPR signals of Cu(II)–DNA systems in aqueous solution, Ropars and Viovy[256] have demonstrated the existence of two types of binding sites for the copper ion. The same authors have also shown[257] that the various complexes formed in the Cu(II)–DNA systems are in dynamic equilibrium; the relative concentrations of these complexes vary with the pH and ionic strength of the solution.

Differential UV spectrophotometric measurements have confirmed the direct interaction of the copper ions with the bases of DNA,[8] although the kinetic study of the ascorbic acid oxidation, whose activation by Cu(II) ions is inhibited in the presence of DNA, has indicated that a Cu(II)–DNA complex is formed in an intermediate step of the reaction,[8] with copper bound only to partially dehydrated phosphate groups.

The fixation of the copper ion to DNA seems to be favored by the degrading action of hydrogen peroxide, and in this case the highly preferred binding sites are on the DNA bases. The process which induces the break of the two DNA strands has been studied by several authors.[7,258-261] In any case, the equilibrium binding constants polarographically determined[10] for both the Cu(II)–native DNA and Cu(II)–denatured DNA systems vary with the ionic strength of the solution and with the charge of the DNA molecule. These effects could be calculated from the dependence of the electrostatic potential on the charge density for a cylindrical polyelectrolyte in excess of electrolyte.[262]

On the other hand, the results of gel filtration indicate that the average molar ratio of DNA nucleotides bound to a Cu(II) ion is 2:1, what could imply a binding to purine nucleotides only.[9] Some changes can be induced on the UV and CD spectra of the Cu(II)–DNA system by several chemical and physical agents; in this respect the spectra seem to be very sensitive to Cu(II) concentration, to temperature, and to the reaction time.[9] The appearance of a new absorption band in this system by the action of some reducing agents has been attributed to the formation of a Cu(I)–DNA complex with the binding site on a DNA base.[263] The mechanism for the formation of the new complex includes a proton transfer, along the hydrogen bond, from guanine to cytosine together with the fixation of Cu(I) on guanine. The measurement of the anisotropic components of the CD spectrum of the Cu(I)–DNA complex, made possible by a flow orientation of DNA,[264] has given evidence for a complexation at the level of a base pair (GC), which is tilted with respect to the DNA helix axis.[265] On the other hand, it has been observed that the new bands which appear in the IR spectra of D_2O solutions of DNA upon addition of Cu(II) ions are the same as those detected in a D_2O solution of a mixture of guanosine and cytidine in the presence of Cu(II) ions.[266] This observation gives an indication that the Watson–Crick base pairing of guanine and cytosine is being altered when the Cu(II) ion is complexing with DNA.

The differential spectra of copper complexes of GC-rich DNA reveal a particularly pronounced displacement of the UV absorption maxima;[267] the magnitude of the effect depends on the GC content of DNA. This spectral shift is much reduced by a specific methylation of DNA at the N(7) site of the guanine residues as well as by a protonation of the GC pairs; the effect has been interpreted as coming from the formation of a chelate between the G and C bases.[267] This type of chelation becomes feasible if a conformational change of one of the bases from the anti to the syn form occurs.[268]

The importance of the GC pairs for the binding of Cu(II) has also been revealed by the negative effect of the N(7) methylation of the guanine residues on the electrolyte-induced reversion of the thermal denaturation of DNA in the presence of Cu(II) ions.[135] On the other hand, ORD and CD experiments have shown that the complexation of Cu(II) with the GC base pairs brings about a change of the tertiary structure of DNA, consisting of an increase of flexibility as well as a folding of the macromolecule.[269] The extent of this folding depends on the GC content of the DNA molecule.[135] The conformational changes of the DNA structure when interacting with Cu(II) ions at low ionic strength have also been confirmed by dilatometric studies.[270]

The N(7) nitrogen as well as the oxygen at C(6) in guanine and the N(3) nitrogen as well as the oxygen at C(2) in cytosine may be involved in the binding of Cu(II) ions on DNA. The coordinating metal ion would thus be situated between the G and C bases of the opposite strands (Figure 30). However, an interaction of the type G–Cu(II)–G could not be excluded,[269] which implies that the N(7) nitrogen has a dominant role in the intercalation of the metal ion between two GC pairs. Such a

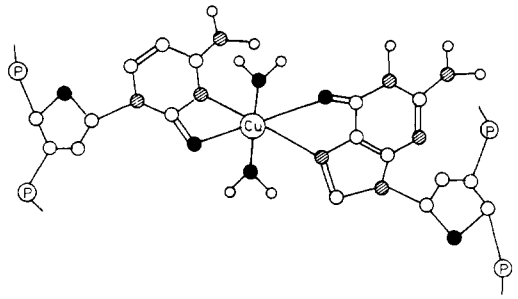


Figure 30. Schematic representation of the complexing of the Cu(II) ion between the guanine and cytosine moieties (after Zimmer, Luck, Fritzsche, and Triebel[135]).

complex would be of a charge-transfer type, with two adjacent G's in the same strand as the donors and Cu(II) as the acceptor. UV differential spectra as well as absorption spectroscopic data in the 400–900-nm region have also shown that the interaction between Cu(II) ions and DNA is of an electron donor–acceptor character.[271] Hydrogen-bonded water molecules act as the barriers for the charge transfer.

EPR experiments have also confirmed the preferential interaction of the Cu(II) ion with the DNA bases, since it was found that the variations of the EPR spectral parameters for Cu(II), under various conditions, are similar if the metal ion is in the presence of either native or denatured DNA.[272] However, studies on the melting temperature (T_m) of DNA under various conditions of ionic strength and Cu(II) concentration have suggested a simultaneous binding of the Cu(II) ion to the phosphate and base moieties.[273]

Also, the decrease of the binding ability of the DNA bases to Cu(II) ions, which occurs at high ionic strength and is evidenced in the differential UV spectra, has been interpreted as a confirmation of the existence of a chelate formed by the metal ion with both the base and phosphate sites.[274] The more accessible N(7) nitrogen of guanine as well as the closest phosphate group on the same strand would be involved in such chelation.[275] All the Cu(II), Co(II), Fe(II), and Zn(II) ions seem to have the capacity to form chelates of this type when in solutions of low ionic strength.

The electrophoretic mobility data for native and denatured DNA, when determined in the presence of various concentrations of metal ions by the method of Rastogi and Misra,[276] can give an insight on the electric properties of the macromolecule and on the influence of the pH in the metal ion–DNA interaction,[277,278] as shown by the studies carried out by Chattoraj et al.[279] Very sharp breaks in the curves representing the electrophoretic mobility of either native or denatured DNA vs. the Cu(II):DNA ratio, as observed by Srivastava and Banerji,[280] have indicated that the copper ion contributes to conformational changes of the DNA molecule. (In the method of Rastogi and Misra,[276] with the DNA molecules adsorbed on glass particles, the electrokinetic motion between solid and liquid possibly occurs in the diffused portion of the double layer.[277])

On the other hand, the direct implication of the DNA nucleotide sequences in the formation of metal ion–DNA complexes has been revealed by the buoyant densities of the latter (as measured in density gradients) since, apparently, they do not depend on the mean GC content of DNA.[281] This observation suggests a direct involvement of nucleotidic sequences of DNA in the interaction with metal ions.

The recent EPR and UV studies performed on both unoriented samples of DNA (solutions) and in partially oriented DNA fibers[282] have shown that a relevant reduction in the amount of Cu(II) which binds to the bases can be caused by the presence of Mn(II) ions. The effect is instead small when Mn(II) is replaced by Li(I) or Na(I); it is therefore suggested that the effect has to be proportional to the capacity of the ion to stabilize the DNA double helix. Thus, it appears that the helical structure has to be deformed, at least locally, for the Cu(II) binding to be favored. Also, the sedimentation coefficients of Cu(II)-binding DNA samples, which have been insolubilized by electrolysis, reveal a slight cleavage of the DNA molecule.[283]

The matrix rank analysis (MRA), applied by Petri et al.[284] to the CD spectra of DNA in the presence of various concentrations of Cu(II) ions, has led to the conclusion that these CD spectra can be divided into two sets, each of them appearing to be associated with the formation of only one type of Cu(II)–DNA complex. This conclusion is probably related to the two classes of preferred conformations for nucleic acids[285] as well as to the two principal metal ion binding mechanisms, one of which is noncooperative and nondenaturing and the other cooperative and denaturing.[286] Thus a conformational transition of native DNA from the B form to a C-like conformation seems to take place gradually in the presence of increasing concentrations of Cu(II) ions.[287,288] It is worth noting that copper ion effects on the CD and ORD spectra of DNA–poly(Lys) complexes also indicate a conformational transition of the macromolecular structure.[289]

The Cu(II) ion induced denaturation of DNA brought about by the fixation of the metal ion between a base and a phosphate group is a highly cooperative process[290] since the corresponding Scatchard plots show that an interaction between occupied binding sites exists.[291,292] However, an X-ray diffraction study of the Cu(II) ion fixation on DNA fibers with 92% of relative humidity has revealed the existence of the B form, without either a modification of the helical parameters nor a change of the base bound to the sugar residue from the anti to the syn position.[293]

By comparing the oscillopolarogram anodic parts in an oscillopolarographic study of the Cu(II)–DNA system, Prasal[294] has also been able to point to guanine as the binding position of bound Cu(II). The situation, however, is not so well defined, since the use of square-wave polarographic criteria of correctness gives results of instability constants which differ from those obtained by dc polarography.[295] Furthermore, the kinetics of the formation and dissociation of both Cu(II)–nondenatured DNA and Cu(II)–denatured DNA complexes, as investigated by stop-flow and temperature-jump techniques,[296] demonstrates that, under certain conditions, the denaturing action of the Cu(II) ion on native DNA can be a consequence of a strong tendency to bind to cytosine. On the other hand, cross-link structures seem to exist even in the nondenatured complexes.

Recently, some CD spectral data combined with microcalorimetric measurements have indicated[297] the formation, at low ionic strength, of a cooperative denaturing complex between Cu(II) and DNA. The process is slow, although the rate of formation increases with increased Cu(II):DNA base-pair ratios and with increased temperature. Instead, the fast formation of a nondenaturing complex occurs at low Cu(II) concentrations and at low temperatures. It is suggested that the nondenaturing complexation involves a chelation of the Cu(II) ion with nitrogen atoms of purine bases and oxygen atoms of the closest phosphate group, as well as the formation of intrastrand cross-linkages at the GC pairs. On the other hand, a conformational change from the B form of DNA to a C-like form is confirmed by the CD spectra in the case of the formation of the cooperative denaturing complex.[297]

Many of the studies on the complexation of Cu(II) ions with ribonucleic acids (RNA) have been focused on the nature and properties of the ternary peroxo compounds formed in aqueous solution in the presence of hydrogen peroxide. Such complex compounds have peroxidative activity which results in the selective degradation of some of the bases.[298,299] Typical EPR spectra showing a superhyperfine structure due to ligand nitrogen atoms of the exposed bases and indicative of the partially co-

valent nature of the bonding have been observed in H_2O_2-degraded samples of Cu(II)-binding yeast RNA.[300] However, the observation of the melting curves of rat liver tRNA and rRNA in the presence of Cu(II) ions indicates, in both cases, the heterogeneity of the binding sites for the copper ion.[301]

2. Interaction of Metal Ions Other Than Cu(II) with Nucleic Acids

Much evidence for the direct interaction of most first group transition-metal ions with nucleic acids was obtained just before the seventies by using NMR and EPR techniques.[172,302-305] The use of EPR in the case of the Mn(II) ion is straightforward since it is possible to determine the concentration of the free manganese ion from the EPR spectra,[306] which can lead to a direct calculation of the association constant as well as of the number of binding sites.[307] Remarkable and reversible shifts of the EPR spectral peaks, observed on mildly dried, metal ion containing, yeast RNA samples as a function of temperature,[305] are indicative of changes occurring in the inner coordination sphere of the metal ions with change of physical conditions. It is thus probable that each metal ion binds in a specific manner under some well-defined conditions.

The difference in the nature of the interactions Mg(II)–DNA and Mn(II)–DNA has been demonstrated by means of the respective differential UV spectra.[308] The UV data for these systems indicate a modification of the electronic transition of the base by the Mn(II) ion which does not occur with Mg(II). Also, the CD spectra of the same systems show that only the binding of Mn(II) to DNA brings about a significant modification of the local conformation of the macromolecule.[308] However, the conformational effect of the Mn(II) ions could affect, in turn, the binding of the Mg(II) ions by the nucleic acid.[309]

The melting temperature of DNA was observed to decrease with the increase of the Mn(II) concentration, and the effect is more pronounced in GC-rich DNA.[310] However, the cation exchange in DNA films, which can be induced by immersion of the samples into water–ethanol solutions of low molecular weight salts (LiCl, $MgCl_2$, $CaCl_2$, $MnCl_2$, $ZnCl_2$), can occur, as monitored by IR spectroscopy, without denaturation of the nucleic acid.[311] On the other hand, the CD optical measurements in 10^{-3} M $NaClO_4$ (aqueous) is evidence that the Zn(II) ions, in spite of binding primarily to phosphate groups in the native DNA, interact with base sites of denatured DNA.[312] In that case, the protonation of GC pairs is suppressed, with subsequent conformational changes. In contrast, Mg(II) binds almost exclusively to phosphate groups and similar CD effects are not observed. The Zn(II) ions are not effective, however, in producing DNA cleavage, at temperatures just below the melting range of DNA, as demonstrated by sedimentation velocity measurements,[313] while almost any metal ion is, instead, capable of degrading RNA under similar conditions.

In contrast with the insignificant degrading effect of the Zn(II) ion, major structural and conformational changes on the DNA double helix are brought about by the binding of both Cd(II) and Ni(II) ions. These changes could be detected by using the techniques of UV absorption, CD, sedimentation, and ^1H NMR,[314] since the binding of any of these two metal ions produces GC-selective perturbations which induce a transition from the B form to a C-like form of DNA; at the same time, the changes in the helical parameters lead to a compactness of the double helix. The induction of structural effects on DNA by metal ions, other than the local conformational changes in the vicinity of a GC pair, is also illustrated by the fact that the direct involvement of the guanine N(7) nitrogen in the binding of Mn(II) ions can affect the specific recognition mechanism of neighboring binding sites by potential ligands.[315]

Qualitative regularities, such as specific nucleotide sequences, may also be an important factor for the selectivity in the metal ion binding by biological macromolecules.[316] In this context, the Cr(III) ion has been suggested as a useful affinity label,[317] and it has already been tested as an irreversible analogue of Mg(II) in labeling experiments on nucleic acids.[318] These experiments, when carried out on DNA, have not yet been fully successful since the X-ray diffraction experiments on labeled DNA fibers have not indicated any regular arrangement of the Cr(III) ions.

The competition effects of Mg(II), Ca(II), Na(I), and even a number of organic cations in the binding of the Mn(II) ion to DNA have been studied on a quantitative basis by deriving the apparent binding constant of Mn(II) in the presence of the competitive cation.[319] It has been found that the calculated association constant for the Mn(II)–DNA system (on the basis of EPR measurements of free Mn(II)) depends on the degree of saturation of the binding sites by the same or other cations, the corresponding dependence parameter decreasing along the series Mn(II), Mg(II), Ca(II), Na(I).[319] It is thought that, besides the mutual steric hindrance between bound cations and their electrostatic repulsion when their positive charges are not completely neutralized, the binding of Mn(II) by the DNA macromolecule could be affected by local conformational changes induced by any of the competitive cations. The EPR spectrum of Mn(II)–DNA, as was observed in these experiments, appeared to be very similar to those of Mn(II)–nucleotide complexes,[320,321] for which ^1H NMR[122,177] and ^{13}C NMR studies[146,322] have indicated the interaction of Mn(II) with the base N(7) site of purine nucleotides and nucleosides.

The stability constant for the formation of the base pairs from nucleoside molecules in aqueous solution, either in the presence or in the absence of Mn(II) ions, has been calculated by Chatterji and collaborators[323] from chemical shift measurements on the NH_2 protons of guanosine as a function of the concentration of deoxycytidine or thymidine. It has been found that a remarkable increase of the stability of the hybrid G–dC pair occurs in the presence of the Mn(II) ions,[323] but no similar effect could be observed with other base-pair systems, thus indicating the specificity of the Mn(II)–base pair interaction.

The ability of the Co(II), Zn(II), Mn(II), and Ni(II) ions to catalyze the formation of stable internucleotide linkages has been demonstrated in the synthesis of oligonucleotides.[324] On the other hand, the ability of the nucleotides 3′-AMP, 5′-AMP, 3′-CMP, and 5′-CMP to enter in the coordination sphere of a metal ion seems to be paralleled by the possibility of their existence as free acid zwitterions in the solid state,[325] in which case there is protonation at the N(1) site on adenine (or at the N(3) site on cytosine), while the phosphate group is negatively charged.

The subtle conformational changes which could be induced on DNA by the Mg(II), Ca(II), and Na(I) ions by way of their minor relevant coordination to N(7)[319] are unlikely to affect the cation electrostatic interactions with the phosphate groups, but the subsequent interaction of Mn(II) with the bases may be affected. In terms of a cooperative effect, the depression of the positive band in the CD spectrum (above 258 nm) of DNA, caused by monovalent salts of chloride (Na(I), K(I), Li(I), and Cs(I)), has been interpreted by Hanlon and co-workers[326-328] as the result of a partial transconformational reaction from a B-like state to a more C-like state. The interpretation was based on a set of reference spectra for the B, C, and A forms of DNA, in aqueous solution of a monovalent salt, which have been obtained through an appropriate statistical treatment of the CD data.[329]

At low concentrations different monovalent cations show differences in their efficiencies to promote the partial B- to C-form conversion, which suggests a specificity in the cation effect.[327] The conformational transformation at high monovalent salt concentrations (above 0.1 M) is instead mainly determined by a dehydration of the DNA molecule.[330-332]

By trying to differentiate between electronic redistributions in nucleotide units which occur as a consequence of the metal

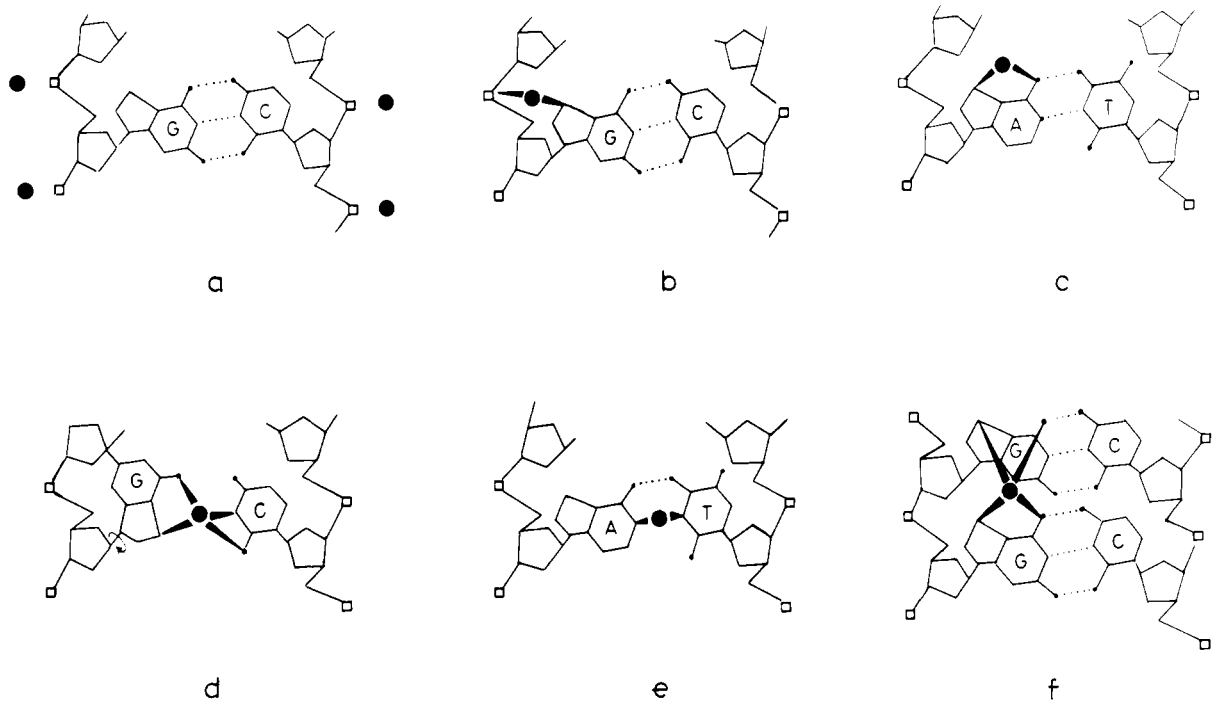


Figure 31. Probable binding sites for metal ions on DNA molecule: (a) electrostatic binding to negatively charged phosphate groups; (b) mixed chelate between N(7) of purine base and phosphate group of the same strand; (c) internal chelate between N(7) and O(6) or N(6) of the same purine base; (d) interstrand complex between reoriented guanine base and its corresponding cytosine base (specific for GC base pairs); (e) interstrand cross-link between N(1) of purine base on one strand and N(1) of pyrimidine base on the opposite strand; (f) charge-transfer complex between two successive guanine bases located on the same strand involving N(7) and O(6) (specific for the GpG sequence) (after Sissoëff, Grisvard, and Guillé[342]).

ion interaction and the effects due to the changes in the secondary structure of DNA, Hanlon and co-workers[333] have studied the variations produced in the CD spectrum of DNA when the concentration of the chloride salts of Mg(II), Ca(II), Mn(II), Zn(II), and Co(II) in the solution is increased. Although the transition cations Mn(II), Zn(II), and Co(II) are known to modify the CD spectrum of DNA at very low concentrations,[334] the results obtained from a spectral component analysis[333] seem to support the hypothesis that the DNA transitions in solutions of $MnCl_2$, $ZnCl_2$, and $CoCl_2$ involve, like those in solutions of monovalent cations, a transformation from a practically unperturbed B-like form to a C state. Further differences observed in the spectral shapes (over those of the reference spectra) during the transition are due to a perturbation of the C-type spectrum. The latter effect can be associated with a change of the chromophores' electronic properties as the divalent ions interact with the bases. The specific effects of the cations and the hydration of the DNA molecule appear to be linked functions, associated with the DNA conformation.

The divalent cations Mn(II) and Zn(II) have also a pronounced effect on the formaldehyde-induced conformational transition of DNA.[335] The theoretical studies on the kinetics of the DNA–formaldehyde interaction as well as the CD measurements carried out on the system led to the conclusion that the binding of the Mn(II) and Zn(II) ions with the chromophoric groups of native DNA decreases the despiralization rate constant of the macromolecule.[336] At the final stage of the process, the Mn(II) or Zn(II) ions are replaced from their sites on the GC pairs by the molecules of formaldehyde.

Organic planar cations (i.e., 3,8-dimethyl-*N*-methylphenanthrolinium (DMP)) are known to intercalate between DNA base pairs, producing a highly remarkable stabilization of the DNA helix.[337] NMR studies on the ternary Mn–DNA–DMP complex have revealed the position of the DMP molecule (its long axis lying almost perpendicular to the H bonds of the DNA base pairs), which brings about the rigidity of the whole structure.[338] By using this observation, Prusik and Geacintov[339] have physically intercalated polycyclic aromatic molecules and dyes (i.e., acridine orange) between the base pairs of DNA and have optically excited them to their triplet states. The quenching of the triplets by Ag(I) and Mn(II) ions (also bound to the DNA molecule) could then be observed. Under these conditions, it was found that the triplet decay curves are exponential, just as in the case of absence of metal ions. This observation can only be satisfactorily explained by the action of a mechanism which involves the free migration of the metal ions along the length of the stiffened DNA–dye complex. Thus, a high probability exists that the metal ions bound to DNA molecules in aqueous solution could diffuse from base pair to base pair along the helical structure.

Two equilibrium constants can, at least, be defined for Mn(II) bound to DNA.[340] However, the Mn(II)–DNA binding constants depend strongly on the concentration of a monovalent salt (i.e., NaCl) in the aqueous solution,[341] which is in agreement with the observation that the effective quenching ability of the Mn(II) ions on the triplet of the bound dye is reduced in the presence of high concentrations of NaCl.[339] Such observation indicates an easy substitution of the mobile metal ions within the DNA helix.

Many specific effects in the metal ion–DNA interactions as well as the biological implications of the presence of metal ions on reiterative DNA molecules have been already described by Sissoëff et al.,[342] who have also illustrated the probable binding sites for metal ions on the DNA macromolecule (Figure 31).

On the other hand, the use of cationic polymers can give an interesting approach for the study of the influence of electrical charges in the binding of metal ions to DNA. The investigations carried out by Mita et al.,[343] using cationic polymers having various distances between charges, have shown that their behavior in binding DNA depends on the charge density of each polycation, which determines the cooperative and noncooperative nature of the binding. Helix–coil transitions can be induced on the highly stable organic polycation–DNA complexes when submitted to thermal treatment. The observed CD effects during the transition are linked to a transformation of the B to a C

form.[344] On the other hand, it has been observed, by means of light-scattering analyses, that polyamine trivalent or tetravalent cations (i.e., spermidine or spermine) cause the molecular condensation of extended coil forms of DNA by means of the almost complete neutralization of the DNA phosphate charges.[345]

The function of bound cations in transfer RNA seems just to be the neutralization of the important electrostatic repulsion of closely neighboring, negatively charged phosphate groups.[346] EPR titration studies on the binding of Mn(II) to tRNA have suggested the existence in tRNA of cooperative binding sites, but also of independent binding sites. The values of the binding constants for the latter are similar to those found for homopolynucleotides.[304]

In order to study selectively the cooperative binding of Mn(II) to RNA, low concentrations of both the Mn(II) ions and the polynucleotide have been used in solutions of low ionic strength (in order to avoid cation competition).[347] The results of the EPR experiments, presented in a Scatchard plot, give a curve with a positive slope and downwards curvature at low concentrations of bound Mn(II). These features of the plot can only be interpreted by assuming an interaction between binding sites which induces some structural change of the macromolecule. At Mn(II) concentrations higher than bound Mn/phosphate = 0.05, it follows in the plot a negative slope and upward curvature, while above bound Mn/phosphate = 0.25 the plot is linear. The last two regions would indicate another type of binding site (noncooperative) and correspond respectively to strong and weak independent sites. By studying the possible contributions to the Mn(II) EPR line broadening,[347] a minimal distance of about 1.5 nm has been estimated between the strong binding sites.

On the other hand, some variations observed in the CD spectra of tRNA in the presence of Mn(II) ions have been interpreted as due to a local conformational change near the 4-thiouracil residue, taking place when Mn(II) is bound by the tRNA molecule.[348] Also, equilibrium dialysis experiments have confirmed the existence of a cooperative as well as a noncooperative phase in the binding of Mn(II) ions to intact molecules of yeast phenylalanine-specific tRNA.[349] Some of the strong binding sites are associated with the cooperative phase, which is instead lacking when the Mn(II) ions bind to fragments of the original tRNA molecules. The cooperative sites seem to arise from the tRNA tertiary structure since the cooperative phase is also lacking in the binding of Mn(II) to the poly(A):poly(U) and poly(I):poly(C) helixes as well as in the single-stranded poly(A) and poly(U). It must be noted that the Mg(II), which can be substituted by the Mn(II) ion in many biological reactions, also induces slow structural changes in transfer RNA when binding to cooperative sites, as was demonstrated by the use of a fluorescent probe.[350] It appears, however, that there are no so well-defined strong binding sites for the Zn(II) and Cu(II) ions on both tRNA and rRNA, as shown by equilibrium dialysis studies.[301] In both cases there is a heterogeneity of binding sites, and experiments carried out in competitive binding conditions indicate that the two metal ions compete for the same binding sites.

The binding of the Mn(II) ion to tRNA has been found to be also highly affected by the presence of Co(III) ions,[351] which seems to indicate that Co(III) binds at sites normally occupied by Mn(II). The maximum number of exchangeable divalent ions in tRNA is 30,[352] and, among them, six are strong binding sites for Co(III). The use of cobaltic labels for structural studies in the vicinity of specific binding sites has been proposed.[353] As an example, the five cooperative sites for Mn(II) in tRNA[349] are no longer available when six Co(III) ions are bound to tRNA.

Also, highly stable complexes formed by a transition-metal ion and a neutral ligand (i.e., $Co^{III}(NH_3)_6$) can effectively replace the Mg(II) and Mn(II) ions in the tRNA binding sites.[354] In this case, kinetics and equilibrium studies indicate that about five complex cations can bind strongly to yeast tRNA, stabilizing its "native" tertiary structure. The order of relative effectiveness for a series of complex cations has been found to be consistent with general electrostatic considerations.

An EPR spin-label method has been used by Vocel and coworkers[355] to detect the conformational changes of tRNA which are induced by the coordination of Mn(II) ions. These investigators have found that the first and second Mn(II) ions produce a sharp increase of the spin-label mobility. These observations are indicative of significant conformational changes, which are simultaneous with the appearance of new coordination sites for Mn(II) ions. The latter can arise from the exclusion of nitrogen bases from the system of hydrogen bonds. The data[355] also indicate that the coordination sites for Mn(II) are occupied in a well-defined order.

The electrostatic effects seem to be very important in the binding of Mn(II) to tRNA, as suggested by its strong dependence on the concentration of monovalent cations in solution.[356] This observation has been confirmed by renaturation experiments of the native structure of tRNA by monovalent ions.[357] The study of the renaturation process, followed by a fluorescent probe, can afford an explanation of the cation binding to tRNA in terms of almost exclusively electrostatic interactions.

However, the specific tertiary interactions in transfer RNA would be stabilized by Mn(II) at the five strong sites, as was demonstrated by the selective broadening of resonances in the NMR spectra of yeast tRNA.[358] These sites must be located close to the tertiary interactions, and three of them are probably the same for Mn(II) and Mg(II). The probable locations of the strong binding sites have been assigned by examining the models for yeast tRNA, proposed from X-ray diffraction studies,[359] but the crystallographic studies undertaken by Jack and collaborators[360] on cation-binding tRNA, crystallized in the presence of Mg(II) ions, have shown that the Co(II) and Mn(II) ions prefer to bind primarily to the bases, as in the transition metal–nucleotide complexes. Some of the most remarkable crystal structures of metal complexes of transfer RNA, besides those of the metal complexes of nucleic acid constituents, have been recently described by Swaminathan and Sundaralingam.[361] It is finally worth mentioning the conclusions recently proposed by Walters et al.[362] after a theoretical reconsideration of several binding studies of Mg^{2+} and Mn^{2+} ions to tRNA. They had suggested a simple model for interpreting both curved and bell-shaped Scatchard plots reported in such studies. It has been shown that these plots can be accounted for quantitatively if appropriate corrections are made for electrostatic interactions and for the effect of conformational changes on these interactions. According to this model there is no need to invoke more than one class of binding sites on tRNA, and all phosphate groups would actually have the same intrinsic binding affinity for either magnesium or manganese.

3. *Electrostatic Theories*

These theories are mainly based on the chemical–physical properties of polyelectrolytes[363] and give a model for the ionic association between single ions and polyelectrolytes in terms of the linear charge density of the polyelectrolytic molecules and the charges of the counter- and co-ions in the limiting case of infinite dilution,[364-366] but the model seems to be appropriate even in the case of high counterion concentration.[367] The concept of counterion condensation, as outlined by the electrostatic theories, is based on the principle that counterions will condense on a polyelectrolyte to lower its linear charge density to a limiting value.[368] This limiting law has been verified in several cases concerning univalent counterions and some others concerning bivalent cations.[369,370] The situation would be, however, more complicated in the case of a mixture of counterions with different valences, because of the competition between cations for

condensation; this case has also been treated by Manning,[368] in its two-variable theory, for two types of simple cations.

An extension of the basic electrostatic theories is given by the work of Magdelenat et al.,[371] which treats the formal relationships between the Scatchard chemical model for the association between ions and polyelectrolytes[372] and the Manning approach in order to get a physical meaning and a precise structural interpretation of the Scatchard plots. On the other hand, De Marky and Manning[373,374] have also given a theoretical tool for calculating the dependence of the DNA double-helix stability on the excess univalent (or bivalent) cation concentration as well as on the polynucleotide phosphate concentration. Also, formal relationships for the extent of incompleteness of counterion condensation on a polyelectrolyte when a strong electric field is applied to the system have been developed;[375] they show the dependence of the stability of ordered polynucleotide structures on field strength. An interesting application of the principles of Manning's counterion condensation has been afforded by the calculation that 89 to 90% of the DNA phosphate charges need to be neutralized by condensed counterions to get a molecular collapse of the native polynucleotide.[345]

The dielectric behavior of metal–DNA complexes, as investigated by Srivastava et al.,[376] indicates that there are considerable effects, due to structural alterations, of the metal ions on the static dielectric constants and dipole moments of the DNA molecule. On the other hand, theoretical studies by Pullman and Berthod[377] show that the interaction between mono- and divalent cations (treated as point charges placed at appropriate distances) and the anionic phosphodiester unit results in a specific screening of the phosphate electrostatic potential. Also, molecular orbital calculations on the specific interaction of Cu(II) with nucleic acid bases[378] have precisely defined the alteration of the charge distribution patterns for practically all the atoms of adenine, guanine, uracil, and cytosine when the metal ion is fixed at different binding sites. All these experimental and theoretical studies give a proof of the complication of a problem which simultaneously involves electrostatic charge distributions and molecular conformations.

IV. Considerations and Conclusions Derived from the Body of Information Obtained by Physical Methods

The electrostatic theories which have been developed to account for a general explanation of the metal ion effects on polyelectrolytes of such structural complexity as the nucleic acids represent remarkable steps in the way of rationalizing the mechanisms of these interactions. The counterion condensation theory (so far the most accepted general theory) is, however, approximate when considering some aspects of the problem. One of its greatest simplifications is to treat all ions as simple point charges in such a way that they can only be distinguished on the basis of charge. However, specific ion effects have been observed in the experimental studies with nucleic acids, and they become even more evident when studying the interaction of metal ions with mononucleotides, nucleosides, and nucleic bases. It must be expected that some metal ion specific properties such as size, degree of hydration, and geometric complexity will have some effect on the mode of interaction. This situation is even emphasized in the case of transition-metal ions, which have the capacity of forming inner-sphere complexes. Furthermore, the phenomenon of interaction is usually complicated by cooperative effects.

If any conclusion had to be derived from the bulk of information that the physical methods have presented to us, it would be in the direction that only the most systematic classification and rational organization of the available material could afford a fairly good picture of the whole situation by linking together complementary results.

V. Perspectives

In summary, it can be said that, at the present time, the actual possibilities of filling the gap still existing in the scientific literature concerning the interaction of metal (particularly, of transition metal) ions with nucleic acids and their constituents come from still-developing methods. Many signals, indicative of this promising situation, can be found in the latest literature, some of them being theoretical approaches.[244,245,377,379] Many reports are also concerned with sophisticated techniques not yet fully employed in this research, such as laser Raman spectroscopy for the study of molecular conformation,[380–384] oscillopolarography,[294] solid-state proton magnetic relaxation,[385] natural-abundance ^{15}N NMR spectroscopy,[386] and ^{19}F NMR,[387] as well as the use of modified molecules.[388,389]

VI. References

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