Available online at www.sciencedirect.com
ScienceDirect
Coordination Chemistry Reviews 252 (2008) 2362–2380
COORDINATION CHEMISTRY REVIEWS
www.elsevier.com/locate/ccr

Review

Multinuclear NMR studies of the interaction of metal ions with adenine-nucleotides

Zoltán Szabó *

Department of Chemistry, Inorganic Chemistry, Royal Institute of Technology (KTH), S-10044 Stockholm, Sweden

Received 2 December 2007; accepted 2 March 2008
Available online 14 March 2008

Contents

Abstract

It is well-known that metal ion complexes are essential in various biological systems, including those with adenosine nucleotides which are substrates for a large number of enzymatic processes. The interactions of various metal ions with adenosine nucleotides have been intensively studied by multinuclear NMR spectroscopy. Nucleotides are polydentate ligands with various potential binding sites, including nitrogen atoms on the purine base, hydroxyl groups on the ribose sugar, and negatively charged oxygen atoms in the phosphate group. Depending on the experimental conditions (*e.g.* pH, concentration range, etc.) and on the size and nature of the metal ions, monodentate, or multidentate coordination to these donor atoms are possible. The review focuses on the applications of different NMR techniques in identifying the stoichiometry and the mode of metal binding in complexes formed with the most important adenosine nucleotides, like adenosine-5′-mono-, di- and triphosphates (AMP, ADP and ATP). Ligand exchange dynamics for some metal ion complexes are also presented.

Keywords: Metal ions; Nucleotides; NMR spectroscopy; Solution coordination chemistry; Complex formation

1. Introduction

1.1. Interaction of metal ions and nucleotides

Metal ions (either as isolated ions or in clusters) play an important role in almost all biological processes. Thirteen metals are presently known and accepted to be essential or beneficial

* Tel.: +46 8 7908144; fax: +46 8 212626.
E-mail address: zoltan@kth.se.

0010-8545/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ccr.2008.03.002

components in all-living systems. Four of these, Na, K, Mg and Ca are present in large quantities and known as the *bulk metals*. The remaining nine are present in smaller quantities and known as *trace metals*. These belong to the d-block elements V, Cr, Mo, Mn, Fe, Co, Ni, Cu and Zn. The interactions of the metal ions and the surrounding neighbors (ligands) in biological systems play a key role in biological processes, both natural processes and human induced processes (*e.g.* drugs, pollution and cleaning). Although there are many well-documented examples [1], still little is understood about the modes of action of metal ions at the molecular level.

It is well-known that metal ion complexes are essential in various biological processes, including those with orthophosphoric acid esters, as the information-carrying nucleic acids (DNA and RNA) or nucleotides [2–10]. Nucleotides occur as coenzymes, intermediates and are also the building blocks for nucleic acids. Adenosine, guanosine, cytidine, thymidine and uridine-5′-monophosphates are the five most important nucleotides found in nature. As shown in Fig. 1, they consist of a heterocycle (nucleobase) bound to a sugar moiety that is converted to nucleotides upon phosphorylation.

These esters are widely distributed and one of the most important classes of ligands in living systems. The most prominent example is adenosine-5′-triphosphate (ATP) with its immense role in the transfer of biochemical energy. Enzymatic reactions involving nucleotides have a general dependence on metal ions. This also applies to the biosynthesis of nucleic acids. Many enzymes require one or more metal ions as a cofactor in catalyzing phosphate ester hydrolysis and trans-esterification. Nucleotides, especially the adenine-nucleotides being substrates for a large number of enzymes, are at the "crossroad" of many biological reactions, and the transfer of phosphoryl or nucleotidyl groups occurs in the presence of divalent metal ions. Tri- and diphosphorylated adenosine (ATP and ADP) provide the energy for activating many enzymatic transformations. The 3′,5′-cyclic monophosphate adenosine (cAMP) plays an important roll in controlling and mediating the actions of peptide hormones. Formation, replication and cleavage of nucleic acid polymers (DNA and RNA), as well as their structural integrity, *e.g.* the double-helical arrangement of conventional DNA require the presence of metal ions. Currently there is considerable interest in understanding the role of the metal ions in these metalloenzymes, and in developing more reactive chemical systems that efficiently and specifically hydrolyze the phosphate diester bonds of DNA and RNA sequences. An understanding of the origin of the stability of these complexes and of their structure in solution can lead to a better understanding of important biological processes and may lead to ideas for the development of new drugs [1–3]. The presence of positively charged metal ions can affect the hydrogen-bond interactions which are essential for DNA base pairing and may result in mispairing of nucleobases, hence an altered genetic information transfer (resulting mutagenic or carcinogenic effects of metal ions). These observations have led to the development of artificial nucleotide analogues, which are among the most promising novel compounds with antiviral properties. Some of them exhibit a cytostatic effect as well.

Considering this, it is not surprising that the research of the interactions of metal ions with nucleotides has drawn great attention. Detailed investigations of these interactions have been made in aqueous solutions, and a remarkable amount of thermodynamic data is available on the metal ion-binding properties of nucleotides [11–13]. Nevertheless, in order to have a deeper insight of the mechanism of these enzymatic reactions, it is essential to know the detailed molecular structure and geometry of the metal–nucleotide complexes. In spite of the extensive studies in the last decade, relatively few data are available concerning the structure of the complexes. It is obvious from the literature that NMR spectroscopy is one of the most powerful analytical tools to gain information about the structure and ligand exchange dynamics of the complexes formed in the reactions of metal ions and nucleotides. A large variety of NMR techniques can be successfully applied to study these interactions. Characteristic changes in the spectral parameters (chemical shifts and

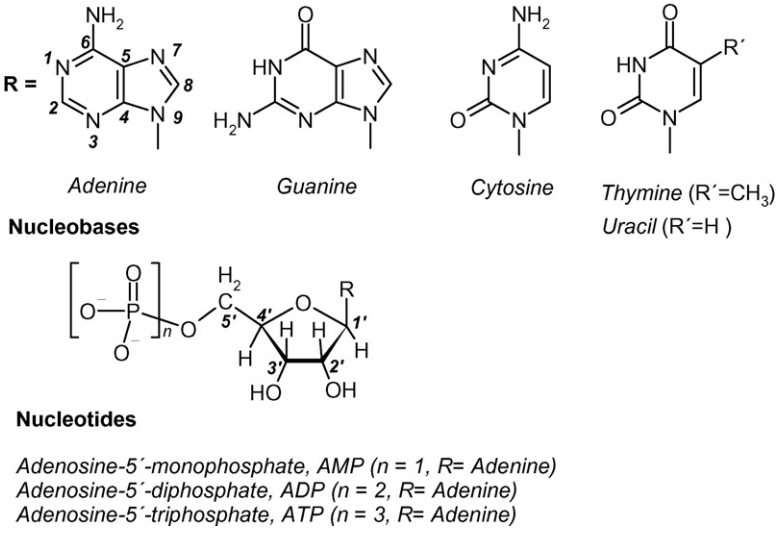

Fig. 1. Structures for the most important nucleotides found in nature.

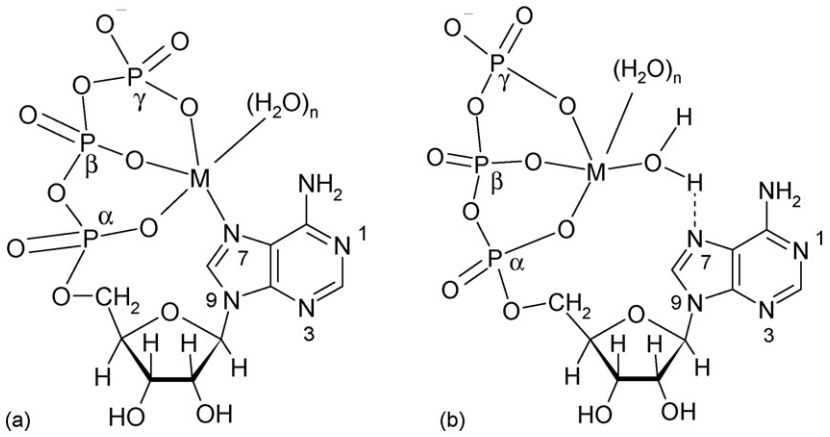

Fig. 2. Structures for macrochelated ATP complexes formed by inner- (a) and outer-sphere (b) coordination of the purine residue (M = metal-ion).

line widths) or changes in diffusion and relaxation rates caused by complex formation have been measured in various systems and used to deduce the structure of the complexes [14–32]. This review offers a brief survey of multinuclear NMR studies in adenosine–nucleotide–metal ion systems. The details of the NMR methodologies are out of the scope of this paper, the focus is on the significance and applicability of NMR spectroscopy.

1.2. Coordination mode of metal ions with nucleotides

Metal ions are bonded through coordination bonds with the donor atoms in the ligands. In biological systems the metal ions are able to coordinate to a variety of biomolecules, *e.g.* to proteins, to nucleic acids and to carbohydrates or lipids. In spite of the great variety of coordinating sites, the metal ions and the biomolecules cannot be treated using the "small" ligand concept in the sense of "classical" coordination chemistry. The *in vivo* reactions between metal ions and ligands are part of a complex system of equilibria, transport and storage. Hence, the use of small molecules, like nucleotides as active-site analogues for nucleic acids (keeping in mind that these molecules themselves as monomers have a key of importance in several biological processes) is a widely used approach in the study of these systems. The purpose of this type of model is not necessarily to duplicate natural properties but to sharpen or focus on certain questions. The goal is to elucidate fundamental aspects of structure, reactivity and chemical mechanism.

Nucleotides are polydentate ligands, with various potential binding sites, including nitrogen and oxygen donors on the bases, hydroxyl groups on the ribose sugar, and negatively charged oxygen atoms in the phosphate group. Depending on external conditions (*e.g.* pH) and on the size and nature of the metal center, monodentate, or multidentate coordination is possible to these donor atoms. The metal ion binding properties of the nucleotides present a true challenge to coordination chemists. The so-called "hard" metal ions like Na(I), Mg(II), Al(III), Mn(II) or Fe(III), preferably interact with the "hard" oxygen sites of the phosphate groups; these metal ions have only a low affinity for the base residues. The "soft" metal ions, *e.g.*, Cd(II), Hg(II), Pd(II), Pt(II), have a rather pronounced affinity for the aromatic N-sites of the nucleobase residues. Nevertheless, in most of the interactions, the coordination with the phosphate group as primary coordination site determines a large part of the stability of the complexes. However, the enhanced stability of adenosine nucleotide complexes with certain metal ions (*e.g.*, with Cu(II), Zn(II), Cd(II) or Pb(II)) in comparison to that expected for the coordination *via* the phosphate group alone indicates that macrochelate formation must occur *via* binding to a nitrogen atom in the heteroaromatic ring [11]. A key question regarding the structure of these complexes is, whether the phosphate-coordinated metal ions bind directly to the N-7 nitrogen in the purine residue (innersphere-type of complex, Fig. 2a), or interact with N-7 *via* a water molecule forming an outersphere-type of complex (Fig. 2b).

2. NMR spectroscopic studies of the interaction of various metal ions with adenine-nucleotides

2.1. Magnesium(II) and calcium(II) complexes

2.1.1. Bonding in magnesium(II) complexes

Magnesium is one of the most abundant metal-ions in biology. It is an essential cofactor for various enzymatic reactions involving nucleotides. Hence, it is not surprising that its complex formation with nucleotides and the mode of coordination in binary ADP, ATP and in various ternary systems (*e.g.* using aromatic amines or other biomolecules as additional ligands) have been intensively studied [33–37]. Unfortunately, the proposals for the structure of the complexes are often controversial. One can find different suggestions concerning the interaction with the purine nitrogens, formation of inner- or outersphere complexes, or even on the stoichiometry of the complexes. The different conclusions are partly due to the differences in the experimental conditions, like the pH and/or total concentration ranges studied in the systems. Nevertheless, all studies agree that the phosphate chain is the primary binding site of magnesium and that the hydroxyls on the sugar unit do not play a role in the complex formation. The chelation pattern of Mg(II) with the phosphate chain in ATP has been studied with various experimental techniques and a controversial issue in these studies is whether the

α- and β-phosphates are coordinated to magnesium. A large variety of NMR nuclei has been used as probes to answer these questions.

In an early ^{15}N NMR study Happe and Morales [38] studied the interaction of Mg(II) with the purine base nitrogens of ATP. In order to increase the sensitivity of the experiment, the ligand and the metal ion concentrations were relatively high, in the range of 0.5–0.9 M, and with a pH range of 7.0–9.6. In the ^{15}N spectrum of ^{15}N enriched ATP five well-separated nitrogen signals were observed. By the addition of equal amounts of Mg(II) to ATP no significant ^{15}N chemical shifts changes were observed indicating that these atoms do not interact with the Mg(II)-ion and that a complex is formed only through phosphate coordination.

The interaction of Mg(II) with ATP was recently studied in a much broader pH range by Jiang and Mao [39]. The pH dependence of the ^{1}H, ^{15}N and ^{31}P chemical shifts of the complex and the free ATP confirmed the results of Happe and Morales [38], however, surprisingly large differences were observed in the ^{15}N shift of the N-1 atom in a narrow pH range at around 3.7 as shown in Fig. 3a. The ^{15}N signals of the ATP ligand were unambiguously assigned by two-bond ^{1}H–^{15}N HMBC spectra measured without and in the presence of $MgCl_2$ (Fig. 3b).

This study was also performed at relatively high concentrations of ATP and magnesium (0.2 M) when self-association of the free and coordinated ligands very likely occurs that may affect the chemical shifts. However, as the authors stated, the aggregation of ATP cannot induce a chemical shift change selectively to N-1. Therefore, it seems very likely that a complex is formed by the interaction with N-1. Since the ^{31}P chemical shifts do not change at lower pH, it was concluded that the binding ability of P_β and P_γ phosphates in this pH range is low, and that significant coordination of Mg(II) only takes place above pH 3.5. By increasing the pH, the phospate oxygens become deprotonated and donate electrons to the metal ion. Consequently, the binding of N-1 becomes weaker and when the pH is above 5 it does not contribute to the complex formation. Based on the ^{31}P chemical shift changes and diffusion NMR measurements the authors suggested that a 1:1 Mg(II)–ATP complex is formed above pH 5 by the coordination of P_β and P_γ phosphates only.

Huang and Tsai [40] have used ^{17}O isotope enriched nucleotides and studied the line broadening effect of the coordination on the phosphate oxygens by ^{17}O NMR spectroscopy. Their conclusion is that Mg(II) interacts with both the β- and α-phosphates and γ- and β-phosphates in ADP and ATP, respectively. Beside this, the α-phosphate in ATP is at least partially involved in the complex formation.

Cowan [41] has used ^{25}Mg NMR to study the degree of coordination of the phosphate chain. The line broadening of the ^{25}Mg signals measured in various systems, together with the differences in the stability constants support bidentate coordination to α- and β-phosphates in ADP and β- and γ-phosphates in ATP. At lower pH, when the α- (in ADP) and β-phoshates (in ATP) are protonated the magnesium preferentially binds only to the terminal phosphate dianion in these ligands.

2.1.2. Ligand exchange dynamics in Mg(II)–, Ca(II)–ATP complexes

The choice of the optimal experimental technique in NMR spectroscopy to study various exchange reactions depends on the characteristics of the exchange system. In systems which are in slow exchange on the time scale determined by the chemical shift differences of the exchanging species, individual signals can be observed for all sites, and kinetic information can be obtained by one- or two-dimensional (EXSY) magnetization transfer experiments [42,43]. When the exchange rate is fast enough to affect

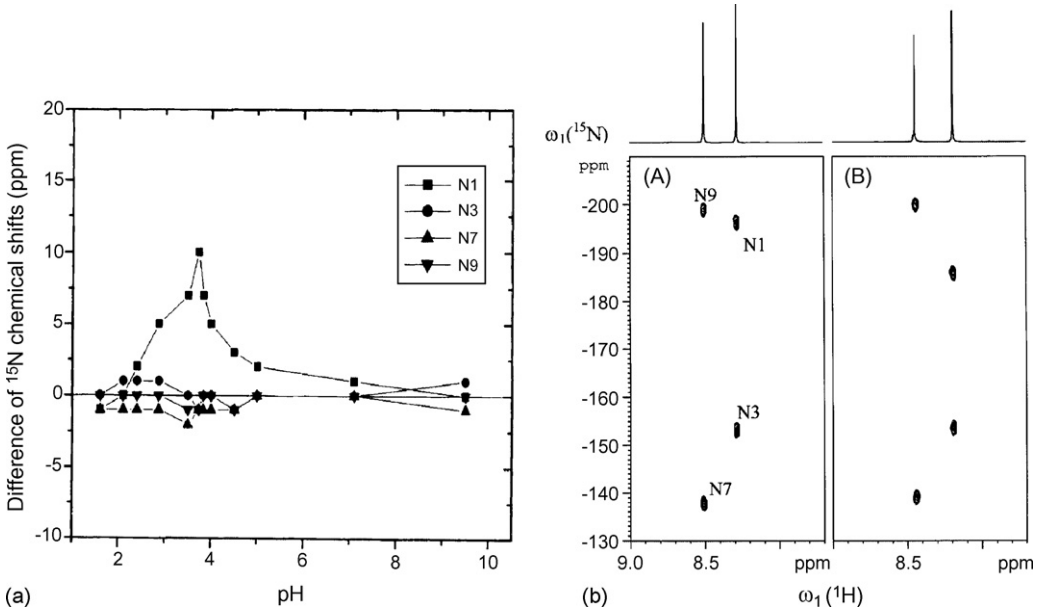

Fig. 3. Plots of the chemical shift changes of ^{15}N of ATP as a function of pH at 298.2 K after $MgCl_2$ is added (A); two-bond ^{1}H–^{15}N HMBC spectra of ATP (A) and ATP solution containing equimolar amount of $MgCl_2$ (B) at pH 3.7 (298.2 K) (B). The figure was reproduced from Ref. [39], with permission of the copyright holders.

the line shape, but still too slow on the chemical shift scale to result in a coalescence of peaks, the pseudo-first-order rate constants may be calculated from the line widths of exchanging species:

$$\pi\Delta\nu_{1/2}(i) = \pi\Delta\nu^{\circ}_{1/2}(i) + \sum_{j=1}^{n} k_{i,j} \quad (1)$$

where $\Delta\nu^{\circ}_{1/2}(i)$ is the non-exchange line width for the ith species and k_{ij} the pseudo-first-order rate constant for the chemical exchange process between sites i and j.

When the exchange rate is fast on the chemical shift scale, as a result of overlapping or coalescence of the peaks, only one peak is observed in the spectrum. The line shape can then be calculated from the individual chemical shifts and the relative populations of the exchanging species by a matrix-formalism suggested by Reeves and Shaw [44]. In this case a quadratic rate matrix contains the linear combination of pseudo-first-order rate constants between the sites involved in the exchange. The determination of the kinetic parameters is based on a comparison between the measured and the calculated spectra.

Vasavada et al. studied the ligand exchange dynamics in Mg-ATP and Ca-ATP complexes by ^{31}P NMR spectroscopy [45]. The ATP exchange for both metal ions was fast on the time scale determined by the largest shift differences of the β-phosphates in the free and the coordinated ATP. Only one exchange averaged signal was observed for each phosphate in the spectra containing equal amounts of the complex and free ATP. However, as shown in Figs. 4 and 5, characteristic differences can be observed in the signals of the β-phosphates. In the case of calcium, the exchange is fast enough to result in a very sharp exchange averaged triplet, while for magnesium, due to the slower exchange, the corresponding signals are merged resulting in a very broad signal without fine structure. Both signals appear in the spectrum as a weighted average of the chemical shifts which can be measured for free and coordinated ATP in the absence of chemical exchange.

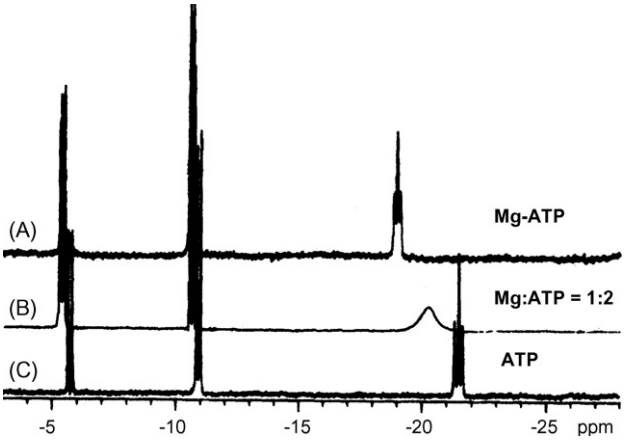

Fig. 4. ^{31}P NMR spectra (121 MHz) of buffered solutions of ATP, MgATP + free ATP and MgATP recorded at 20 °C and pH 8.0. *Concentrations*: (A) $MgCl_2$, 8 mM; $(Na)_2$ATP, 5 mM; (B) $MgCl_2$, 2.7 mM; $(Na)_2$ATP 5 mM; (C) $(Na)_2$ATP, 5 mM. The figure was reproduced from Ref. [45], with permission of the copyright holders.

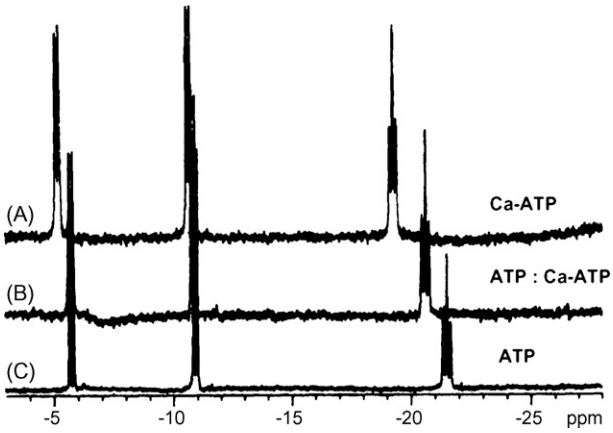

Fig. 5. ^{31}P NMR spectra (121 MHz) of buffered solutions of ATP, Ca-ATP + free ATP and Ca-ATP recorded at 20 °C and pH 8.0. *Other concentrations*: (A) $CaCl_2$ 6 mM, $(Na)_2$ATP 1.5 mM; (B) $CaCl_2$, 0.75 mM; $(Na)_2$ATP, 1.5 mM; (C) $(Na)_2$ATP, 1.5 mM. The figure was reproduced from Ref. [45], with permission of the copyright holders.

From a dynamic NMR point of view the exchange between the free and coordinated ATP can be considered as a two-site exchange in these systems. The exchange between the two sites could occur by the following two reactions, *via* dissociation of a coordinated ligand (2)

$$\text{M(II)-ATP} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{M(II)} + \text{ATP} \quad (2)$$

and/or *via* a bimolecular reaction of the complex and the free ligand without net chemical change (3)

$$\text{M(II)-ATP} + \text{ATP}^* \underset{k_3}{\overset{k_3}{\rightleftharpoons}} \text{M(II)-ATP}^* + \text{ATP} \quad (3)$$

M(II) is either Mg(II) or Ca(II), k_1, k_2 and k_3 are the corresponding rate constants. The star indicates the two different sites for the ATP ligand. The latter reaction would result in a concentration dependence of the exchange rate on the free ATP ligand. However, since this was not observed in the experiments performed at various ATP concentrations, the contribution of this reaction was not considered in the analyses. The exchange rates were determined with the aid of spectrum simulation as a function of the exchange rate using the quadratic rate-matrix formalism mentioned above. The comparison of the measured and the simulated line shapes resulted in a rate constant of $2.1 \times 10^3\ s^{-1}$ for the dissociation rate of the magnesium complex. For the Ca(II)–ATP complex only a lower limit of the rate constant, $3 \times 10^5\ s^{-1}$, could be estimated because the calculated line shape did not change by using larger rates than this value in the simulation procedure. The experiments proved that the ATP dissociation is at least two orders of magnitude faster for calcium than that for magnesium. The Arrhenius-plot of the rate constants in a temperature range of 10–50 °C resulted in an activation energy of 8.1 kcal mol^{-1} for the dissociation of ATP in the Mg(II)–ATP system.

A significantly larger metal–ligand concentration range of the Mg(II)–ATP system was studied later also by ^{31}P NMR spectroscopy [46]. The magnesium to ATP ratio in the test solutions was kept constant at 0.5, and the total ATP concentration was decreased from 100 to 0.2 mM. In the ^{31}P NMR spectra measured at higher than 10 mM ATP concentrations only one set of signals was observed for the α, β and γ phosphates. However, the spectra recorded at 10 mM and below showed an increase of the line width for all signals, and finally the spectrum measured at 0.2 mM total ATP concentration showed two signals for both the β and γ phosphates and one for the α phosphate as shown in Fig. 6.

This indicates that at least two ATP species are in fast exchange equilibrium with each other at higher total ATP concentrations than 10 mM. The chemical shift differences between the corresponding phosphate sites in the two species are different, they are larger for the β and γ phosphates than that for the α phosphates (similarly to the study discussed before), resulting in a different NMR time scale for the exchange reactions. Consequently, the exchange rate at the lowest concentration is still fast for the α phosphates and only one broad peak can be observed, while it is slow enough for the β and γ phosphates to result in individual peaks for the exchanging sites. These changes in the spectra can be related to two different processes; dilution slows intermolecular exchange or results in dis-aggregation of aggregated species. However, the latter can be excluded because changes in the spectra can be observed even at the concentration (10 mM and below) where the purine rings are no longer stacked. Hence, similar to the study discussed before, the exchange between the two sets of signals may be due to the ATP exchange between the coordinated ATP in the complex and the free ATP (or Na_2ATP). However, based on the analysis of the characteristic changes of the chemical shifts and couplings, the author stated that this is plausible and favored the following mechanism. At higher concentrations the solutions contain aggregates of $Mg(ATP)_2$ complexes in which there are two conformations of the ATP residues in fast equilibrium. The exchange between the conformers is fast on the NMR shift time scale and results in one set of time-averaged signals for the phosphorous sites. Upon dilution, the aggregates dissociate and the exchange between the phosphates become slow, finally individual signals for the two distinct ATP residues in the $Mg(ATP)_2$ complex can be observed. The spectral effects of dilution were also investigated on samples analogous to the above but containing Ca(II) in the same study [46]. At 1 mM ATP concentration, where five signals were observed for the corresponding Mg(II) complex, the Ca(II) complex showed only three signals, one for each phosphate group. These signals showed some line broadening indicating exchange. In order to slow down the exchange the spectrum was recorded at 0.02 mM ATP concentration. In this solution two broad signals, one for each α- and γ-phosphate were observed without any sign of splitting, while the β-phosphate peak was similar to that observed for the magnesium complex at the 10 mM ATP concentration (see Fig. 6). This, in accordance with the study discussed before [45] confirmed that the ligand exchange reaction is much faster in the Ca(II)–ATP complex than that in the Mg(II)–ATP complex.

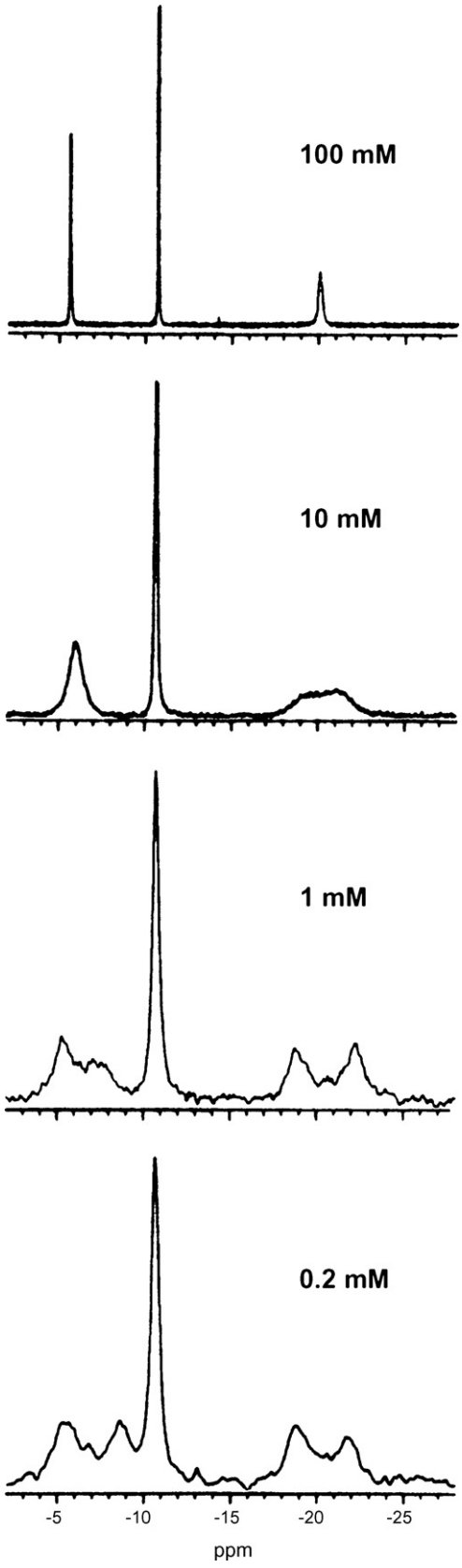

Fig. 6. Proton-decoupled ^{31}P NMR spectra of aqueous Mg(II)–ATP complexes corresponding to the composition $Na_6Mg(ATP)_2$ at pH 7.2 and 24 °C, demonstrating the effects of dilution. The Mg(II):ATP ratio is 0.5, and mM's indicated correspond to the ATP concentration. The figure was reproduced from Ref. [46], with permission of the copyright holders.

2.2. Aluminum(III) complexes

It is known that several human diseases can be linked to the presence of aluminum(III) [47]. Although little is known about the molecular base of the effects of this metal in biological systems, it is clear that the aluminum coordination to phosphate bearing biomolecules, including nucleotides, plays an important role in various biological processes. Because of the similar size, aluminum(III) may interfere and replace magnesium(II) in ATP complexes in living systems. The differences in binding affinity, stability and ligand exchange dynamics of the complexes may all contribute to the differences in the biological effects of aluminum(III) and magnesium(II). The structural and the biological aspects of the interaction of Al(III) with nucleotides and other biomolecules have been reviewed in the recent past [47–49]. Therefore, only a brief summary of the most important conclusions concerning the structure of Al(III)–nucleotide complexes is given below.

Some studies reported macrochelate formation of Al(III) with ATP *via* coordination to the N-7 nitrogen based on the ^{1}H NMR chemical shift changes of the purine ring. Others ruled this out and agreed that Al(III) exclusively interacts with the phosphate oxygens in the nucleotides [47,48]. The first binding constants ($\log K_1$) of Al(III) with completely deprotonated phosphate groups in AMP, ADP and ATP increase modestly (6.17, 7.82 and 7.92) [50]. This indicates that both ADP and ATP ligands are coordinated in bidentate fashion to the phosphate chain, and that the α-phosphate in ATP is not involved in the complex formation. From ^{1}H, ^{13}C, ^{31}P and ^{27}Al NMR measurements the formation of two main species was established with ADP and ATP [51]. In the pH range of 3.8–7.3 two dimeric complexes are formed in which the Al(III) to nucleotide ratio is 2:2. In the presence of the excess of the ligands in the pH range of 4.2–8.1, a biscomplex is formed with 1:2 metal/ligand ratio. Above pH 8, Al(III) does not interact with the nucleotides and the formation of $Al(OH)_4^-$ becomes dominant. The exchange reactions between the free and coordinated nucleotides in these complexes are slow as indicated by their separated ^{1}H and ^{31}P NMR signals [52].

2.3. Complexes with d-block metal ions

2.3.1. Complexes with potential antitumor activity

Platinum is the most widely studied metal concerning the complex formation with nucleotides due to the potential antitumor activity. The application of platinum complexes in cancer therapy is, perhaps the best-known example of the use of metal complexes in the treatment of a disease. After the discovery of the antitumor activity of platinum(II) complexes, the interactions of other metal ions and nucleotides, among others, palladium(II), ruthenium(II) and organotin(IV) have been intensively studied. Although the mechanism of their anticancer activity is not fully understood, the results provide the base for an intensive area of research.

2.3.1.1. Platinum(II) complexes. The kinetics of the complex formation between *cis*-diamminedichloroplatinum(II), *cis*-$[Pt(NH_3)_2Cl_2]$, and adenine-nucleotides have been studied at different platinum/nucleotide ratios by multinuclear NMR spectroscopy [53,54]. In these studies the reactions were followed by the time dependence of the NMR signals. The experiments indicated the existence of several consecutive reactions and the formation of different intermediates. These reactions proved to be slow and the formation of the final products took several hours.

In one of the studies, the kinetics of the reaction of AMP with an excess of *cis*-$[Pt(NH_3)_2Cl_2]$ was followed by ^{1}H NMR, and the structure of the species formed was further characterized by their ^{195}Pt spectra [53]. The final product of the reaction is formed by consecutive reactions *via* two intermediates. In the intermediates one chloride of the *cis*-$[Pt(NH_3)_2Cl_2]$ complex is replaced by an AMP ligand, which is coordinated either by the N-7 or N-1 nitrogen of the purine ring as shown in Fig. 7, structures IA, IIA. Subsequently, these intermediates react with the excess of *cis*-$[Pt(NH_3)_2Cl_2]$ resulting in the final product in which two platinum atoms are bound to N-7 and N-1 nitrogen atoms of an AMP molecule (Fig. 7, structure IIIA).

Different intermediates and final products were reported a few years' later for the reactions of *cis*-$[Pt(NH_3)_2Cl_2]$ and an excess of AMP, ADP and ATP [54]. The reactions were studied by ^{1}H, ^{13}C, ^{31}P NMR spectroscopy and spectrophotometry. Both experimental techniques confirmed consecutive, first-order kinetics in the system. As shown in the proton-decoupled ^{31}P spectrum, two sets of two doublets were observed for the coordinated ADP molecules in the final products, beside the two doublets for the free ligand (Fig. 8).

Similarly, two ^{31}P signals for the corresponding product with AMP and two sets of ^{31}P multiplets for the product with ATP were observed. These observations and the integral of the signals confirm the formation of two products with 1:1 stoichiometry. The characteristic ^{1}H and ^{31}P chemical shift differences support the coordination of both the N-7 nitrogen and one of the phosphate groups for all nucleotides. Based on these, the authors suggested that initially a phosphate bound intermediate is formed in the reactions of either *cis*-$[Pt(NH_3)_2Cl_2]$ or the aquated *cis*-$[Pt(NH_3)_2(H_2O)Cl]$ complex and the nucleotides, which is then followed by the substitution of the second chloride through inter- and intramolecular coordination of the N-7 nitrogen of the purine ring. Three structures were proposed for the products as shown in Fig. 9, although due to the proximity of the adjacent phosphate groups in structure III, the authors disregarded this one as major product of the reaction.

Briand et al. studied the pH dependence of the complex formation between *cis*-$[Pt(NH_3)_2Cl_2]$ and AMP and ATP by multinuclear NMR and did not find evidence for N-1 binding of the nucleotides [55]. They identified two complexes with 1:1 stoichiometry, *cis*-$[Pt(NH_3)_2$(AMP-N7)Cl] at acid pH, and *cis*-$[Pt(NH_3)_2$(AMP-N7)OH] at neutral and basic pH, and two other complexes with 1:2 stoichiometry, *cis*-$[Pt(NH_3)_2$(AMP-N7)$_2$] and *cis*-$[Pt(NH_3)_2$(AMP-N7)(AMP-PO)]. AMP-N7 and AMP-PO indicates that the AMP ligands are coordinated in a monodentate fashion through the N-7 nitrogen and the phosphate group, respectively. In the complexes formed with ATP the phosphate moiety proved to be the most reactive bind-

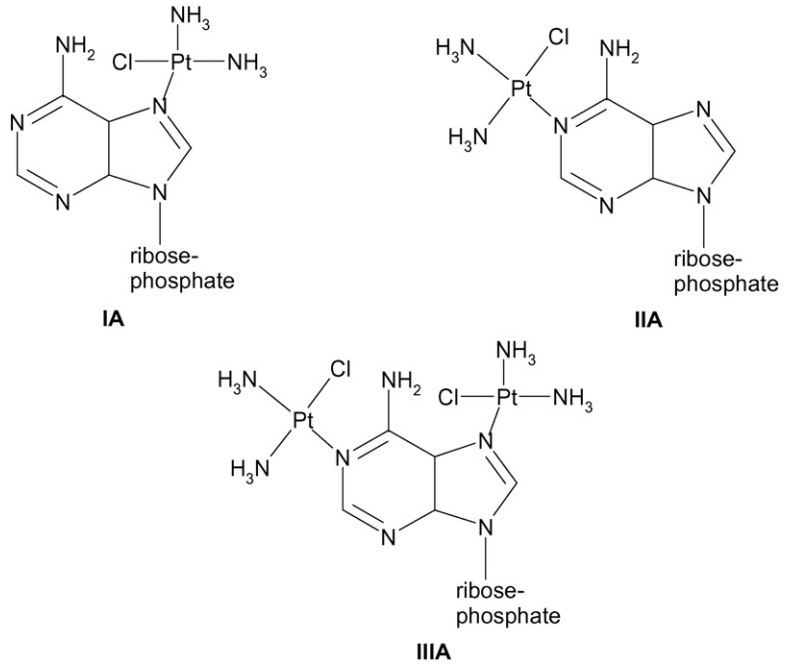

Fig. 7. Proposed structures of species IA, IIA, and IIIA formed in the reaction of AMP with excess *cis*-$[Pt(NH_3)_2Cl_2]$. The figure was reproduced from Ref. [53], with permission of the copyright holders.

ing site and the phosphate coordinated intermediates play an important role in the formation of N-7 coordinated complexes, *cis*-$[Pt(NH_3)_2(AMP\text{-}N7)H_2O]^+$ and *cis*-$[Pt(NH_3)_2(ATP\text{-}N7)_2]$ at different pH values.

Competitive complex formation of *cis*- and *trans*-$[Pt(NH_3)_2Cl_2]$ and $[Pt(en)(H_2O)_2](CF_3SO_3)_2$ complexes (en = ethylenediamine) with a large excess of the mixture of four nucleotides was studied by Raman and ^1H NMR spectroscopy [56]. The mixture of four nucleotides AMP, guanosine- (GMP), uridine- (UMP) and cytidine-monophosphate (CMP) served as a reasonable binding model for denatured polynucleotides. Both experimental techniques indicated complex formation with purine nucleotides (AMP and GMP), but not with the pyrimidine nucleotides (UMP and CMP) at pH 7. The ^1H spectra showed the formation of complexes with both purine nucleotides $[Pt(en)(AMP)_2]^{2-}$, $[Pt(en)(GMP)_2]^{2-}$ and a mixed complex $[Pt(en)(AMP)(GMP)]^{2-}$. The signal intensities showed a relatively small difference in selectivity to the benefit of AMP, hence this molecule can coordinate through either N-7 or N-1, while GMP can coordinate only by N-7 under the experimental conditions studied. This specificity of the complex formation with the mononucleotides seemed to be valid for larger molecules. IR experiments proved that the reaction of *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ with calf-thymus DNA led to a selective binding to the purine bases, but essentially no binding to either pyrimidine bases was observed, similar to that observed in the mixture of mononucleotides.

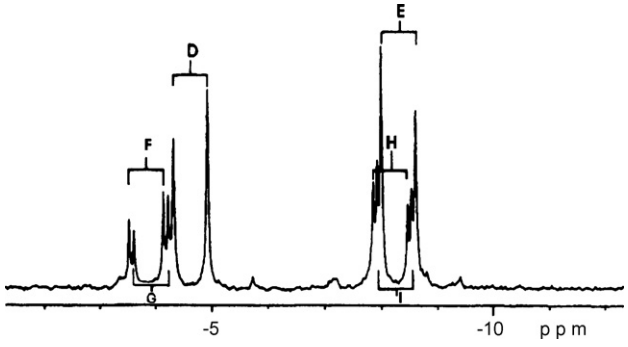

Fig. 8. Ambient-temperature 36.3-MHz proton-decoupled ^{31}P NMR spectrum of the reaction mixture of *cis*-diamminedichloroplatinum(II) (5 mM) and ADP (40 mM) at pH 6.8 after 48 h. Doublets D and E are due to free ADP. The four sets of smaller doublets are due to two products. The figure was reproduced from Ref. [54], with permission of the copyright holders.

The reaction of the platinum complex containing a tridentate ligand [Pt(L)Cl]Cl (L: ethylenetriamine, $NH_2\text{-}CH_2CH_2\text{-}NH\text{-}CH_2CH_2\text{-}NH_2$) with various nucleotides was studied by ^1H NMR [57]. In the reaction with AMP two 1:1 complexes are formed through N-7 and N-1 coordination. By increasing the platinum concentration a third complex became dominant in which two platinum moieties are bridged by one AMP molecule through N-7 and/or N-1 binding.

In a combined ^1H and ^{31}P NMR study the coordination of the purine rings through N-7 was proposed for *cis*-$[Pt(NH_3)_2L_2]$ complexes (L: AMP, ADP, ATP and GMP) [58]. The appearance of two resolved NMR signals for most of the nuclei at ambient temperature indicated the existence of two diastereomers (rotamers) due to the restricted rotation about the Pt–N7 bond. The two isomers exist in head-to-tail conformations in which the two six-membered ring moieties of the purine base are on the opposite sides of the square plane. As shown in Fig. 10, the cross peaks observed in the two-dimensional EXSY spectrum between the ^{31}P signals of the *cis*-$[Pt(NH_3)_2(AMP)_2]$ indicated exchange between the rotamers.

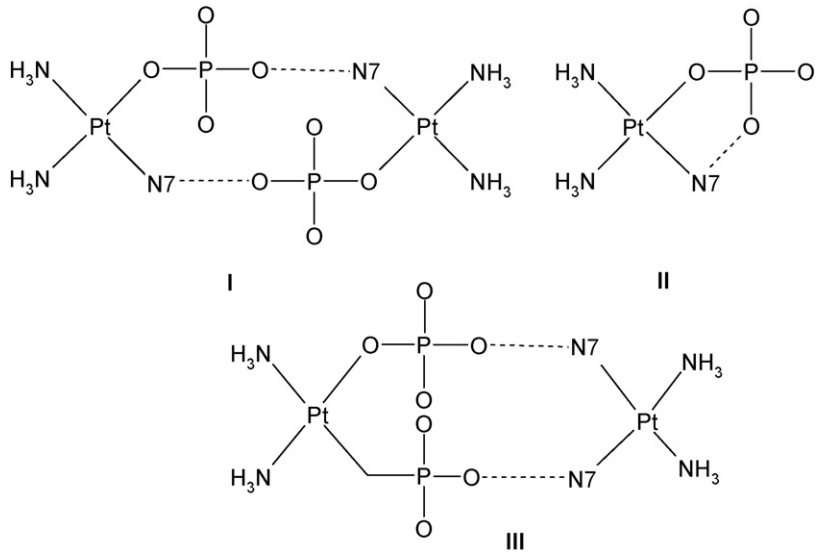

Fig. 9. Proposed structures of species I, II, and III formed in the reaction of AMP with *cis*-[Pt(NH$_3$)$_2$Cl$_2$]. The figure was reproduced from Ref. [54], with permission of the copyright holders.

By increasing the temperature the line widths of the ^{31}P signals of the rotamers formed with AMP increased and merged at 85 °C (Fig. 11). For the ADP and ATP complexes coalescence of the signals were not observed even at this temperature.

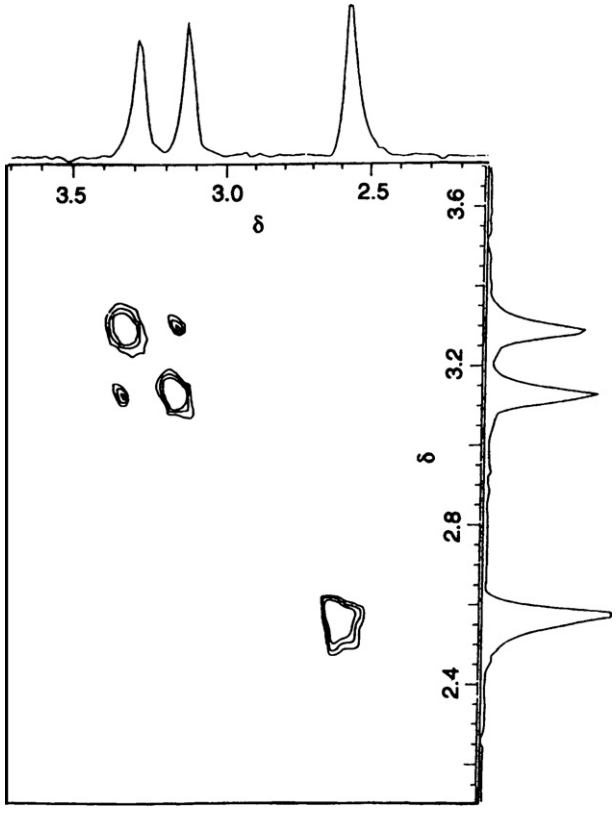

Fig. 10. 121.5 MHz ^{31}P two-dimensional EXSY contours of *cis*-[Pt(NH$_3$)$_2$Cl$_2$] (5.0 mM) and AMP (15.0 mM) mixed in D$_2$O at 25 °C and pD 5.3. The two downfield ^{31}P signals at 3.28 and 3.13 ppm are for the *cis*-[Pt(NH$_3$)$_2$(AMP)$_2$] rotamers and the peak at 2.55 ppm for the unreacted nucleotide. The figure was reproduced from Ref. [58], with permission of the copyright holders.

Only one phosphorous and one H-8 proton signal was observed in the NMR spectra of [Pt(NH$_3$)$_2$(GMP)$_2$] at 25 °C indicating a faster rotation about the Pt–N7 bond on the NMR time scale in this complex. The activation energies for the rotation about the Pt–N7 purine bonds in various nucleotide complexes were evaluated from the quantitative analysis of the ^{31}P EXSY spectra measured at different temperatures and from the line-shape analysis of the exchange broadened ^{31}P signals of the rotamers. The calculations resulted in the following activation energy values for adenosine nucleotides complexes, [Pt(NH$_3$)$_2$(AMP)$_2$]: 70 ± 5 kJ mol^{-1}, [Pt(NH$_3$)$_2$(ADP)$_2$]: 89 ± 5 kJ mol^{-1}, [Pt(NH$_3$)$_2$(ATP)$_2$]: 95 ± 10 kJ mol^{-1} and a much smaller value for the complex with a guanosine nucleotide, [Pt(NH$_3$)$_2$(GMP)$_2$]: 25 ± 5 kJ mol^{-1}. According to the authors, the increased rotational energy barrier in the adenosine nucleotide complexes compared to that of the GMP complex is attributed to a direct interaction of the lone-pair electrons of both 6-NH$_2$ groups (positioned above and below the platinum atom) with the σ-bonding platinum orbitals.

The existence of rotamers was observed by ^{1}H and ^{195}Pt NMR for [Pt(en)(dAMP-N7)$_2$] (en: ethylenediamine) complex too [59]. The line-shape analysis of the temperature dependent ^{1}H signals resulted in high activation energy ~71 kJ mol^{-1} for the rotation between the rotamers similar to the values calculated above.

Recently, the reaction of a platinum–iminoether complex, *trans*-[PtCl$_2${*E*-HN=C(OCH$_3$)CH$_3$}$_2$] (*trans-EE*) with AMP and GMP was studied by ^{1}H and ^{15}N NMR [60]. The formation of four complexes was observed, [Pt(*trans-EE*)$_2$(NMP-N7)Cl], [Pt(*trans-EE*)$_2$(NMP-N7)H$_2$O]$^+$ at lower, and [Pt(*trans-EE*)$_2$(NMP-N7)$_2$] and [Pt(*trans-EE*)(*cis-EE*)(NMP-N7)$_2$] at higher platinum to nucleotide ratios where NMP-N7 indicates N-7 coordination of AMP and GMP. The ^{1}H-detected ^{1}H–^{15}N HMQC spectra measured at natural ^{15}N abundance turned out to be very sensitive probe for detecting the nitrogen binding sites on the nucleobase. The ^{1}H–^{15}N HMQC spectra of

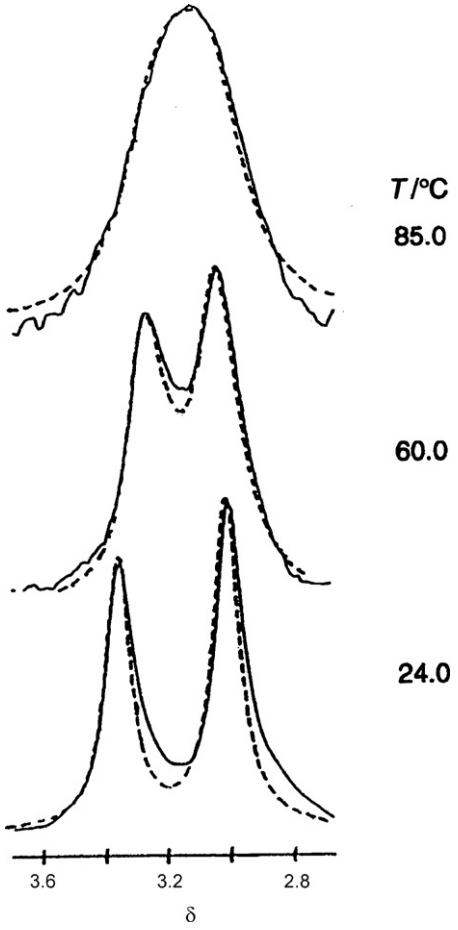

Fig. 11. Experimental (solid lines) and simulated (dashed lines) ^{31}P signals of *cis*-[Pt(NH$_3$)$_2$(AMP)$_2$] rotamers measured at the indicated temperatures. The figure was reproduced from Ref. [58], with permission of the copyright holders.

[Pt(*trans-EE*)$_2$(AMP-N7)Cl] showed a significant upfield shift of N-7 (−247.7 ppm compared to −150.8 ppm measured for N-7 in free AMP) while N-3 and N-9 were only shifted downfield by 10 ppm. The N-1 nitrogen is partly protonated in this complex, hence its signal is shifted to high field by 22.3 ppm. Similar observations proved N-7 coordination of AMP in the other complexes formed.

Non-covalent interactions of planar platinum(II) complexes possessing large heteroaromatic ligands with DNA are also well recognized [61]. The structures of the adducts formed in the intercalation of different platinum(II)-complexes with nucleotides have been extensively studied because of their potential antitumor activity. A number of NMR studies reported that Pt(II) complexes, such as [Pt(phen)(en)]$^{2+}$, [Pt(terpy)(Cl)]$^{+}$, [Pt(bpy)(en)]$^{2+}$ (phen: 1,10 phenantrolin; terpy: 2,2′,2″terpyridine; bpy: 2,2′-bypyridine; en: ethylenediamine) form stable adducts in dilute solutions with nucleotide-5′-monophosphates through aromatic ring stacking [62–64]. The upfield shifts for the purine and ribose protons of AMP in the presence of [Pt(bpy)(en)]$^{2+}$ clearly indicated the stacking interactions [62]. The largest shift change observed for the H-2 signal of the purine ring in AMP indicated that this proton is located above the heteroaromatic rings in the complex. The corresponding experiment with [Pt(en)$_2$]$^{2+}$ did not change the signals of AMP due to the absence of intercalation. The proton spectra also indicated that the [Pt(L)(en)] complexes, where L is a large heteroaromatic base, retain their coordination structure before and after mixing with the nucleotides [63]. The chemical shift changes with the ionic strength of the test solutions indicated that electrostatic interactions, due to the phosphate group and the positively charged platinum center, also contribute to the adduct formation. The effect of aromatic ring stacking has also been investigated by ^{195}Pt NMR [64]. The downfield ^{195}Pt shifts observed in various nucleotides complexes were attributed to the decrease of the electron density in the metal center as a result of electron delocalization over the coordinated and stacked aromatic rings.

2.3.1.2. Palladium(II) complexes. The coordination chemistry of platinum and palladium are very similar. However the ligand exchange reactions are much faster for the palladium(II) complexes. Most of the reactions of various platinum(II) and palladium(II) complexes in biological systems begin with an analysis of their aquation. This is much faster for palladium(II), which makes the NMR study of its reactions more feasible. A number of NMR studies on the coordination behavior of palladium(II) towards nucleotides has been carried out in the presence of dipeptides as the simplest models for more complicated DNA–protein systems [65–69]. Kozlowski et al. have studied the coordination of adenosine nucleotides to different palladium(II)dipeptide complexes, such as Pd(II)-glycyl-L-aspartic acid, Pd(II)-glycyl-L-histidine and Pd(II)-glycyl-L-tyrosine by ^{1}H and ^{13}C NMR spectroscopy [66–68]. The complex formation of Pd(II)-glycyl-L-aspartic acid with adenosine and ATP was studied by ^{1}H NMR [66]. The largest chemical shift changes observed for the H-2 and H-8 protons indicated the coordination of only the purine nitrogens in both ligands. Three modes of coordination were established, two mononuclear complexes through either N-7 or N-1 coordination, where the N-1 coordinated species is more stable, and dimer formation where two palladium-dipeptide moieties are bridged by an AMP molecule *via* N-1 and N-7 bindings. The phosphate chain of ATP did not interact with palladium; the nitrogen atoms are coordinated to the fourth position of these square planar complexes and the other three positions are occupied by the tridentate peptide ligands. At pH values above 11, in contrast to that observed for ATP, the adenosine molecule is not able to coordinate to the palladium-dipeptide moiety and a hydrolyzed complex [Pd(II)(glycyl-L-aspartate)(OH)$_2$] is formed with a bidentate coordination of the dipeptide. The formation of similar complexes was observed in the reactions of Pd(II)-glycyl-L-histidine [67] and Pd(II)-glycyl-L-tyrosinate [68] with ADP and ATP. A few years later a ^{1}H NMR study reported that the N-7 coordinated complexes formed in the reactions of AMP and ATP with three Pd(II)-dipeptides possess higher stability [69].

A palladium(II) complex containing the tripeptide glycylhistidyllysine (GHK), [Pd(gly-L-his-L-lis)Cl], (Pd(GHK)Cl) was prepared, and its reactions with various 5′-deoxyribonucleotides were studied by a variety of one- and two-dimensional techniques [70]. Three products were observed

in the reaction with 5′-d(AMP), two complexes formed with 1:1 stoichiometry through N-1 and N-7 bindings, and a third one [{Pd(gly-L-his-L-lis)}$_2$d(AMP)] in which the nucleotide is bridging two palladium-tripeptide moieties by N-7 and N-1 coordination. In the case of the N-1 coordinated mononuclear complex two H-2 and H-8 proton signals were observed due to the hindered rotation about the Pd–N1 bond. For the other two complexes only one set of signals appeared, indicating either fast rotation or no rotation at all about the Pd–adenosine nitrogen bond.

The reaction product of the chloride bridged cysteinato-*O*-methylester Pd(II) dimer, {Pd(CH_3OCys)Cl}$_2$ and AMP was characterized by NMR and IR spectroscopy [71]. Based on the ^{1}H, ^{13}C and ^{31}P spectra, chelate formation was proposed for AMP through bidentate coordination of the N-7 atom and the phosphate group. Monodentate N-7 coordination was suggested for AMP in the ternary complexes formed in the reactions with Pd complexes possessing tridentate dicarboxylates, like *N*-methyliminodiacetate and pyridyl-2,6-dicarboxylate [72].

The binding sites and the relative binding strength of the complexes formed in the reactions Pd(II)–diethylenetriamine complex and a mixture of various mononucleotides were studied by ^{1}H NMR [73–75]. At pH 7 the following order of binding strength was found, N7(GMP) > N3(TMP) > N3(CMP) > N7(AMP), N1(AMP) [73]. The pH dependence of the chemical shift of the H-8 proton indicated characteristic pH dependence of the ratio for the N-7/N-1 coordination in the AMP complex.

Non-covalent π–π interactions of nucleotides with palladium(II) complexes possessing large heteroaromatic bases have been also studied. In a recent paper the interactions of two homochiral cyclic trinuclear metallacalix[3]arene species, [((*R*,*R*)-1,2-diaminocyclohexane)Pd(phen)]$_3$(NO_3)$_6$, [((*S*,*S*)-1,2-diaminocyclohexane)Pd(phen)]$_3$(NO_3)$_6$ (phen: 4,7-phenanthroline) and mononucleotides (AMP, TMP) were studied by ^{1}H NMR in aqueous solution [76]. It turned out that these trinuclear complexes possess suitable cavities for the inclusion of the mononucleotides. The twice larger association constant for the inclusion of AMP relative to TMP indicates that these trinuclear complexes are potent ligands in supramolecular recognition processes, hence they are potential candidates for DNA targeting drugs.

2.3.1.3. Ruthenium(II) complexes. In the research field of anticancer drugs containing transition metals, some of the Ru(II) complexes are the most promising alternatives to the well-known platinum drugs. Therefore the investigations of both the covalent and the non-covalent interactions of various ruthenium complexes with nucleotides and nucleic acids have attracted considerable interest. An emerging field is to study the interactions of Ru(II) polypyridyl complexes with nucleotides. These complexes are able to bind DNA stereoselectively by intercalation; hence these are excellent probes for nucleic acid sequence and structure. Their interactions have been extensively studied by various experimental techniques, among others by different one- and two-dimensional NMR methods to gain information about the secondary structure of DNA, especially to map the bulge sites of DNA which are potential targets for new drugs [77–79].

Sadler et al. has recently studied the interaction of [(η^6-arene)Ru(en)X] complexes (en: ethylenediammine, arene: tetra-, di-hydroanthracene, biphenyl, *p*-cymene and benzene, X: Cl or H_2O) with various mononucleosides and mononucleotides (AMP, GMP, TMP, IMP and CMP) by ^{1}H, ^{31}P and ^{15}N NMR spectroscopy [80]. The studied Ru(II) complexes showed high selectivity in their recognition of nucleic acid bases. The reactivity of the various binding sites of nucleobases toward the [(η^6-biphenyl)Ru(en)]$^{2+}$ complex at neutral pH decreased in the order: N7(GMP) > N7(IMP) > N1(IMP), N3(TMP) > N3(CMP) > N7(AMP), N1(AMP). In addition, significant amounts of the 5′-phosphate-coordinated species (40–60%) were present at equilibrium for 5′-TMP and 5′-CMP. Only phosphate coordination and negligible nucleobase binding was observed in the reaction with 5′-AMP over the pH range of 3.1–9.6. As shown in Fig. 12a–c, the phosphate binding with 5′-AMP is sensitive to pH, reaching a maximum at pH 7.2. At lower pH the complex formation is inhibited by the protonation of the phosphate group, while at higher pH competitive binding of OH^- reduces the formation of 5′-phosphate-coordinated adducts.

The competitive binding of [(η^6-biphenyl)Ru(en)Cl]$^+$ with GMP versus AMP or TMP or CMP was followed also in this study by ^{1}H and ^{31}P NMR, and the formation of only [(η^6-biphenyl)Ru(en)N7-GMP] was observed. Based on the time dependence of the NMR signal intensities it was concluded that the complex formation proceeds via aquation of [(η^6-biphenyl)Ru(en)Cl]$^+$, followed by a rapid phosphate coordination and then rearrangement to give the final nucleobase-bound products. The high site-selectivity between guanine and adenine bases was explained by the formation of a hydrogen bond between the NH_2 group in ethylenediammine and the exocyclic carbonyl oxygen of the nucleobases in GMP or IMP. This interaction is repulsive toward the exocyclic amino groups of the nucleobases in AMP and CMP.

Different coordination modes have been found in a recent study of the interaction of [(η^6-C_6H_6)Ru(D_2O)$_3$]$^{2+}$ with AMP, ADP and ATP [81]. The pH dependence of the complex formation was followed by ^{1}H and ^{31}P NMR. For AMP no evidence for phosphate coordination was observed, diastereomeric μ-1κN^1:2κ$^2N^6$,N^7 coordinated cyclic trimers of the type [{Ru(5′-AMP)(η^6-C_6H_6)}$_3$] were formed in the pH range of 3.3–9.2. The position of the bridging Ru atoms in the trimer is the same as was found in the corresponding adenosine complex [82] shown in Fig. 13.

In contrast to AMP, the formation of a mononuclear species is dominant with ATP, and the cyclic trimer, analogous to the AMP complex, remains a relatively minor species. The pronounced shift changes of the β- and γ-phosphates and the H-2, H-8 protons indicates macrochelate formation through the coordination of the phosphates and the N-7 nitrogen atom of the purine ring. The time dependence of the NMR spectra measured in the corresponding ADP system showed similar initial macrochelate formation. However, this step was followed by

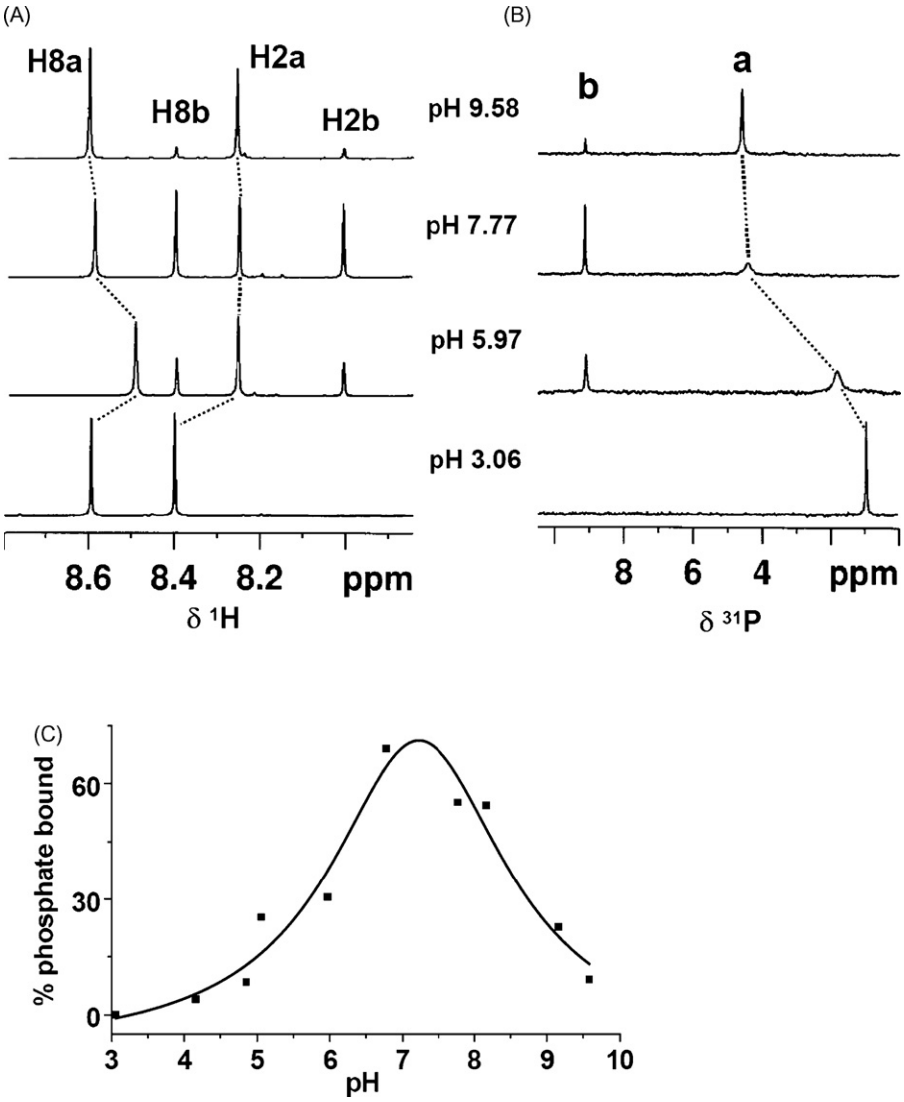

Fig. 12. (A) ^{1}H, and (B) ^{31}P NMR spectra for the reaction of 5′-AMP with [(η^6-biphenyl)Ru(en)Cl]$^{+}$ (1:1, 5 mM) after 24 h at 310 K at various pH values. *Peak assignments*: a, free 5′-AMP; b, Ru-*O*(PO_3)AMP. (C) Plot showing the variation with pH of species "b" as a percentage of the total 5′-AMP present, based on ^{31}P NMR peak intensities. The figure was reproduced from Ref. [80], with permission of the copyright holders.

the cleavage of the β-phosphate group and the formation of the cyclic trimer as for AMP.

Two neutral octahedral ruthenium(II)–dimethyl sulfoxide complexes, *cis*- and *trans*-$RuCl_2(DMSO)_4$ are also considered as potential antitumor drugs, and their interactions with nucleotides have been intensively studied. The structure of the complexes formed with mono- and dinucleotides were studied lately by ^{1}H and ^{31}P NMR in aqueous solutions at physiological pH [83,84]. A comparative study of interaction of *cis*-$RuCl_2(DMSO)_4$ with AMP and GMP showed significant differences between the coordination mode of the two nucleotides [83]. It was found that GMP coordinates through both the phosphate group and the N-7 nitrogen of the nucleobase, and forms two diastereomeric chelate complexes, while AMP coordinates only through the phosphate group.

The structure of the complexes formed in the reaction of *cis*- and *trans*-$RuCl_2(DMSO)_4$ with four dinucleotides possessing purine bases ApG (adenylyl (3′–5′) guanosine monophosphate), GpA (guanylyl (3′–5′) adenosine monophosphate) and their deoxy derivatives d(ApG) and d(GpA) were studied by one and two-dimensional ^{1}H NMR [84]. One major product is formed in an equimolar mixture (1–1.5 mM) of the dinucleotides and the *cis*- or *trans*-Ru(II) complex in an unbuffered aqueous solution at pH 5.6–5.9. The proton spectra for the products formed with *cis*- and *trans*-$RuCl_2(DMSO)_4$ were identical indicating the same binding mode of ruthenium in the complexes. The large downfield shifts of both adenine and guanine H-8 protons of the dinucleotides in the products indicated a bifunctional coordination through the N-7 nitrogen atoms of the purine moieties. Based on the cross peak between the H-8 protons observed in the ROESY spectra, a head-to-head arrangement was suggested for the two bases coordinated to ruthenium.

2.3.1.4. Organotin(IV) complexes. A number of organotin(IV) derivatives, especially dialkyltin(IV) derivatives also exhibit significant antitumour activity. In contrast to platinum(II) com-

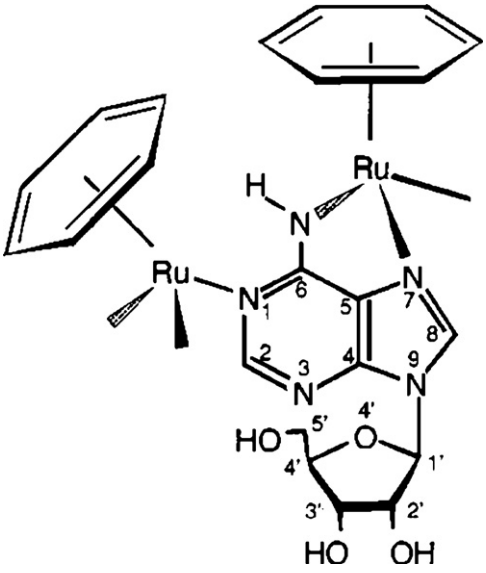

Fig. 13. μ-1κN^1:2κ$^2N^6$,N^7 bridging in the cyclic trimer [{Ru(μ-AdoH$_{-1}$)(η^6-C_6H_6)}$_3$]$^{3+}$. The figure was reproduced from Ref. [81], with permission of the copyright holders.

plexes, little is known about the origin and the mechanism of their activity. In order to gain more information a number of diorganotin(IV) derivatives have been investigated with regards to their interactions with nucleosides, nucleotides and nucleic acids [85–87]. The pH dependence of the coordination of $Me_2Sn(IV)^{2+}$ to AMP, GMP, ATP and different sugar-phosphates in aqueous solutions have been recently studied by various experimental techniques, among others by ^{1}H and ^{31}P NMR spectroscopy [88–90]. Based on the pH dependence of the NMR spectra, it was concluded that Sn(IV) did not interact with the purine nitrogens. In the pH range of 2 and 6.5, the formation of mixed nucleotide–Sn(IV)–hydroxo complexes was observed for AMP and GMP through mono- or bidentate coordination of the phosphate group [88,89]. Around pH 8 the phosphate binding is not strong enough to prevent the hydrolysis of the organotin(IV) cation. Based on the ^{31}P spectra the coordination of the phosphate group of AMP and GMP could be excluded, and the formation of $[Me_2Sn(OH)_2(H_2O)]$ was observed around this pH.

The phosphate chain in ATP provides the possibility for much stronger complex formation in comparison to the monophosphate nucleotides due to the larger negative charge and the possible formation of a six-membered chelate ring [90]. The coordination of $Me_2Sn(IV)^{2+}$ had only a slight effect on the H-2 signal of ATP, but resulted in a more pronounced upfield shift of the H-8 proton signal. This is in contrast to the observed downfield shifts of H-8 protons for those complexes in which the N-7 nitrogen of the purine ring is directly coordinated. However, a macrochelate formation by direct coordination of the metal to the γ- and β-phosphates and trough-water coordination to N-7 cannot be excluded. By increasing the pH, new complexes are formed with all nucleotides studied (AMP, GMP, ATP) *via* deprotonation and the coordination of the two sterically favourable 2′- and 3′-OH groups of the sugar moiety with replacement of one OH^- from the organotin(IV) species, $[Me_2Sn(OH)_2(H_2O)]$.

The reaction products of Bu_2SnCl_2 or Bu_3SnCl (Bu = *n*-C_4H_9) and mononucleotides (AMP and GMP) were characterized by ^{1}H, ^{13}C and ^{31}P NMR by Walmsley and co-workers [91]. The stoichiometry of the complexes formed with Bu_3SnCl was 1:2, while that with Bu_2SnCl_2 was 1:1. The $(Bu_3Sn)_2$AMP and $(Bu_3Sn)_2$GMP complexes are monomeric, and one Bu_3Sn moiety is coordinated to the phosphate group, while the other one is bound to the 3′-oxygens of the ribose unit in the nucleotide. The structure of (Bu_2Sn)AMP and (Bu_2Sn)GMP complexes proved to be polymeric through phosphate bridging, and differ in that in the GMP complex the two butyl groups are in different chemical environments while in the AMP complex they are not.

2.3.2. Complexes with paramagnetic metal ions

The interaction of divalent paramagnetic metal ions, like Cu(II), Mn(II), Ni(II) and Co(II) with nucleotides has been extensively studied. These metal ions show significant effect on the spin–spin (T_2) and spin–lattice (T_1) relaxations of the nuclei in the proximity of their binding sites due to the interactions between the nuclei and the unpaired electrons of the metal ions. Different NMR techniques have been applied to study their coordination in various binary and ternary nucleotide systems. In general, it can be concluded that the phosphate moiety and the N-7 nitrogen of the purine base are the key binding sites in most of the complexes formed with adenine-nucleotides. Two types of macrochelates, inner- and outer-sphere complexes can be formed which differ in the coordination modes of N-7 [92] as mentioned earlier.

2.3.2.1. Copper(II) complexes. The paramagnetic Cu(II) induced line broadening of the ^{13}C signals of 2′- and 5′-AMP indicated N-7 and phosphate bindings irrespective of the position of the phosphate in the ribose ring [93]. The stoichiometry and the structure of Cu(II)–ATP complexes formed in D_2O were studied by the line broadening effect in the ^{1}H NMR spectra [94]. At lower pD (below 5.4), the formation of one 1:1 and one 1:2 Cu(II)–ATP complexes was observed, while at higher pD two hydrolyzed species $CuATP(OD)^{3-}$ and $CuATP(OD)_2^{4-}$ were formed. In the three 1:1 complexes the ATP is coordinated through the phosphate chain and the N-7 nitrogen atom. In the 1:2 complex one ATP is coordinated similarly, while the other ATP is bound only by ring stacking of the adenine moieties. The complex formation of Cu(II) with 2′-, 3′- and 5′-AMP was studied by ^{1}H NMR [95,96]. It was found that by increasing the Cu(II)–nucleotide ratio in the test solutions the H-8 proton signal is broadened preferentially to the H-2 signal in 3′- and 5′-AMP, while these signals were broadened to the same extent in 2′-AMP. The results with 3′- and 5′-AMP were explained by the formation of a binuclear 2:2 complex in which the two purine bases are stacked with each other and the Cu(II) ions bound to a phosphate of one AMP and N-7 nitrogen of the other. With 2′-AMP a 1:1 complex is formed by the coordination of N-3 and the phosphate group of the ligand.

The stability and the structure of ternary Cu(II) complexes formed with adenosine monophosphates and heteroaromatic N-

bases were investigated by potentiometry and ^{1}H NMR [97]. Mononuclear, 1:1:1, Cu(II)(N-base)(NMP) complexes (N-base: 2.2′-bipyridyl or 1,10-phenantroline, and NMP: 2′-, 3′- or 5′-AMP) were observed, in which the Cu(II)-ion formed a bridge between the bidentate coordinated heteroaromatic base and the monodentate coordinated (through only the phosphate group) nucleotides. The result showed that the steric orientation of the metal ion bridge has a large effect on the degree of the intramolecular stacking of the purine and the heteroaromatic bases in the complexes. In two recent ^{13}C and ^{31}P NMR studies, similar monodentate phosphate coordination was reported for 5′-AMP in ternary Cu(II)–polyamine–nucleotide complexes [98,99]. It was found that all nitrogen atoms of the polyamine and the phosphate group of the nucleotide were coordinated to the metal center, while the purine nitrogen atoms of AMP were in the outer coordination sphere.

2.3.2.2. Manganese(II) complexes. ^{31}P NMR relaxation measurements indicated that the phosphate moiety is the primary binding site of adenine-nucleotides and nucleic acids in their complexes formed with Mn(II) and Co(II) in aqueous solutions [100,101]. The Mn(II) induced paramagnetic line broadening of the C-5 and C-8 NMR signals of 2′-, 3′- and 5′-AMP in their Mn(II) complexes indicated direct phosphate and inner- or outer-sphere N-7 coordination of the nucleotides [102]. A few years later, based on ^{15}N, ^{13}C and ^{31}P relaxation measurements, direct phosphate and N-7 coordination was proposed for Mn(II)–AMP complexes [103]. In a ^{1}H NMR study of the Mn(II)–ATP system [104], the formations of mono and bis macrochelated complexes were reported. In the biscomplex, one of the ATP molecules is directly bound by three oxygen atoms, one from each α-, β- and γ-phosphate groups, and the N-7 nitrogen is indirectly coordinated through a water bridge. The second ATP is bound only by the N-7 nitrogen through a water bridge and its purine ring is stacked to the other one in such a way that the two H-8 protons are about equally distant from the central metal ion.

Mn(II)–ADP complexes were studied by ^{17}O NMR, and similar to the ATP system above, the formation of mono and bis complexes was observed [105]. From ^{17}O NMR line broadening and shift measurements it was concluded that four water molecules are coordinated in the mono complex, which is consistent with macrochelate formation of ADP through the coordination of one phosphate oxygen and the N-7 nitrogen. In the bis complex, $Mn(ADP)_2^{4-}$ three or four water molecules are present. This is possible only if the second ADP ligand is bounded by non-covalent interactions through a head-to-tail stacking of the purine bases similar to that observed for the bis Mn(II)–ATP complex.

Ternary complex formation of different paramagnetic metal ions, Mn(II), Co(II) and Ni(II), with catecholamine and ATP was investigated by ^{1}H NMR [106]. Complexes with the same 1:1:1 stoichiometry were formed with each metal, in which the metal ions bind simultaneously to the three phosphates of ATP and may directly or indirectly interact with the N-7 nitrogen of the purine ring. There was no direct interaction observed between the catecholamine molecule and the metal ions; the catecholamine associated with ATP alone by non-covalent interactions.

2.3.2.3. Nickel(II) and cobalt(II) complexes. In the reaction of Ni(II) and AMP in aqueous solutions the formation of a macrochelated complex with 1:2 stoichiometry was observed by ^{13}C and ^{31}P NMR paramagnetic relaxation measurements [107,108]. N-7 nitrogen bindings were found for both coordinated AMP ligands, while the phosphates are outer-sphere coordinated by the nickel ion. Several structural models were proposed in which the relative orientations of the adenine rings with respect to the metal center and their conformations are about the same, and the Ni(II) is out of the plane of the adenine rings.

Early ^{1}H and ^{31}P paramagnetic studies indicated macrochelate formation between Ni(II), Co(II) and ATP through the interactions with the phosphate groups and possibly the N-7 nitrogen of the adenine ring [109,110]. A decade later, wide Ni(II), Co(II) and ATP concentration ranges were studied by ^{1}H NMR. The same structures were proposed for the mono- and bis-complexes formed with both metal ions [111]. In the biscomplex the metal ion binds directly to the three phosphate groups of one ATP and to three water molecules. The adenine moiety of the same ATP is outer-sphere coordinated to one of the water molecules. The second ATP molecule is solely outer-sphere coordinated through a water bridge and by ring stacking of the two-adenine rings. However, two ATP molecules turned out to be magnetically equivalent due to the fast ligand exchange reactions.

The stability and the structure of ternary Ni(II) and Co(II) complexes formed with ADP or ATP and two tetradentate polyamines have been recently studied by potentiometry, ^{13}C and ^{31}P NMR spectroscopy [112]. In Ni(II)–nucleotide–tetramine systems, the nucleotides bind solely through the N-1 or N-7 nitrogens of the purine ring. Surprisingly, the phosphate oxygen atoms are not coordinated; they are in non-covalent interactions with the protonated amine groups of the polyamines which then blocks their direct metal binding. The comparison of ternary Ni(II)-tetramine-AMP, -ADP and -ATP complexes showed that the increasing number of phosphate groups had no effect on the stability of the complexes and the mode of coordination of the nucleotides. In contrast to Ni(II), Co(II) is coordinated to the phosphate oxygens and N-7 nitrogen in the corresponding complexes, whereas the N-1 nitrogen atom interacts with the amino groups of the polyamines. The same stability of the ternary Co(II) complexes formed with ADP and ATP indicated that not all phosphate groups interact with the metal center.

2.4. Complexes with f-block metal ions

2.4.1. Lanthanide complexes

Although, the f-block elements, lanthanides and actinides do not belong to the essential elements in living systems, some of them are known to have multiple target organs or tissues. The research of lanthanides related to living systems is based on their paramagnetic properties and focused on finding contrast agents in magnetic resonance imaging, first of all with gadolinium(III) complexes. Recently, there has been much interest in the development of lanthanide complexes as nucleic acid cleavage agents

[9,10,113,114]. It has been known for decades that lanthanides, *e.g.* cerium(IV) are highly reactive for hydrolysing phosphate ester bonds, including the phosphate bonds in DNA. This reflects the high Lewis acidity associated with tri- or tetravalent lanthanide ions, however neither the structure of these complexes or the mechanism of these reactions are fully understood. On the other hand, trivalent lanthanide ions possess a large variety of spectroscopic properties which provide outstanding possibilities to study their interactions with nucleotides and enzymes. For example Eu(III) complexes can be easily studied by laser-induced luminescence spectroscopy [115]. Paramagnetic ions, like Gd(III) increases the NMR relaxation rate of the surrounding nuclei in the complexes, therefore they have been most widely studied by relaxation measurements [115]. For complexes containing diamagnetic ions, like Pr(III), the ^{31}P NMR chemical shift differences or line broadening effects can be measured to deduce structural information [115,116]. Beside these, lanthanide metal ions have been used as NMR probes to study the conformations of various nucleotides in aqueous solutions [116–118].

Despite the large range of the available experimental techniques, the results concerning the metal binding and the stoichiometry of the lanthanide(III) complexes formed by various nucleotides are often controversial. The structure of ATP complexes with Pr(III), Nd(III), Eu(III) and Yb(III) ions was studied by ^{1}H and ^{31}P chemical shift and ^{1}H relaxation measurements. A metal to ligand ratio of 1:1 was proposed for the complexes formed, in which the lanthanide ions bind predominantly to the β- and γ-phosphate oxygens of the ligand and may interact with the heterocyclic base through a water bridge [116]. A few years later Tsai et al. studied the complex formation of Sc(III), La(III) and Lu(III) with ATP by ^{17}O, ^{31}P and ^{1}H NMR spectroscopy [119]. Based on the concentration dependence of the various spectral parameters, the formation of Ln(III)(ATP)$_2$ complexes was proposed for the three metal ions studied. From the line broadening effects in ^{17}O NMR spectra recorded by ^{17}O enriched nucleotides, predominant α-, β- and γ-phosphate coordination was proposed for Sc(III)(ATP)$_2$ and Lu(III)(ATP)$_2$ complexes. In the case of the La(III)(ATP)$_2$ complex, the effect on γ-^{17}O was larger than that of the α- and β-^{17}O. This was explained by α-, β-, γ-tridentate, but somehow stronger γ coordination, or by the formation of a mixture of complexes by tridentate and γ-monodentate coordination. The large upfield shift of the H-8 protons in the coordinated ligands was explained by the ring current effects due to the heteroaromatic ring stacking of the two ATP molecules in La(III)(ATP)$_2$. The ^{1}H and ^{31}P spectra measured at various Sc(III)/ATP ratios in the same study showed that the ligand exchange is slow on the NMR time scales, and separated signals were observed for the coordinated and free ATP (see Fig. 14).

From the smallest ^{1}H shift difference observed in case of the H-2′ protons in the coordinated and free ATP, an upper limit of the exchange rate, 12 s^{-1} was estimated for the Sc(III) complex. In the Lu(III) and La(III) systems only one set of exchange averaged phosphorus signals was observed indicating faster ligand exchange in the complexes. In another study, the ATP complexes of Eu(III), Gd(III) and Pr(III) were investigated by two independent experimental techniques, by laser induced Eu(III) luminescence spectroscopy and NMR chemical shift and relaxation measurements [115]. Independent from the metal to ligand ratios, the same type of 1:2 complex formation was found with these metals. Both experimental techniques confirmed that approximately two water molecules are coordinated in the complexes. This low number of hydration supported the suggestion that the metal ions are highly coordinated by the phosphate oxygens. The ATP ligand exchange proved to be fast even on the relatively large ^{31}P shift time scale determined by the paramagnetic Pr(III) ion. The largest line broadening of the signals was observed when the complex and the free ligand were present in equimolar amounts, that is when 0.2–0.27 equivalents of Pr(III) was added. This is an independent confirmation of the 1:2 stoichiometry for the Pr(III)(ATP)$_2$ complex.

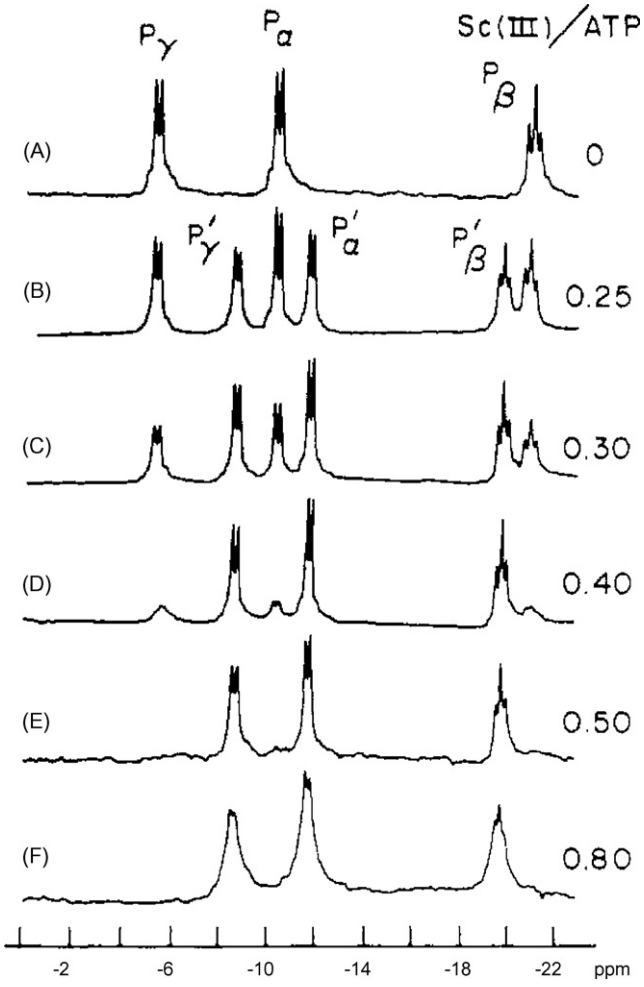

Fig. 14. ^{31}P NMR (81 MHz) spectra of ATP (10 mM, pH 8.0) with varying concentrations of Sc(III). The signals P_α, P_β, and P_γ are due to free ATP, whereas P'_α, P'_β, *and* P'_γ are due to complexed ATP. The figure was reproduced from Ref. [119], with permission of the copyright holders.

2.4.2. Actinide complexes

The chemistry of f-block metals, especially the chemistry of actinides, is in many aspects very different from the d-block elements. Examples are the large number of chemically accessible oxidation states for the pre-curium elements and the formation of

linear dioxo-actinoid (V or VI) ions. These elements have very similar chemical properties in the same oxidation state and vary in a predictable way within their series. Some chemical characteristics such as the stoichiometry and the structure of chemical compounds are very near the same. Within the actinides, it is reasonably easy to obtain quantitative experimental information on thorium and uranium systems. Therefore, one can combine the experimental data collected for just a few actinides to predict the properties of those systems, which are difficult to be accessed by experiments.

The interactions between uranium(VI) and nucleotides and nucleic acids have been intensively studied; two aspects are of particular importance. A number of studies focused on the application of uranium(VI) as catalyst in the synthesis of 2′–5′-linked oligonucleotides with high regio- and stereoselectivity [120–125]. Another intensively studied field is the application of uranyl ion as a photochemical agent for cleavage of nucleic acids [126–131]. The uranyl ion, UO_2^{2+}, has two oxygen ligands, so-called "yl"-oxygens, that are chemically inert under most circumstances, while the exchangeable ligands are all located in a plane perpendicular to the linear UO_2-unit as shown in Fig. 15.

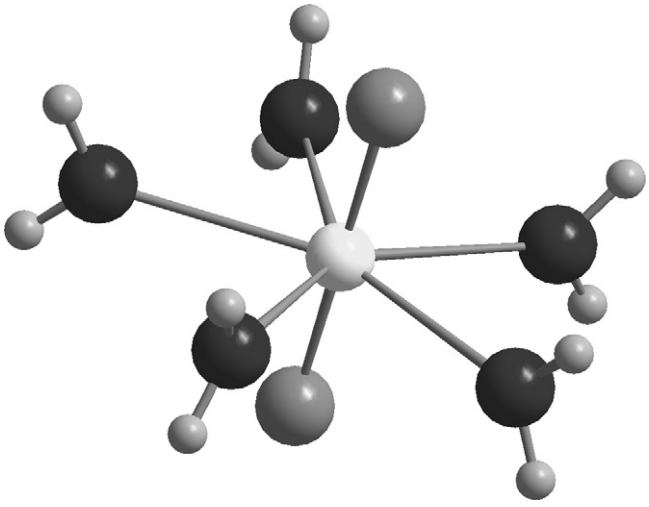

Fig. 15. Pentagonal bipyramid coordination geometry for $UO_2(H_2O)_5^{2+}$.

Hence the steric requirements in ligand substitution reactions and in template and catalytic reactions are strict, one of the pre-requisites for selectivity in these types of reactions. The UO_2^{2+}-unit polarizes the coordinated ligands strongly, and may enhance the nucleophilicity of an OH group of a sugar moiety and thereby organize the ligands through coordination to promote internucleotide bond formation from activated nucleotides. Hence, it can be a very effective catalyst in oligonucleotide synthesis [120].

The "yl"-oxygens in the uranyl ion can be labilized in two ways, either by coordination of a strong Lewis-base, or by photochemical activation. The photo-excited uranyl ion is a strong oxidant for a variety of substrates, among others nucleic acids. The uranyl-mediated photocleavage of nucleic acids is an important method to probe the tertiary structure of various DNA and RNA molecules and to identify metal ion binding sites in these ligands [129–131]. Although the mechanism of these processes has not yet been fully elucidated, the coordination of the phosphate group to the uranyl ion and the coordination geometry of the formed complexes are of key importance in these reactions. A common observation for both processes is that the reactions are practically absent at above pH 8.5, thereby indicating a dramatic change in the structure of the complexes [120,131].

The physiological importance of the uranyl-adenosine triphosphate complex was already shown 50 years ago [132]. According to this, the inhibition of cellular metabolism by uranium is due to its replacement of Mg(II) from the active ATP–Mg(II)–hexokinase complex where the uranyl complex does not phosphorylate glucose. This observation served as the basis for the first studies of the interaction of uranium(VI) with adenine-nucleotides [132–134]. Agarwal and Feldman studied the uranium(VI)–AMP system by ^{1}H NMR and suggested the formation of three complexes in aqueous solutions at pH above 8: one dimer with a uranium to ligand ratio of 2:2 and two other with the ratio 4:2 [133]. One year later they reinvestigated and modified their originally proposed structure of the 2:2 dimer [134] to the one shown in Fig. 16a.

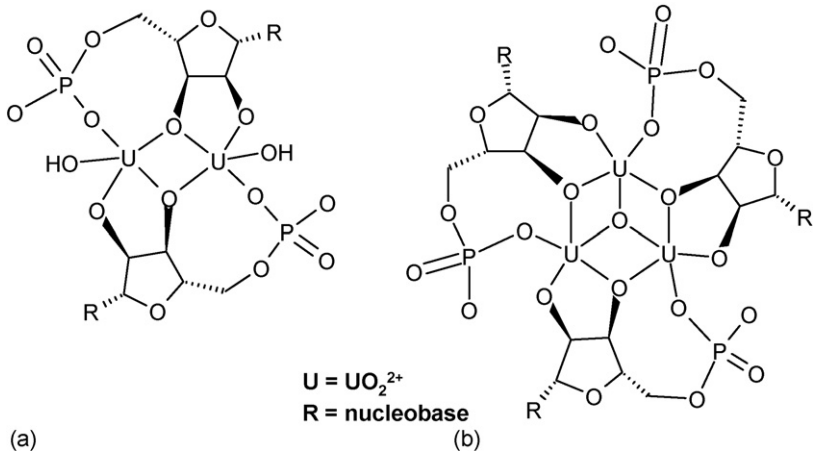

Fig. 16. Structures for one of the complexes formed in the uranium(VI)–nucleotide systems. Structure proposed by Feldman et al. (a), structure identified by Szabo et al. (b). (Charges are neglected for simplicity.) The figure was reproduced from Ref. [138], with permission of the copyright holders.

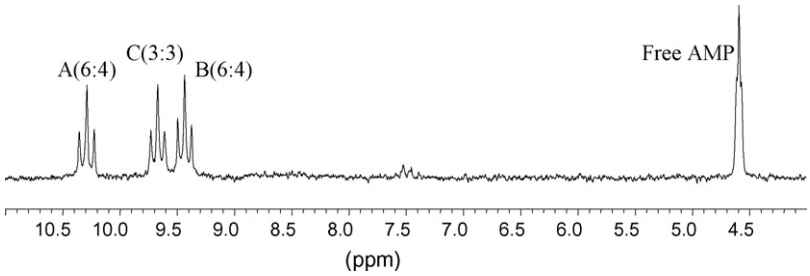

Fig. 17. ^{31}P NMR spectrum measured in the binary uranium(VI)–AMP system at pH 9.2. The triplets indicate the coupling with the neighboring CH_2-protons. A, B are the signals for the 6:4 (metal:ligand), and C is the signal for the 3:3 complexes. The figure was reproduced from Ref. [138], with permission of the copyright holders.

This proposal served thereafter as a model for other metal–nucleotide complexes with *e.g.* molybdenum and was later cited as "*sandwich-type*" or "*Feldman-complex*" [135]. Later, two research groups re-investigated the uranium(VI)–AMP system by ^{1}H and ^{31}P NMR and confirmed the formation of three uranium(VI)–AMP complexes. One group reported [136] the same structures proposed by Feldman, however the other group [137] proposed the formation of two tetranuclear and one octanuclear complex with hydroxo bridges between the uranyl units. In order to find the correct structures for the uranium(VI) complexes, the complex formation of uranium(VI) with four monophosphate nucleotides has been recently re-investigated by Szabó et al. in the pH region of 8.5 and 12. [138]. Based on various multinuclear spectra (^{1}H, ^{13}C, ^{31}P and ^{17}O), two-dimensional correlation experiments, and relaxation and diffusion measurements, it was proved that neither of the structures mentioned above are correct. According to this study, only two complexes are formed in the system. In one of the complex the metal to ligand ratio is 3:3 (in the previous papers called "*Feldman-complex*"), as shown in Fig. 16b. In the other complex the metal to ligand ratio is 6:4. In this complex there are two symmetric phosphorous sites, with two phosphorous atoms in each site. These sites appear as two ^{31}P signals, A and B in the NMR spectra (see Fig. 17). In the earlier studies, these signals were assigned to two different molecules.

However, diffusion measurements confirmed unambiguously that the two signals arising from phosphorous atoms are located in one molecule. In the investigated pH region the two complexes are inter-converting, and at higher pH (10–12) only the 3:3 complex exists.

In general, the variation of the pH and/or the ratio of the concentration of the metal and ligand provide information about the composition of the complexes formed. However, this is not the case in systems where multinuclear, symmetric complexes are formed, *e.g.* the metal to ligand ratio of 1:1 may correspond to a stoichiometry of 2:2; 3:3 and so on. In order to resolve these issues in the uranium(VI) system, the NMR spectra were recorded by a mixtures of two nucleotides instead of using only one. By this method, the symmetry of the complexes was destroyed, and several signals were observed (instead of only one signal in the simplest case), reflecting the formation of the structural isomers. From the number of the signals the number of the isomers, hence the number of the ligands coordinated in one molecule can be deduced. Using an equimolar mixture of AMP and CMP the following ^{1}H decoupled ^{31}P NMR spectrum was recorded (Fig. 18).

The spectrum shows two sets of signals (one from AMP and the other from CMP molecules, each consisting of four lines) for the 3:3 complex. For the other (6:4) complex, four sets of signals, altogether 16 signals, can be observed for one of the sites (A), in which 4 + 4 signals are arising from AMP and 4 + 4 signals are from CMP molecules. For the other site (B) one can also observe four groups of signals. Considering all probabilities of the occupation of the coordination sites in the various structural isomers, it was concluded that one of the complexes is a cyclic trinuclear complex (and not a dimer as proposed by Feldman and Rich [134]), while the other is a symmetric tetranuclear complex

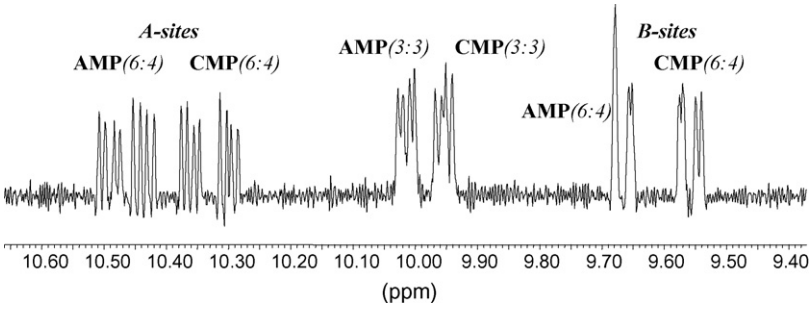

Fig. 18. Proton decoupled ^{31}P NMR spectrum measured in the ternary U(VI) (50 mM)–AMP (25 mM)–CMP (25 mM) system at pH 9.4. The spectrum is resolution enhanced using Lorentz–Gauss weighting functions. The number of peaks indicates the formation of several structural isomers. The figure was reproduced from Ref. [138], with permission of the copyright holders.

with a uranium to ligand ratio of 6:4. The structure of the 3:3 complex has been determined by single crystal diffraction as well, and the results confirm the structure proposed by NMR in aqueous solution.

3. Conclusions

The works discussed in this review highlight that a large variety of NMR techniques can be successfully applied to study the structure and ligand exchange dynamics of the complexes formed in the reactions of metal ions and adenine-nucleotides. As presented through this review adenine-nucleotides are polydentate ligands, with various potential binding sites, including nitrogen and oxygen donors on the bases, hydroxyl groups on the ribose sugar, and negatively charged oxygen atoms in the phosphate group. The metal ion binding properties of the nucleotides present a true challenge to a coordination chemist. A major advantage of NMR spectroscopy is that a large variety of nuclei can be successfully used as probes to study these interactions. Characteristic changes in the spectral parameters (chemical shifts, line widths) measured for the ligands and the metal centers can be used to deduce structural information of the complexes. As demonstrated, NMR spectroscopy is very powerful tool to study the coordination behavior of certain metal ions towards nucleotides in ternary systems too. The interactions with dipeptides as the simplest models for more complicated DNA–protein systems have been studied by different one- and two-dimensional NMR methods. The non-covalent π–π interactions of metal–nucleotide complexes with ligands possessing large heteroaromatic bases have also been extensively studied. Investigations of the time dependence of the NMR signal intensities can contribute to a better understanding of the kinetics of the complex formation in various systems.

The proposals for the structure and the stoichiometry of the complexes are often controversial. However, the conflict between them can be resolved by applying the latest NMR techniques, *e.g.* high-resolution multinuclear spectra (^{1}H, ^{13}C, ^{31}P and ^{17}O), very sensitive multidimensional inverse correlation experiments, relaxation and diffusion measurements. For example, the ^{1}H-detected ^{1}H–^{15}N HMQC spectra measured at natural ^{15}N abundance turned out to be very sensitive probe for detecting the nitrogen binding sites on the nucleobase and may contribute to the success of further research of these systems.

References

[1] W. Kaim, B. Schwederski, Bioinorganic Chemistry: Inorganic Elements in the Chemistry of Life, Wiley, Chichester, 1994.
[2] F. Ciardelli, E. Tsuchida, D. Wöhrle (Eds.), Macromolecule–Metal Complexes, Springer, Berlin, 1996.
[3] M.J. Kendrick, M.T. May, M.J. Plishka, K.D. Robinson, Metals in Biological Systems, Ellis Horwood, Chichester, 1992.
[4] E.J. Baran, J. Inorg. Biochem. 80 (2000) 1.
[5] D.M. Mustafi, J. Telser, M.W. Makinen, J. Am. Chem. Soc. 114 (1992) 6219.
[6] J. Petersen, K. Fisher, C.J. Mitchel, D.J. Lowe, Biochemistry 41 (2002) 13253.
[7] D.P. Smith, E. Kohen, M.F. Maestre, R.H. Fish, Inorg. Chem. 32 (1993) 4119.
[8] W. Wirth, J.B. Baltronat, U. Kleinkes, W.S. Sheldrick, Inorg. Chim. Acta 339 (2002) 14.
[9] A. Boraei, S.A. Ibrahim, A.H. Mohamed, J. Chem. Eng. Data 44 (1999) 907.
[10] C. Liu, M. Wang, T. Zhang, H. Sun, Coord. Chem. Rew. 248 (2004) 147.
[11] H. Sigel, Chem. Soc. Rev. 22 (1993) 255.
[12] E.M. Bianchi, S. Ali, S. Sajadi, B. Song, H. Sigel, Chem. Eur. J. 9 (2003) 881.
[13] H. Sigel, R. Griesser, Chem. Soc. Rev. 34 (2005) 875.
[14] J.L. Bock, J. Inorg. Biochem. 12 (1980) 119.
[15] Y. Liu, E. Sletten, J. Inorg. Biochem. 93 (2003) 190.
[16] G. Crisponi, R. Caminiti, S. Biagini, M. Casu, A. Lai, Polyhedron 3 (1984) 1105.
[17] K.H. Scheller, F. Hofstetter, P.R. Mitchell, B. Prijs, H. Siegel, J. Am. Chem. Soc. 103 (1981) 247.
[18] J.A. Happe, R.L. Ward, J. Am. Chem. Soc. 91 (1969) 4906.
[19] G. Brewer, C.M. Grisham, Inorg. Chim. Acta 106 (1985) 37.
[20] S.H. McClaugherty, C.M. Grisham, Inorg. Chem. 21 (1982) 4133.
[21] M. Hediger, R.M. Milburn, J. Inorg. Biochem. 16 (1982) 165.
[22] I. Muro, I. Morishima, T. Yonezawa, Chem. -Biol. Interactions 3 (1971) 213.
[23] J. Torreilles, A. Crastes de Paulet, Biochimie 55 (1973) 845.
[24] H. Sigel, K.H. Scheller, Eur. J. Biochem. 138 (1984) 291.
[25] M. Brauer, B.D. Sykes, Biochemistry 21 (1982) 5934.
[26] Sh. Nafisi, N. Mohajerani, A. Hadjiakhoondi, M. Monajemi, F. Garib, J. Mol. Struct. 562 (2001) 35.
[27] J.B. Orenberg, B.E. Fischer, H. Sigel, J. Inorg. Nucl. Chem. 42 (1980) 785.
[28] H. Sigel, F. Hofstetter, R.B. Martin, R.M. Milburn, V. Scheller-Krattiger, K.H. Scheller, J. Am. Chem. Soc. 106 (1984) 7935.
[29] J.M. Rifkind, G.L. Eichhorn, J. Am. Chem. Soc. 94 (1972) 6526.
[30] H. Sigel, C.P. Da Costa, R.B. Martin, Coord. Chem. Rev. 219–221 (2001) 435.
[31] I. Stokkeland, P. Stilbs, Biophys. Chem. 24 (1986) 61.
[32] I. Stokkeland, P. Stilbs, Biophys. Chem. 23 (1985) 65.
[33] H.C. Graham, R.J.P. Williams, Eur. J. Biochem. 197 (1991) 81.
[34] J. Granot, D. Fiat, J. Am. Chem. Soc. 99 (1977) 4963.
[35] N.H. Kolodny, L.J. Collins, J. Biol. Chem. 261 (1986) 14571.
[36] D.M. Freitas, L. Amari, C. Srinivasan, Q. Rong, R. Ramasamy, A. Abraha, C.F.G.C. Geraldes, M.K. Boyd, Biochemistry 33 (1994) 4101.
[37] L. Amari, B. Layden, Q. Rong, C.F.G.C. Geraldes, D.M. Freitas, Anal. Biochem. 272 (1999) 1.
[38] J.A. Happe, M. Morales, J. Am. Chem. Soc. 88 (1966) 2078.
[39] L. Jiang, X. Mao, Spectrochim. Acta A 57 (2001) 1711.
[40] S.L. Huang, M.-D. Tsai, Biochemistry 21 (1982) 951.
[41] J.A. Cowan, Inorg. Chem. 30 (1991) 2740.
[42] K.G. Orrell, V. Sik, D. Stephenson, Prog. Nucl. Magn. Reson. Spectrosc. 22 (1990) 141.
[43] J. Sandström, Dynamic NMR Spectroscopy, Academic Press, London, 1982.
[44] L.W. Reeves, K.N. Shaw, Can. J. Chem. 48 (1970) 3641.
[45] K.V. Vasavada, B.D. Ray, B.D. Nageswara Rao, J. Inorg. Biochem. 21 (1984) 323.
[46] T. Glonek, Int. J. Biochem. 24 (1992) 1533.
[47] T. Kiss, P. Zatta, B. Corain, Coord. Chem. Rev. 149 (1996) 329.
[48] P. Rubini, A. Lakatos, D. Champmartin, T. Kiss, Coord. Chem. Rev. 228 (2002) 137.
[49] D.J. Nelson, Coord. Chem. Rev. 149 (1996) 95.
[50] T. Kiss, I. Sóvágó, R.B. Martin, Inorg. Chem. 30 (1991) 2130.
[51] J.P. Laussac, G. Commenges, Nouveau J. Chim. 7 (1983) 579.
[52] J.L. Bock, D.E. Ash, J. Inorg. Biochem. 13 (1980) 105.
[53] G.M. Clore, A.M. Gronenborn, J. Am. Chem. Soc. 104 (1982) 1369.
[54] R.N. Bose, R.D. Cornelius, R.E. Viola, J. Am. Chem. Soc. 108 (1986) 4403.
[55] M. Sarrazin, V. Peyrot, C. Briand, Inorg. Chim. Acta 124 (1986) 87.
[56] S. Mansy, G.Y.H. Chu, R.F. Duncan, R.S. Tobias, J. Am. Chem. Soc. 100 (1978) 607.
[57] P.-C. Kong, T. Theophanides, Bioinorg. Chem. 5 (1975) 51.

[58] D. Li, R.N. Bose, J. Chem. Soc., Dalton Trans. (1994) 3717.
[59] M.D. Reily, L.G. Marzilli, J. Am. Chem. Soc. 108 (1986) 6785.
[60] Y. Liu, M.F. Sivo, G. Natile, E. Sletten, Metal-Based Drugs 7 (2000) 169.
[61] T. Yajima, G. Maccarrone, M. Takani, A. Contino, G. Arena, R. Takamido, M. Hanaki, Y. Funahashi, A. Odani, O. Yamauchi, Chem. Eur. J. 9 (2003) 3341.
[62] O. Yamauchi, A. Odani, R. Shimata, Y. Kosaka, Inorg. Chem. 25 (1986) 3339.
[63] A. Odani, R. Shimata, H. Masuda, O. Yamauchi, Inorg. Chem. 30 (1991) 2133.
[64] A. Odani, T. Sekiguchi, H. Okada, S. Ishiguro, O. Yamauchi, Bull. Chem. Soc. Jpn. 68 (1995) 2093.
[65] S. Kasselouri, A. Garoufis, M. Lamera-Hadjiliadis, N. Hadjiliadis, Coord. Chem. Rev. 104 (1990) 1.
[66] H. Kozlowski, Inorg. Chim. Acta 24 (1977) 215.
[67] H. Kozlowski, E. Matczak-Jon, Inorg. Chim. Acta 32 (1979) 143.
[68] H. Kozlowski, S. Wolowiec, B. Jezowska-Trzebiatowska, Biochim. Biophys. Acta 562 (1979) 1.
[69] P.I. Vestues, R.B. Martin, Inorg. Chim. Acta 55 (1981) 99.
[70] I. Rombeck, B. Lippert, Inorg. Chim. Acta 273 (1998) 31.
[71] G. Pneumatikakis, Inorg. Chim. Acta 80 (1983) 89.
[72] D. Chatterjee, A. Mitra, A. Sengupta, S. Basak, Inorg. Chim. Acta 358 (2005) 2900.
[73] P.I. Vestues, R.B. Martin, J. Am. Chem. Soc. 103 (1981) 806.
[74] K.H. Scheller, V. Scheller-Krattiger, R.B. Martin, J. Am. Chem. Soc. 103 (1981) 6833.
[75] U.K. Häring, R.B. Martin, Inorg. Chim. Acta 80 (1983) 1.
[76] M.A. Galindo, J.A.R. Navarro, M.A. Romero, M. Quirós, Dalton Trans. (2004) 1563.
[77] J.G. Collins, A.D. Sleeman, J.R. Aldrich-Wright, I. Greguric, T.W. Hambley, Inorg. Chem. 37 (1998) 3133.
[78] J.A. Smith, J.G. Collins, B.T. Patterson, F.R. Keene, Dalton Trans. (2004) 1277.
[79] J.L. Morgan, D.P. Buck, A.G. Turley, J.G. Collins, F.R. Keene, J. Biol. Inorg. Chem. 11 (2006) 824.
[80] H. Chen, J.A. Parkinson, R.E. Morris, P.J. Sadler, J. Am. Chem. Soc. 125 (2003) 173.
[81] S. Korn, W.S. Sheldrick, J. Chem. Soc., Dalton Trans. (1997) 2191.
[82] S. Korn, W.S. Sheldrick, Inorg. Chim. Acta 254 (1997) 85.
[83] Y.-N. Tian, P. Yang, Q.-S. Li, M.-L. Guo, M.-G. Zhao, Polyhedron 16 (1997) 1993.
[84] A. Anagnostopoulou, E. Moldrheim, N. Katsaros, E. Sletten, J. Biol. Inorg. Chem. 4 (1999) 199.
[85] L. Ghys, M. Biesemans, M. Gielen, A. Garoufis, N. Hadjiliadis, R. Willem, J.C. Martins, Eur. J. Inorg. Chem. (2000) 513.
[86] Z. Yang, T. Bakas, A. Sanchez-Diaz, C. Charalampopoulos, J. Tsangiris, N. Hadjiliadis, J. Inorg. Biochem. 72 (1998) 133.
[87] P. Yang, M. Guo, Metal-Based Drugs 5 (1988) 41.
[88] H. Jankovics, L. Nagy, N. Buzás, L. Pellerito, R. Barbieri, J. Inorg. Biochem. 92 (2002) 55.
[89] F. Gharib, E. Farzad, M.M. Amini, Can. J. Chem. 84 (2006) 1534.
[90] A. Jancsó, L. Nagy, E. Moldrheim, E. Sletten, J. Chem. Soc., Dalton Trans. (1999) 1587.
[91] A. Atkinson, M.D. Rodriguez, T.E. Shewmaker, J.A. Walmsley, Inorg. Chim. Acta 285 (1999) 60.
[92] H. Siegel, Eur. J. Biochem. 165 (1987) 65.
[93] G. Kotowycz, O. Suzuki, Biochemistry 12 (1973) 5325.
[94] I. Feldman, V. Wee, Biochemistry 13 (1974) 1836.
[95] N.A. Berger, G.L. Eichhorn, Biochemistry 10 (1971) 1847.
[96] N.A. Berger, G.L. Eichhorn, Biochemistry 10 (1971) 1857.
[97] S.S. Massoud, R. Tribolet, H. Sigel, Eur. J. Biochem. 187 (1990) 387.
[98] A. Gasowska, R. Jastrzab, R. Bregier-Jarzebowska, L. Lomozik, Polyhedron 20 (2001) 2305.
[99] L. Lomozik, A. Gasowska, G. Krzysko, J. Inorg. Biochem. 100 (2006) 1781.
[100] R.G. Shulman, H. Sternlicht, B.J. Wyluda, J. Chem. Phys. 43 (1965) 3116.
[101] G.K. Jarori, B.D. Ray, B.D. Nageswara Rao, Biochemistry 24 (1985) 3487.
[102] G. Kotowycz, K. Hayamizu, Biochemistry 12 (1973) 517.
[103] G.C. Levy, J.J. Dechter, J. Am. Chem. Soc. 102 (1980) 6191.
[104] V. Wee, I. Feldman, P. Rose, S. Gross, J. Am. Chem. Soc. 96 (1974) 103.
[105] M.S. Zetter, G.Y.-S. Lo, H.W. Dodgen, J.P. Hunt, J. Am. Chem. Soc. 100 (1978) 4430.
[106] J. Granot, J. Am. Chem. Soc. 100 (1978) 2886.
[107] J. Lang, K. Chmelová, J. Štěpánek, J. Kowalewski, A. Holý, J. Mol. Struct. 480/481 (1999) 363.
[108] J. Štěpánek, J. Kowalewski, J. Lang, P. Mojzeš, J. Biol. Inorg. Chem. 3 (1998) 543.
[109] H. Sternlicht, R.G. Shulman, E.W. Anderson, J. Chem. Phys. 43 (1965) 3123.
[110] H. Sternlicht, R.G. Shulman, E.W. Anderson, J. Chem. Phys. 43 (1965) 3133.
[111] J. Granot, D. Fiat, J. Am. Chem. Soc. 99 (1977) 70.
[112] A. Gasowska, Z. Anorg. Allg. Chem. 632 (2006) 2281.
[113] B. Zhu, Y.-J. Wu, D.-Q. Zhao, J.-Z. Ni, BioMetals 12 (1999) 11.
[114] B. Zhu, X. Li, Y.-J. Wu, D.-Q. Zhao, J.-Z. Ni, Polyhedron 16 (1997) 3415.
[115] C.D. Eads, P. Mulqueen, W.W. Horrocks, J.J. Villafranca, J. Biol. Chem. 259 (1984) 9379.
[116] P. Tanswell, J.M. Thornton, A.V. Korda, R.J.P. Williams, Eur. J. Biochem. 57 (1975) 135.
[117] C.F.G.C. Geraldes, R.J.P. Williams, Eur. J. Biochem. 97 (1979) 93.
[118] C.F.G.C. Geraldes, M.H. Mendonça-Dias, J. Magn. Reson. 45 (1981) 394.
[119] Y.-J. Shyy, T.-C. Tsai, M.-D. Tsai, J. Am. Chem. Soc. 107 (1985) 3478.
[120] H. Sawai, K. Kuroda, T. Hojo, Bull. Chem. Soc. Jpn. 62 (1989) 2018.
[121] H. Sawai, T. Shibusawa, K. Kuroda, Bull. Chem. Soc. Jpn. 63 (1990) 1776.
[122] M. Shimazu, K. Shinozuka, H. Sawai, Angew. Chem. Int. Ed. Engl. 32 (1993) 870.
[123] H. Sawai, H. Katsutaka, K. Kuroda, J. Chem. Soc., Perkin Trans. (1992) 505.
[124] H. Sawai, T. Ito, K. Kokaji, M. Shimazu, K. Shinozuka, H. Taira, Bioorg. Med. Chem. Lett. 6 (1996) 1785.
[125] H. Sawai, A. Hirano, H. Mori, K. Shinozuka, B. Dong, R.H. Silverman, J. Med. Chem. 46 (2003) 4926.
[126] P.E. Nielsen, C. Jeppesen, O. Buchardt, FEBS Lett. 235 (1988) 122.
[127] A.R. Hill Jr., L.E. Orgel, Bioconjugate Chem. 2 (1991) 431.
[128] P.E. Nielsen, C. Hiort, S.H. Sönnichsen, O. Buchardt, O. Dahl, B. Nordèn, J. Am. Chem. Soc. 114 (1992) 4967.
[129] P.E. Nielsen, N.E. Møllegaard, J. Mol. Recognit. 9 (1996) 228.
[130] S.H. Sönnichsen, P.E. Nielsen, J. Mol. Recognit. 9 (1996) 219.
[131] D. Wittberger, C. Berens, C. Hammann, E. Westhof, R. Schroeder, J. Mol. Biol. 300 (2000) 339.
[132] I. Feldman, J. Jones, R. Cross, J. Am. Chem. Soc. 89 (1967) 49.
[133] R.P. Agarwal, I. Feldman, J. Am. Chem. Soc. 90 (1968) 6635.
[134] I. Feldman, K.E. Rich, J. Am. Chem. Soc. 91 (1969) 4559.
[135] C.F.G.C. Geraldes, M.M.C.A. Castro, J. Inorg. Biochem. 28 (1986) 319.
[136] M.M.C.A. Castro, C.F.G.C. Geraldes, Inorg. Chim. Acta 140 (1987) 377.
[137] M. Kainosho, M. Takahashi, Nucl. Acid Res. Symp. Ser. 12 (1983) 181.
[138] Z. Szabó, I. Furó, I. Csöregh, J. Am. Chem. Soc. 127 (2005) 15236.