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A Carbon-13 Nuclear Magnetic Resonance Study of Binding of Copper(II) to Purine Nucleotides†

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ABSTRACT: The influence of paramagnetic Cu^{2+} ions on the proton-decoupled ^{13}C nuclear magnetic resonance spectra of purine nucleotides has been studied. For 5′-AMP and 2′-AMP, the C-4 and C-5 resonances broaden first, followed by the broadening of the C-8 resonance upon the addition of Cu^{2+} ions. The C-2, C-6, and ribose resonances are unaffected over the metal ion concentration range studied. These results indicate that the metal ion is held near the N-7 position of the base, irrespective of the position of the phosphate group on the ribose ring. Possible models for a complex between 5′-AMP or 2′-AMP with Cu^{2+} are discussed. With 5′-GMP and 5′-IMP the C-4, C-5, and C-8 resonances are nearly equally affected by the Cu^{2+} ions, indicating that the metal ion is also held near the N-7 position of the base.

The interactions between nucleosides, nucleotides, and polynucleotides with Cu^{2+} ions have been extensively studied and more experimental work has been reported for the interaction of these ligands with Cu^{2+} than with any other single metal ion (Izatt *et al.*, 1971). Cu^{2+} ions have an influence on the melting temperature of DNA (Eichhorn, 1962) and the effect of Cu^{2+} ions on DNA denaturation has now been extensively studied (Eichhorn and Clark, 1965; Hiai, 1965; Coates *et al.*, 1965; Venner and Zimmer, 1966; Eichhorn and Shin, 1968; Liebe and Stuehr, 1972a, b; Holman and Jordan, 1972; Richard *et al.*, 1973) and occurs because the Cu^{2+} ions sever the bonds between the double helix. Recent work on Cu^{2+}–DNA denaturation (Liebe and Stuehr, 1972a) implies that Cu^{2+} ions bind to both phosphate and base moieties of DNA simultaneously. Cu^{2+} ions have significant and different effects on polyribonucleotides which are degraded into small oligonucleotides when heated with copper ions by the cleavage of phosphate bonds (Butzow and Eichhorn, 1965).

^{31}P Nuclear magnetic resonance (nmr) studies confirm the binding of Cu^{2+} ions to the phosphate portion of the nucleotides (Cohn and Hughes, 1962; Eichhorn *et al.*, 1966; Kan and Li, 1972; Missen *et al.*, 1972). Metal interactions with the base portion of the nucleotides have also been extensively studied. Proton magnetic resonance studies indicate that the H-8 resonance is much more strongly affected than the H-2 resonance for D_2O solutions of ATP (Cohn and Hughes, 1962; Schneider *et al.*, 1964), 5′-dAMP (Eichhorn *et al.*, 1966), 5′-AMP (Berger and Eichhorn, 1971a; Missen *et al.*, 1972), and 3′-AMP (Berger and Eichhorn, 1971a) indicating that the metal ion interacts with the adenine base at the N-7 position.

In 1958, Frieden and Alles (1958) proposed that Cu^{2+} ions form five-membered ring complexes with guanosine derivatives by coordinating with N-7 and the carbonyl oxygen O-6. The H-8 proton resonance is broadened in 5′-dGMP by Cu^{2+} ions indicating that the metal ions are bound at N-7 (Eichhorn *et al.*, 1966). Tu and Friederich (1968) by means of conductometric, potentiometric, and spectrophotometric titrations found that Cu^{2+} ions combined with guanosine, 5′-GMP, inosine, and 5′-IMP on a mole to mole basis. Infrared spectra indicate that the oxygen atom at C-6 was also involved so the pentacyclic complex involving the C-6 oxygen and the N-7 nitrogen was favored. Sletten (1971), however, has shown by X-ray analysis of the 9-methylhypoxanthinecopper crystal that the N-7 nitrogen is the only binding site and the chelate structure involving N-7 and O-6 is not observed.

Cu^{2+} ions broadened the H-8 and H-2 resonances of 5′-IMP equally and simultaneously (Berger and Eichhorn, 1971b) indicating either chelation to N-7 and the O-6 positions or binding of Cu^{2+} ions to nitrogen atoms on both rings. In the polymer poly(I), the H-8 proton resonance is preferentially broadened indicating that the metal binds to N-7 only (Berger and Eichhorn, 1971b). These studies were extended (Berger and Eichhorn, 1971c) to Cu^{2+} ion interactions with

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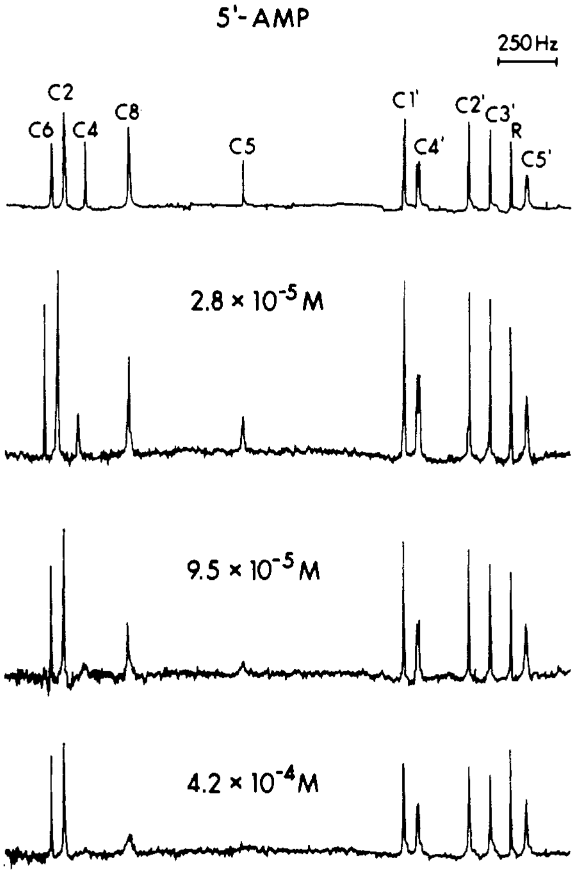

FIGURE 1: The effect of Cu^{2+} ions on the natural abundance, Fourier transform, proton-decoupled ^{13}C nmr spectra of 5'-AMP in D_2O (pD 7.4) at 25°. The top spectrum is for the metal-free solution and the Cu^{2+} ion concentration is indicated for the remaining spectra. R is the reference dioxane resonance.

inosine in D_2O and the results can best be accounted for by multiple binding sites on the inosine base. Some coordination occurs at the N-1, O-6 area of the inosine base but N-7 is the predominant coordination site.

Berger and Eichhorn (1971a) have interpreted their proton magnetic resonance (pmr) data of Cu^{2+} interactions with 5'-AMP and 3'-AMP in terms of an M_2L_2 species (L = ligand) with each Cu^{2+} ion bound to a phosphate of one ligand and the N-7 position of the neighboring molecule. With 2'-AMP the results were interpreted by an intramolecular chelate (ML) involving the phosphate group and the N-3 nitrogen of the same molecule. The ligand: metal ion ratio is about 500:1 in these studies. However, Frey and Stuehr (1972) have measured stability constants for complexes of 5'-AMP and 2'-AMP with Ni^{2+} ions and found that, in addition to ML species, there was a substantial amount of bis complexation present (ML_2 species). By analogy, Cu^{2+} ions which also form strong complexes with nitrogen-containing compounds might be expected to form bis complexes.

In view of the above results, we wanted to study the nature of the Cu^{2+} ion–nucleotide interaction in aqueous solutions. The Fourier transform, natural abundance, ^{13}C nmr approach is used for these studies. We thus have a large number of nuclear probes which can be used to yield information about the metal binding site.

Experimental Section

The nucleotides were obtained from Sigma Chemical Co., and were used without further purification. The solutions for the ^{13}C nmr experiments were prepared in D_2O (obtained from Columbia Organic Chemicals) and the pH was adjusted to 7.0 (pD 7.4) (Glasoe and Long, 1960). The nucleotide concentrations were between 0.50 and 0.55 M. Stock solutions of $CuCl_2$ were prepared and were added to the above solutions using H. E. Pedersen micropipets.

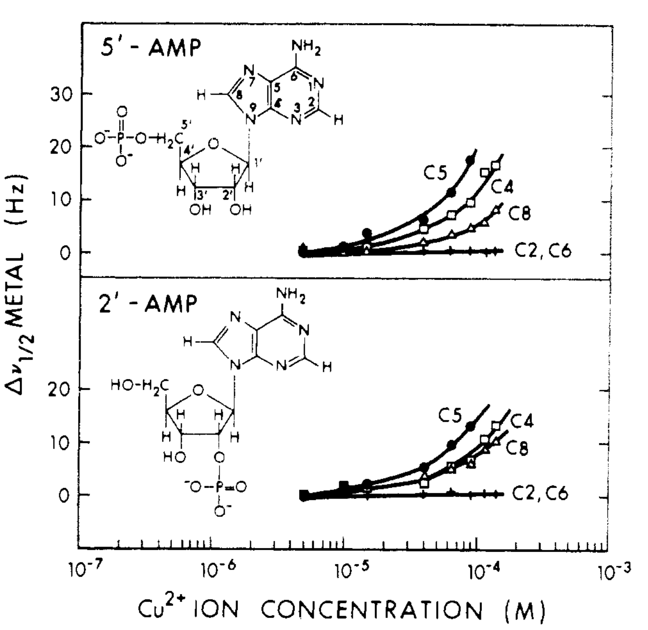

FIGURE 2: The dependence of $\Delta\nu_{1/2}$ metal on the Cu^{2+} ion concentration for the base carbon nuclei of 5'-AMP and 2'-AMP.

The nmr spectra were obtained using a Bruker HFX-90 nmr spectrometer operating in the Fourier transform mode and equipped with a Nicolet 1085 computer. The ^{13}C nmr spectra were obtained at a frequency of 22.63 MHz with proton noise decoupling and the deuterium resonance from the solvent D_2O was used for the heteronuclear lock signal. For 5'-AMP and 2'-AMP the free induction decay signals were accumulated in 16K data points of the computer and 1024 accumulations were carried out. The frequency range of the Fourier-transformed spectra was 3000 Hz (0.37 Hz/point). For 5'-GMP and 5'-IMP, the free induction decay signals were accumulated in 8K data points and 2048 accumulations were carried out. The frequency range of the Fourier-transformed spectra was 2500 Hz (0.61 Hz/point). The ^{13}C chemical shifts are calibrated with respect to the internal dioxane resonance. The ^{13}C spectra were recorded on an expanded scale from which the line widths at half-height are determined. For resonances with a line width at half-height less than 10 Hz, the line-width measurements are accurate to ±1 Hz. However, for resonances with a line width greater than 10 Hz, the line width measurements are accurate to ±3 Hz.

The temperature for all measurements except for 5'-IMP was maintained at 25°. For solubility purposes, 5'-IMP was measured at 35°.

Results

The proton-decoupled ^{13}C nmr spectrum of 5'-AMP is shown in Figure 1 together with the effect of progressive addition of Cu^{2+} ions. The results of the metal ion line broadening experiments on the adenine nucleotides are seen in Figure 2, and the results with 5'-GMP and 5'-IMP are shown in Figure 3.

The ^{13}C nmr line assignments are based on those reported in the literature for nucleosides (Jones *et al.*, 1970a–c), 5′-nucleotides (Dorman and Roberts, 1970), uridine monophosphates, and polyuridylic acid (Mantsch and Smith, 1972). The C-4 resonance was assigned to low field of the C-2 resonance of 5′-IMP (Kotowycz and Suzuki, 1973). The ^{13}C chemical shifts have been reported previously together with the observed $^{31}P-^{13}C$ coupling constants (Kotowycz and Hayamizu, 1973; Kotowycz and Suzuki, 1973).

Discussion

The Cu^{2+} ions bind to the phosphate groups of the nucleotides. In addition, the interaction with the rest of the molecule must be considered. The approach that is used in this study has been described previously (Kotowycz and Hayamizu, 1973; Kotowycz and Suzuki, 1973) and involves the measurement of the broadening of specific lines in the nmr spectrum of the molecules upon the addition of the paramagnetic ions (Li *et al.*, 1962). The Cu^{2+} ion concentration is much less than that of the nucleotide and the rapid exchange of molecules in solution causes all nucleotides to be equally affected by the Cu^{2+} ions.

The influence of paramagnetic ions on nuclear relaxation times has been extensively studied (Solomon, 1955; Bloembergen and Morgan, 1961; Bernheim *et al.*, 1959). Two mechanisms, namely the dipole–dipole and the hyperfine interaction, are important for a transverse relaxation time process. For the dipole–dipole interaction, relaxation times are extremely sensitive to the distance between the metal ion and the nucleus under study. The scalar interaction is sensitive only to the nature of the intervening bonds. The line widths and therefore the T_{2p} of each ^{13}C resonance were studied upon the progressive addition of the Cu^{2+} ions. The line width at half-height arising from the presence of the metal ion, $\Delta\nu_{1/2}$ metal, may be expressed as

$$\Delta\nu_{1/2}\text{ metal} = \Delta\nu_{1/2,\text{obsd}} - \Delta\nu_{1/2,0} = 1/\pi T_{2p}$$

where $\Delta\nu_{1/2,\text{obsd}}$ is the observed line width at half-height for the metal ion solution and $\Delta\nu_{1/2,0}$ is the line width at half-height for the same resonance without metal ions. T_{2p} is the effective transverse relaxation time due to the presence of the metal ion.

A temperature study of the line widths of the ^{13}C resonances of 5′-AMP in the presence of Cu^{2+} ions was carried out. The temperature dependence of the relaxation processes in the presence of the metal ion is determined by the temperature dependence of the average lifetime of a molecule coordinated at any site (τ_M) and the relaxation time of the nuclei when the nucleotide is coordinated to the metal ion ($1/T_{2M}$). The $1/T_{2M}$ term has, indeed, dipolar and hyperfine contributions (Swift and Connick, 1962). The temperature behavior for the C-4, C-5, and C-8 carbon nuclei line widths is similar and the relaxation processes are dominated by the $1/T_{2M}$ term.

The effect of Cu^{2+} ions on the ^{13}C nmr spectrum of 5′-AMP is seen in Figure 1. The C-4 and C-5 resonances broaden first followed by the broadening of the C-8 resonance at slightly higher Cu^{2+} ion concentrations. The C-2, C-6, dioxane, and ribose resonances are only very slightly affected over the metal ion concentration range studied. From Figure 2 it is seen that similar line-broadening behavior is observed for both 5′-AMP and 2′-AMP. Berger and Eichhorn (1971a) have shown with their pmr studies on tubercidin that the broadening effect of the paramagnetic Cu^{2+} ion bound to the six-membered ring is not transmitted to the five-membered ring. The very slight broadening of the C-2 and C-6 resonances and the strong broadening of the C-4, C-5, and C-8 resonances indicate that Cu^{2+} ions preferentially bind to the five-membered ring of the base, near the N-7 nitrogen for both nucleotides.

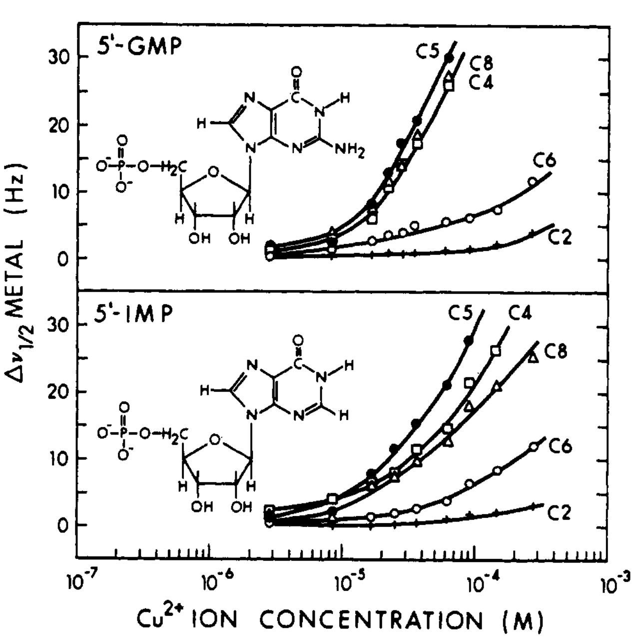

FIGURE 3: The dependence of $\Delta\nu_{1/2}$ metal on the Cu^{2+} ion concentration for the base carbon nuclei of 5′-GMP and 5′-IMP.

The metal ion is located near the N-7 position for both 5′-AMP and 2′-AMP, irrespective of the position of the phosphate group. A 1 : 1 intramolecular complex involving both the phosphate group and the N-7 position of the base is possible between 5′-AMP and Cu^{2+} on the basis of molecular models. However, 2′-AMP must undergo drastic conformational changes for an intramolecular 1 : 1 complex to be formed with the Cu^{2+} ion. Under the present experimental conditions, the nucleotide : Cu^{2+} ion ratio is about 5000 : 1. Under these conditions, the nucleotides stack in aqueous solution (Ts'o *et al.*, 1969; Ts'o, 1970). Hence a possible model for a complex between 2′-AMP with Cu^{2+} ions is very likely the bis complex (ML_2) which involves the binding of the metal ion to the phosphate group of one nucleotide and simultaneouly to the adenine ring N-7 of a second nucleotide. 5′-AMP probably exists in solution in the ML as well as the ML_2 form described above. Unfortunately, stability constants are not known for these complexes. Therefore it is not possible to know the solution compositions as have been tabulated for Mn^{2+}–ATP complexes (Zetter *et al.*, 1973).

The effect of Cu^{2+} ions on 5′-GMP and 5′-IMP is seen in Figure 3. Even though 5′-GMP very likely undergoes some gel formation (Chantot *et al.*, 1971) under our conditions, sharp resonances were obtained. The C-4, C-5, and C-8 resonances are equally affected. The C-6 resonance is broadened slightly at much higher Cu^{2+} ion concentrations. Again the C-2 base carbon resonance and the ribose carbon resonances remain unaffected. These results indicate that the location of the Cu^{2+} ion is near the N-7 nitrogen.

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