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A Carbon-13 Nuclear Magnetic Resonance Study of Nucleotide–Metal Interactions. Binding of Manganese(II) with Adenine Nucleotides†

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ABSTRACT: The influence of paramagnetic Mn^{2+} ions on the proton decoupled ^{13}C nuclear magnetic resonance spectra of adenosine monophosphates has been observed. The C-5 and C-8 resonances are broadened preferentially to the C-2, C-4 and C-6 resonances by Mn^{2+} ions in 5′-AMP, 3′-AMP, and 2′-AMP. The five ribose carbon resonances for the three nucleotides are little affected. The metal ion is therefore held near the N-7 position of the base. The present data indicate that specific line-broadening effects are observed in ^{13}C spectra which can be used in determining the nature of the paramagnetic ion binding site. The line-broadening behavior is similar for the three adenosine mononucleotides. A possible model for the Mn^{2+} complex with the nucleotides is discussed.

Metal ions play an important role in protein chemistry as well as in nucleic acid processes. Numerous investigations have been carried out in studies of the nature of the metal complexes. The recent advances in metal interactions with nucleosides, nucleotides, and related compounds have been summarized (Izatt *et al.*, 1971; Phillips, 1966; Weser, 1968). Nuclear magnetic resonance is one of the powerful techniques that is used in the determination of metal binding sites and the nature of the complex in these systems.

^{31}P nuclear magnetic resonance (nmr) showed that Mn^{2+} and Co^{2+} ions bind to the phosphate group of 5′-AMP (Shulman *et al.*, 1965). The Mn^{2+} ion has been shown using ^{1}H line-broadening experiments of the H-2 and H-8 resonances to affect the H-8 resonance of 5′-AMP most strongly (Chan and Nelson, 1969). Water proton T_2 relaxation time studies showed that 5′-AMP and 2′-AMP have one binding site for the Mn^{2+} ion (Heller *et al.*, 1970).

In this paper we wish to illustrate the potential and the applicability of the ^{13}C nmr approach in the study of adenosine monophosphates (2′-AMP, 3′-AMP, 5′-AMP) in D_2O solutions containing small quantities of Mn^{2+} ions. The ^{13}C nmr spectrum of AMP consists of ten lines, five arising from the base and five from the ribose moiety of the molecules. Therefore with ten nuclear probes, we can expect to obtain detailed information about the nature of the metal binding site. We use an approach that has been extensively applied and involves the measurement of the broadening of specific lines in a spectrum upon the addition of paramagnetic ions. The influence of paramagnetic ions on nuclear relaxation times and hence on line broadening has been analyzed by Solomon (1955), Bloembergen and Morgan (1961), Bernheim *et al.* (1959), and reviewed by Eaton and Phillips (1965). The binding of the paramagnetic ion leads to broadening of the ^{13}C resonances. When the electron-spin, nuclear-spin, dipole–dipole interaction is the predominant relaxation mechanism, then $T_1 = T_2$ and the relaxation times are extremely

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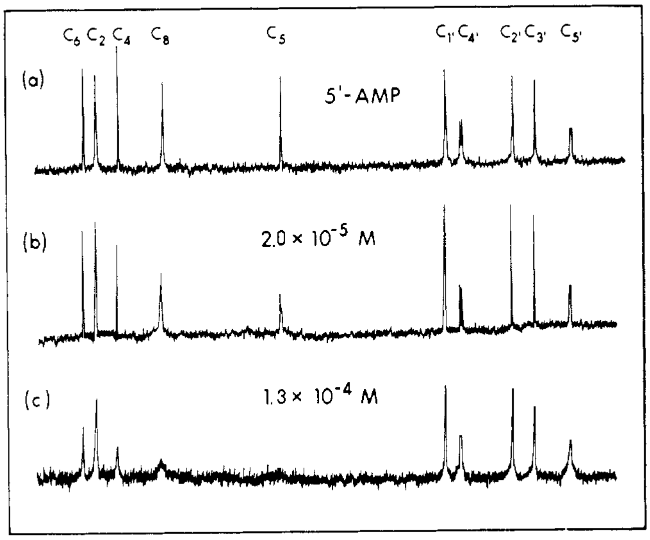

FIGURE 1: The effect of Mn^{2+} ions on the ^{13}C proton-decoupled nmr spectra of 5′-AMP in D$_2$O (pD 7.4) at 27°. Spectrum a is for the metal-free solution and the Mn^{2+} ion concentration is indicated for spectra b and c.

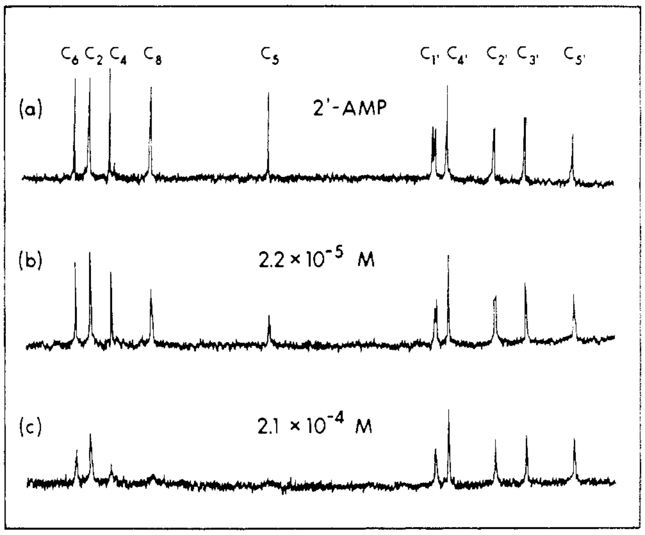

FIGURE 3: The effect of Mn^{2+} ions on the ^{13}C proton-decoupled nmr spectra of 2′-AMP in D$_2$O (pD 7.4) at 27°. Spectrum a is for the metal-free solution and the Mn^{2+} ion concentration is indicated for spectra b and c.

sensitive to the distance ($1/r^6$) between the metal ion and the nucleus under study (Carrington and McLachlan, 1967). Hence the line width and therefore the T_2 of each ^{13}C resonance of 2′-, 3′-, and 5′-AMP was studied with the progressive addition of the paramagnetic Mn^{2+} ions.

Experimental Section

All three nucleotides, 5′-, 3′-, and 2′-AMP were purified using a cation-exchange resin. The acid form of the exchange resin ANGC-244 from J. T. Baker Chemical Co. was neutralized using a 5% aqueous pyridine solution. The nucleotide was passed through the column and the effluent was then freeze-dried. The pyridinium salt was titrated using a dilute NaOH solution and freeze-dried again. Following this purification procedure, reproducible line widths and relative intensities of the ^{13}C resonances were obtained for the nucleotides from different sources. Solutions with the nucleotide concentration of about 0.6–0.7 M were then prepared in D$_2$O for the nmr experiments. Stock solutions of MnSO$_4$ were prepared at various concentrations and were added to the AMP solutions using H. E. Pedersen micropipets. The pH of the solution was checked along with the progressive addition of the paramagnetic ions and remained constant at pH 7.0 (pD = 7.4) (Glasoe and Long, 1960).

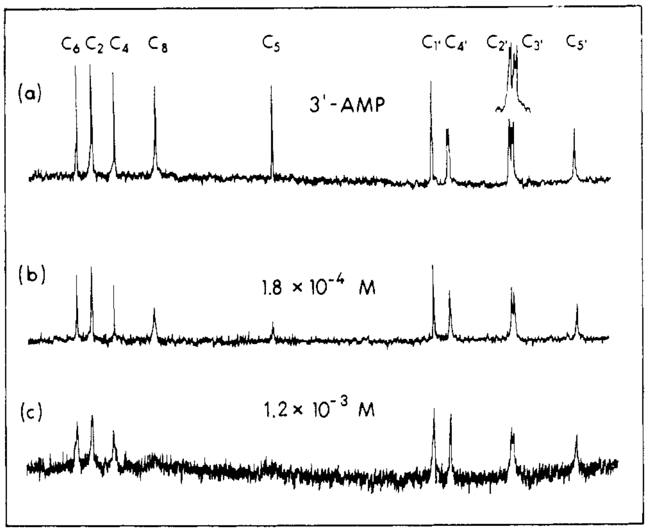

FIGURE 2: The effect of Mn^{2+} ions on the ^{13}C proton-decoupled nmr spectra of 3′-AMP in D$_2$O (pD 7.4) at 27°. Spectrum a is for the metal-free solution and the Mn^{2+} ion concentration is indicated for spectra b and c.

The ^{13}C nmr spectra were obtained using a Bruker HFX-90 spectrometer operating at a frequency of 22.63 MHz and equipped with a Nicolet 1085 computer. The spectra were obtained in the Fourier transform mode with proton noise decoupling. The deuterium resonance from the solvent D$_2$O was used for the heteronuclear lock signal. The free induction decay signals were accumulated in 16K data points of the computer and 1024 accumulations were carried out to enhance the signal to noise ratio. The frequency range of the Fourier transformed spectra was 2500 Hz. The temperature was maintained near 27° by blowing nitrogen gas over the sample tube. Great care was taken to choose the proper pulse conditions to obtain optimum spectra. The same experimental conditions were maintained throughout all the observations of the spectra including the pulse width, the pulse height, the repetition time, and the temperature.

Results

The ^{13}C nmr spectrum of 5′-AMP is shown in Figure 1a and the spectra of 3′-AMP and 2′-AMP are shown in Figures

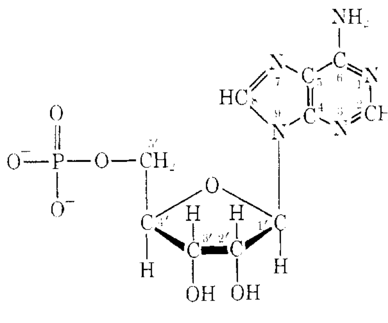

TABLE I: ^{13}C Chemical Shifts of Aqueous Solutions of Adenosine Monophosphates.[a]

Carbon	5′-AMP	3′-AMP	2′-AMP
C_6	−88.4	−88.4	−88.5
C_2	−85.8	−85.6	−85.5
C_4	−82.2	−81.5	−81.8
C_8	−73.6	−73.8	−74.2
C_5	−51.8	−53.3	−52.2
$C_{1'}$	−20.6	−21.9	−21.0
$C_{4'}$	−17.9	−18.7	−18.8
$C_{2'}$	−8.0	−7.3	−9.6
$C_{3'}$	−4.0	−6.8	−4.0
$C_{5'}$	2.6	4.9	5.0

[a] The chemical shifts are in ppm with respect to internal dioxane. A negative value indicates that the resonance is to low field of the reference.

TABLE II: ^{31}P–^{13}C Coupling Constants of Aqueous Solutions of Adenosine Monophosphates.[a]

Coupling Constant	5′-AMP	3′-AMP	2′-AMP
$J_{^{31}P-C1'}$			8.8
$J_{^{31}P-C2'}$		2.8	4.3
$J_{^{31}P-C3'}$		4.7	
$J_{^{31}P-C4'}$	7.9	5.0	
$J_{^{31}P-C5'}$	3.9		

[a] The coupling constants are in Hz.

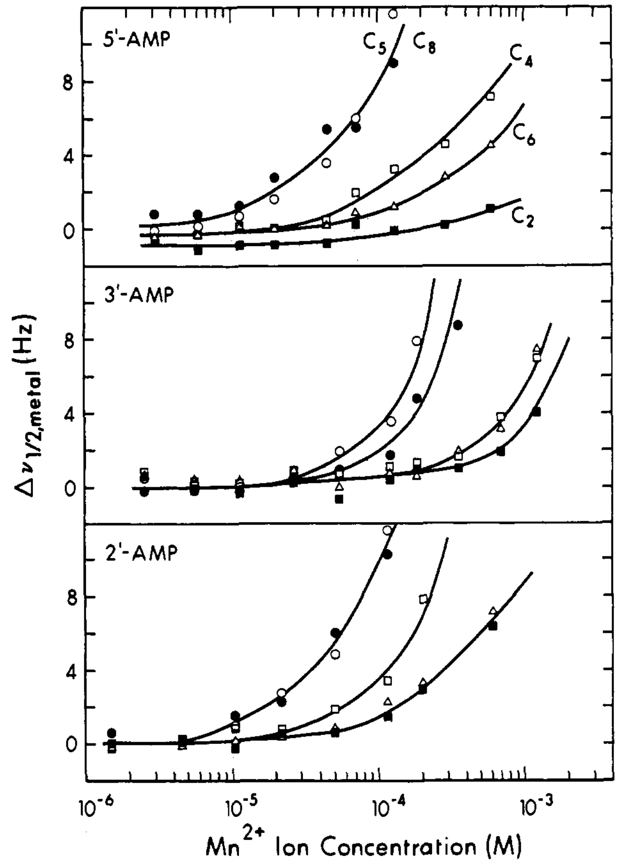

FIGURE 4: The dependence of $\Delta\nu_{1/2}$ (metal) for the five base carbon nuclei on the Mn^{2+} ion concentration. The different carbon nuclei are represented as follows: (■) C_2, (□) C_4, (○) C_5, (△) C_6, (●) C_8.

2a and 3a, respectively. Table I summarizes the ^{13}C chemical shifts for all three nucleotides in aqueous solution. The chemical shifts of the resonances remained unchanged in all the solutions containing the paramagnetic ion. The observed ^{31}P–^{13}C coupling constants are listed in Table II. The 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 poly(uridylic acid) (Mantsch and Smith, 1972). The ^{31}P–^{13}C coupling constants observed for the AMP isomers (Table II) follow the same trends that have been observed for the uridine monophosphate isomers (Mantsch and Smith, 1972).

The effect of progressive addition of Mn^{2+} ions on the ^{13}C nmr spectra of 5′-, 3′-, and 2′-AMP is seen in Figures 1, 2, and 3, respectively. The Mn^{2+} ion concentration for each solution is indicated in the figure and the spectra with varying metal concentrations may readily be compared with the spectra of the pure nucleotide. These figures illustrate the line broadening of selective ^{13}C resonances with the addition of Mn^{2+} ions. The results are summarized in Figure 4.

Discussion

In the ^{31}P nmr study of paramagnetic ion interactions with nucleotides and related systems, Shulman *et al.* (1965) used 1.0–0.5 M aqueous solutions of 5′-AMP and Mn^{2+} ion concentrations of up to 0.06 M to observe the effects of the paramagnetic metal ions. Berger and Eichhorn (1971) observe proton magnetic resonance line-broadening effects on 0.1 M 5′-AMP solutions in D_2O with a Cu^{2+} ion concentration of about 3×10^{-4} M. In the present study, the line broadening effects arising from paramagnetic Mn^{2+} ions are observed for about 0.6–0.7 M solutions of the AMP isomers in the presence of between 10^{-5} and 10^{-4} M Mn^{2+} ions. The data presented above illustrate that ^{13}C nmr spectra are very sensitive to the presence of paramagnetic ions in solution.

The line width at half-height arising from the addition of the metal ion, $\Delta\nu_{1/2}$(metal), may be expressed as: $\Delta\nu_{1/2}(\mathrm{metal}) = \Delta\nu_{1/2}(\mathrm{obsd}) - \Delta\nu_{1/2}(0)$, where $\Delta\nu_{1/2}$(obsd) is the observed line width at half-height for a metal ion solution and $\Delta\nu_{1/2}(0)$ is the line width at half-height of the same resonances without metal ion. In Figure 4, therefore, $\Delta\nu_{1/2}$(metal) is plotted for each of the five adenine base ^{13}C lines as a function of the Mn^{2+} ion concentration. The results indicate that on the addition of Mn^{2+} ions the C-5 and C-8 resonances broaden first and most rapidly for all three nucleotides. The C-4, C-6, and C-2 resonances broaden next whereas the five ribose carbon resonances begin to be slightly affected at much higher metal ion concentrations (about 10^{-3} M). The line-broadening behavior is similar for the three AMP isomers. However, 3′-AMP is relatively insensitive to the paramagnetic ions since greater Mn^{2+} ion concentrations are required to obtain the same effects as are observed for a given metal concentration with 5′-AMP and 2′-AMP.

The effect of paramagnetic species on ^{13}C spectra has been studied from the viewpoint of quenching the nuclear Overhauser effect (LaMar, 1971a,b; Natusch, 1971; Freeman *et al.*, 1971). The results observed for the formic acid resonance as a function of the hexaquochromic perchlorate concentration (Freeman *et al.*, 1971) indicate that the paramagnetic ions begin to induce appreciable line broadening when the metal concentration is about 0.1 M. In the present study, specific line-broadening effects are observed for the adenine carbon resonances at much lower Mn^{2+} ion concentrations (10^{-5}–10^{-4} M) whereas the remaining resonances are only slightly perturbed. The interaction of the metal ion with the AMP isomers is therefore very specific.

The above line-broadening results demonstrate that Mn^{2+} ions interact with the base in preference to the ribose region of the molecule. Preferential binding is also observed for the five-membered ring rather than the six-membered ring of the base. Berger and Eichhorn (1971) have shown that the effect of the paramagnetic Cu^{2+} ion bound to the six-membered ring is not transmitted to the second ring. The foremost broadening of the C-5 and C-8 resonances indicates that the metal either binds to or is located near the N-7 position of the base in all three AMP isomers. However, the phosphate group has been implicated as the primary binding site (Shulman *et al.*, 1965; Heller *et al.*, 1970). This conclusion is supported by the measurements of Anderson *et al.* (1971) showing that little or no broadening of the H-8, H-2, and H-1′ resonances is observed for adenosine and deoxyadenosine in the presence of Mn^{2+} ions. Hence in the AMP isomers, the Mn^{2+} ion may be held in the vicinity of the N-7 position of the base for conformational reasons (Glassman *et al.*, 1971) while bound to the phosphate group.

In postulating a possible model for the AMP complex with Mn^{2+} ions, metal interactions with ATP should also be considered. Metal–ATP interactions have been extensively studied using a variety of techniques (Izatt *et al.*, 1971). Cohn and Hughes (1962) found that the addition of Mn^{2+} ions to ATP in solution broadens all three ^{31}P resonances as well as the H-8 proton resonance. This raised the possibility of attachment of the metal not only to the phosphate groups but also to the N-7 position of the ring. These studies were then extended (Sternlicht *et al.*, 1965a,b) to ATP–metal complexes involving Co^{2+}, Ni^{2+}, as well as Mn^{2+} ions and showed that the degree of interaction is equivalent at both the phosphate groups and the ring position. In the Mn^{2+}–$(ATP)_2$ complex, the metal simultaneously binds to the phosphate group of one nucleotide and to the N-7 of the second nucleotide for solutions with a very large ratio of ATP molecules to Mn^{2+} ions (Sternlicht *et al.*, 1968). Recently (Glassman *et al.*, 1971; Kuntz *et al.*, 1972) a structure has been proposed using nmr techniques for the complex between ATP with Mn^{2+}, Co^{2+}, and Ni^{2+} ions. In this complex a water molecule is coordinated to the phosphate-bound metal ion and is simultaneously hydrogen bonded to N-7 of the adenine ring.

Similar line-broadening behavior for the three nucleotides indicates that the metal–nucleotide complex is independent of the position of the phosphate group on the ribose. Yet the metal is held near the N-7 position of the base and is bound to the phosphate group. From molecular models, a 1:1 intramolecular complex involving both the phosphate group and the N-7 position of the base is possible between 5′-AMP and Mn^{2+}. However, molecular models indicate that 3′-AMP or 2′-AMP must undergo drastic conformational changes for an intramolecular 1:1 complex to be formed with the Mn^{2+} ion. Under the present experimental conditions, the nucleotide:Mn^{2+} ion ratio is very large and about 10,000:1. Under these conditions, a possible model for a complex between 5′-AMP, 3′-AMP, or 2′-AMP with Mn^{2+} may involve the binding of the metal ion to the phosphate group of one nucleotide and simultaneously to the adenine ring N-7 of a second nucleotide.

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