A Carbon-13 Nuclear Magnetic Resonance Study of Binding of Manganese(II) to Purine and Pyrimidine Nucleosides and Nucleotides†

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ABSTRACT: The influence of paramagnetic Mn^{2+} ions on the proton-decoupled ^{13}C nuclear magnetic resonance spectra of pyrimidine nucleosides and nucleotides as well as on purine nucleotides has been studied. For 5′-CMP and cytidine, the C-2 carbon resonance is broadened preferentially to the C-4, C-5, and C-6 base carbon resonances and to the ribose carbon resonances. For 5′-UMP, 5′-TMP, and uridine, the C-2 and C-4 base carbon resonances are equally and preferentially affected by the Mn^{2+} ions. Hence the Mn^{2+} ions are held near the carbonyl oxygen at C-2 in 5′-CMP and cytidine and near the carbonyl oxygens at C-2 and C-4 in 5′-UMP, 5′-TMP, and uridine. For 5′-GMP and 5′-IMP, the C-5, C-6, and C-8 resonances are preferentially and nearly equally affected by the presence of Mn^{2+} ions indicating that the metal binds near the C-6 carbonyl oxygen as well as near the N-7 position of the base. ^{31}P nuclear magnetic resonance data on the nucleotides confirms that the phosphate group is also abinding site for the Mn^{2+} ions. These results indicate the presence of multiple binding sites for the Mn^{2+} ions.

Metal ions play an important rule in nucleic acid processes as well as in protein chemistry and these metal complexes have been extensively studied. Studies of the interactions between metal ions with nucleosides, nucleotides, and related compounds have recently been reviewed (Izatt *et al.*, 1971; Phillips, 1966; Weser, 1968). Nuclear magnetic resonance (nmr) is a powerful technique that has been extensively used in the determination of metal binding sites in these systems.

The interactions between Mn^{2+} ions and adenine nucleosides and nucleotides have been well studied. Cohn and Hughes (1962) observed that the addition of Mn^{2+} ions to ATP in solution broadens the H-8 proton resonance as well as all ^{31}P nmr resonances. Sternlicht and coworkers (1965a,b) extended these studies to metal–ATP complexes involving Co^{2+}, Ni^{2+} as well as Mn^{2+} ions, and studied the nature of the Mn^{2+}–$(ATP)_2$ complex for solutions with a very large ratio of ATP molecules to Mn^{2+} ions (Sternlicht *et al.*, 1968). Recently, a structure has been proposed (Glassman *et al.*, 1971; Kuntz *et al.*, 1972) for the ATP complex with Mn^{2+}, Co^{2+}, and Ni^{2+} ions in which the metal ion binds to three oxygen atoms on the phosphate groups and simultaneously binds to N-7 of the adenine ring *via* a water molecule bridge.

The interactions of 5′-AMP with metal ions have also been extensively studied. ^{31}P nmr showed that Mn^{2+} ions bind to the phosphate group (Shulman *et al.*, 1965; Missen *et al.*, 1972). As well, from ^{1}H line-broadening experiments on the H-8 and H-2 resonances, the Mn^{2+} ion affects the H-8 resonance most strongly (Chan and Nelson, 1969; Kan and Li, 1972; Missen *et al.*, 1972). Similarly, Mn^{2+} ions completely broaden the H-8 proton resonance as well as the ^{31}P nmr signal of 5′-GMP for solutions containing 1.8×10^{-4} M Mn^{2+} ions and 0.2 M 5′-GMP (Kan and Li, 1972). Recently, Kotowycz and Hayamizu (1973) observed specific line-broadening effects due to the influence of paramagnetic Mn^{2+} ions on the proton-decoupled ^{13}C nmr spectra of 5′-AMP, 3′-AMP, and 2′-AMP. The C-5 and C-8 resonances are broadened preferentially to the C-2, C-4, and C-6 resonances whereas the five ribose carbon resonances are little affected. These data indicate that the metal ion binds near the N-7 position of the base in all three nucleotides irrespective of the position of the phosphate group and that specific line-broadening effects on the ^{13}C nmr spectra can be used in determining the nature of the metal–nucleotide interaction.

The interaction between Mn^{2+} ions and pyrimidine nucleosides and nucleotides have not been as extensively studied. Mn^{2+} ions broaden the H-6, H-5, and H-1′ proton line widths of CTP by 64, 76, and 22 Hz, respectively, for a solution with a metal:nucleotide ratio of 0.026 (Glassman *et al.*, 1971). Water proton transverse relaxation time (T_2) studies showed that 5′-CMP, 5′-UMP, as well as 5′-AMP and 5′-GMP have one binding site for the Mn^{2+} ion (Heller *et al.*, 1970).

In this paper we wish to examine the nature of the interaction between Mn^{2+} ions and the purine nucleotides 5′-GMP and 5′-IMP as well as the pyrimidine nucleotides 5′-CMP, 5′-UMP, 5′-TMP, and nucleosides cytidine and uridine. The approach that is used has been extensively applied and involves the measurement of the broadening of specific lines in the nmr spectrum of the molecules upon the addition of paramagnetic ions (Li *et al.*, 1962). The Fourier transform ^{13}C nmr approach together with Fourier transform ^{31}P nmr was chosen for this study.

Solomon (1955), Bloembergen and Morgan (1961), and Bernheim *et al.* (1959) have analyzed the influence of paramagnetic ions on nuclear relaxation times. Two mechanisms, namely the dipole–dipole and the hyperfine interaction, can contribute to the transverse relaxation rate, $1/T_{2M}$, as given in the following expression (Carrington and McLachlan, 1967)

$$\frac{1}{T_{2M}} = \frac{7S(S+1)g^2\beta^2\gamma_I^2}{15r_i^6}\tau_c + \frac{S(S+1)a^2}{3\hbar^2}\tau_e$$

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where r_i is the average electron–nuclear separation, γ_I is the nuclear magnetogyric ratio, S the electron spin, $a/\hbar$ is the contact coupling constant in radians per seconds, g is the g value of the electron, β is the value of the Bohr magneton, τ_c and τ_e are the correlation times for the dipolar and contact interactions, respectively. The first term in the above equation represents the dipolar relaxation and the second is the relaxation rate due to hyperfine interactions. For the dipole–dipole relaxation mechanism, 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. Hence the line widths and therefore the T_2 of each ^{13}C and ^{31}P resonance were studied with the progressive addition of the Mn^{2+} ions.

Experimental Section

All nucleotides (obtained as sodium salts) and nucleosides 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) with dilute NaOD using an Orion 801 digital pH meter. The volume of each solution was determined using a pipet. Stock solutions of $MnCl_2$ were prepared and were added to the above solutions using H. E. Pedersen micropipets. The final Mn^{2+} ion concentration for each solution could thus be calculated. The pyrimidine nucleotides 5′-CMP, 5′-UMP, and 5′-TMP and nucleosides cytidine and uridine were studied at concentrations between 0.75 and 0.80 M. The less soluble purine nucleotides 5′-GMP and 5′-IMP were studied at concentrations between 0.50 and 0.55 M. The nucleotide solutions for the ^{31}P nmr experiments were prepared in distilled water and were adjusted to a pH of 7.0 using dilute NaOH.

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 the pyrimidine nucleotides and nucleosides 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 for cytidine, uridine, 5′-CMP, and 5′-UMP (0.37 Hz/point) and 5000 Hz for 5′-TMP (0.61 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 measured automatically using the computer program and are calibrated with respect to the internal dioxane resonance. The chemical shifts are accurate to ± 0.1 ppm. The ^{13}C spectra were recorded on an expanded scale (Figure 1) from which the line widths at half-height and the ^{31}P–^{13}C coupling constants are determined. The coupling constants are accurate to ± 1.0 Hz. For resonances with a line width at half-height less than 10 Hz, the line-width measurements are accurate to ± 1.5 Hz. However, for resonances with a line width greater than 10 Hz, the signal to noise ratio decreases and the line-width measurements are accurate to ± 3 Hz.

In order to study the line broadening as a function of temperature, a D_2O solution of 0.8 M 5′-CMP with a Mn^{2+} ion concentration of 1×10^{-4} M was prepared. The ^{13}C nmr spectra 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). The temperature was determined directly using the Bruker temperature control unit. The readings on the temperature control unit were calibrated over the temperature interval under consideration by a thermocouple inserted in a sample tube under the same experimental conditions used in this work. The temperatures agree to within ± 1°K.

The ^{31}P nmr spectra were measured at 36.43 MHz and the proton resonance from the solvent H_2O was used for the heteronuclear lock. The free induction decay signals were accumulated in 8K data points and the frequency range of the

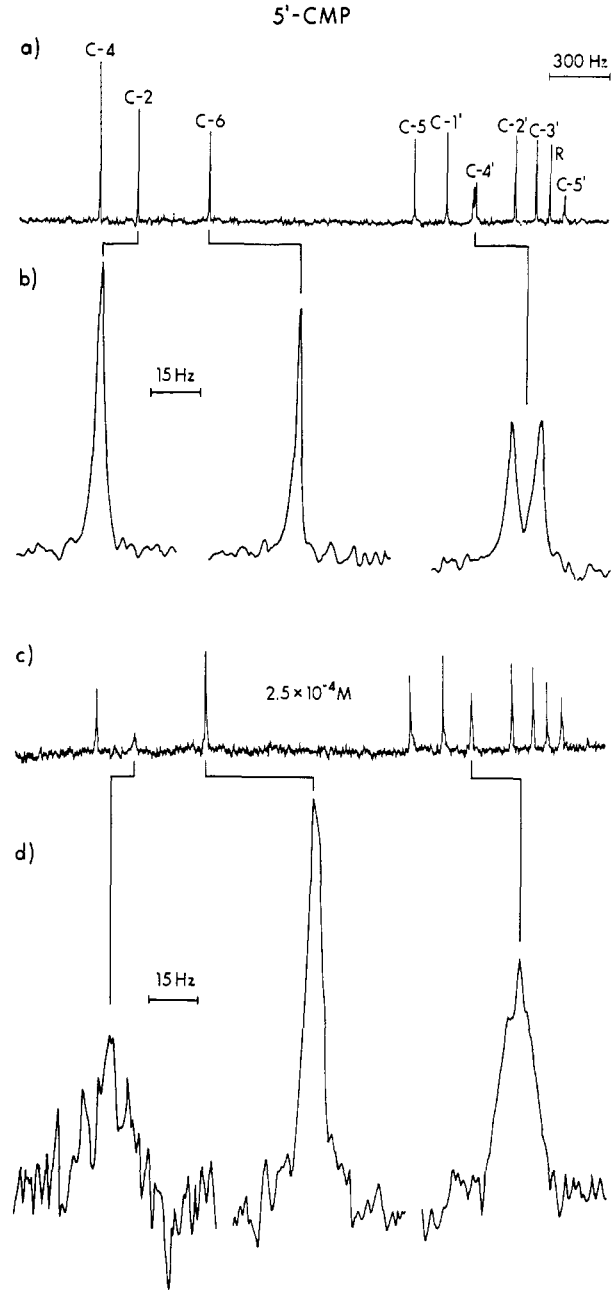

FIGURE 1: The effect of Mn^{2+} ions on the natural abundance, Fourier transform, proton-decoupled ^{13}C nmr spectra of 5′-CMP in D_2O (pD 7.4) at 25°. Spectrum a is for the metal-free solution and the Mn^{2+} ion concentration is indicated for spectrum c. Several resonances are shown on an expanded scale in spectra b and d. The recycle time for the experiment is 2.6 sec. R is the reference dioxane resonance.

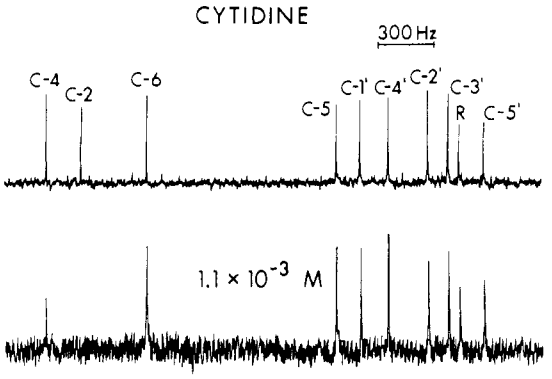

FIGURE 2: The effect of Mn^{2+} ions on the natural abundance, Fourier transform, proton-decoupled ^{13}C nmr spectra of cytidine in D_2O (pD 7.4) at 25°. The top spectrum is for the metal-free solution and the Mn^{2+} ion concentration is indicated for the lower spectrum. The recycle time for the experiment is 2.6 sec. R is the reference dioxane resonance.

Fourier-transformed spectra was 5000 Hz (1.2 Hz/point). The recycle time for the experiment is 0.8 sec. The number of pulses that were accumulated ranged from 512 to 4096, increasing with an increase in the metal concentration of the solutions. The line widths at half-height were measured from the spectra recorded on an expanded scale. The ^{31}P chemical shifts are calibrated with respect to an external P_4O_6 resonance with an accuracy of ±0.3 ppm.

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′-CMP is shown in Figure 1 and the spectra of cytidine and 5′-IMP are shown in Figures 2 and 3, respectively. The ^{13}C chemical shifts for the pyrimidine nucleosides and nucleotides are summarized in Table I and those for the purine nucleotides are tabulated in Table II. The ^{31}P nmr chemical shifts for all the nucleotides occur at 109.2 ± 0.5 ppm to high field of the P_4O_6 capillary. The ^{13}C and ^{31}P chemical shifts are not affected by the presence of the paramagnetic ions. The observed ^{31}P–^{13}C coupling constants are 8.5 ± 1 Hz for $J_{^{31}P,C\text{-}4'}$ and 4 ± 1 Hz for $J_{^{31}P,C\text{-}5'}$. The temperature dependence of the

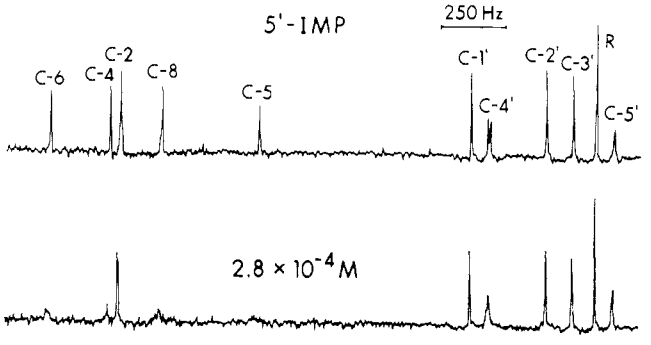

FIGURE 3: The effect of Mn^{2+} ions on the natural abundance, Fourier transform, proton-decoupled ^{13}C nmr spectra of 5′-IMP in D_2O (pD 7.4) at 35°. The top spectrum is for the metal-free solution and the Mn^{2+} ion concentration is indicated for the lower spectrum. The recycle time for the experiment is 1.6 sec. R is the reference dioxane resonance.

TABLE I: ^{13}C Chemical Shifts[a] of Pyrimidine Nucleotides and Nucleosides in Aqueous Solutions.

Carbon	5′-CMP	5′-UMP	5′-TMP	Cytidine	Uridine
C-4	−99.2	−99.6	−99.6	−99.4	−99.4
C-2	−90.8	−85.2	−85.0	−90.8	−84.9
C-6	−75.1	−75.4	−70.9	−75.0	−75.2
C-5	−30.0	−36.0	−44.9	−29.5	−35.6
C-1′	−22.8	−22.0	−18.3	−23.8	−22.8
C-4′	−16.7	−17.4	−19.2	−17.2	−17.6
C-2′	−7.7	−7.4	+28.1	−7.5	−7.2
C-3′	−3.0	−3.4	−4.5	−2.6	−2.8
C-5′	+3.2	+3.2	+2.6	+5.8	+5.8
CH_3			+54.8		

[a] The chemical shifts are in parts per million with respect to internal dioxane and are accurate to ±0.1 ppm. A negative value indicates that the resonance is to low field of the reference.

^{13}C line widths observed for the 5′-CMP solution containing Mn^{2+} ions is seen in Figure 4.

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 poly(uridylic acid) (Mantsch and Smith, 1972). A disagreement in the literature concerning the assignment of the C-2 and C-4 resonances of 5′-IMP (Dorman and Roberts, 1970; Jones *et al.*, 1970a) was resolved by observing the proton-coupled spectrum. Thus the C-4 resonance was assigned to low field of the C-2 resonance, in agreement with the assignment by Dorman and Roberts (1970). The values of $J_{^{31}P,^{13}C}$ observed for the nucleotides follow the same trends that have been observed previously (Dorman and Roberts, 1970; Mantsch and Smith, 1972; Lapper *et al.*, 1972; Kotowycz and Hayamizu, 1973).

The effect of adding Mn^{2+} ions on the ^{13}C nmr spectrum of 5′-CMP is seen in Figure 1, whereas the effect on the spectra of cytidine and 5′-IMP is seen in Figures 2 and 3, respectively. These figures illustrate the selective broadening of

TABLE II: ^{13}C Chemical Shifts[a] of 5′-GMP and 5′-IMP in Aqueous Solution.

Carbon	5′-GMP	5′-IMP
C-6	−92.1	−91.8
C-2	−87.2	−80.0
C-4	−84.6	−81.8
C-8	−70.6	−73.2
C-5	−49.1	−56.8
C-1′	−20.4	−21.0
C-4′	−17.6	−18.0
C-2′	−7.6	−8.3
C-3′	−3.9	−3.9
C-5′	+2.9	+2.9

[a] The chemical shifts are in parts per million with respect to internal dioxane and are accurate to ±0.1 ppm. A negative value indicates that the resonance is to low field of the reference.

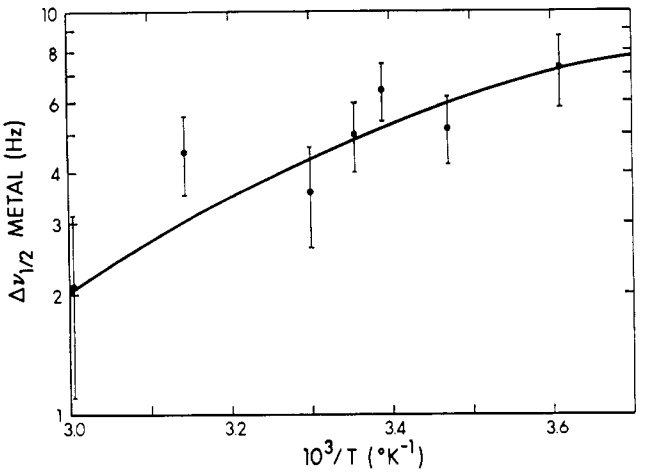

FIGURE 4: The temperature dependence of $\Delta\nu_{1/2}$(metal) for the C-2 carbon nucleus of 5′-CMP. The concentration of 5′-CMP is 0.80 M whereas the Mn^{2+} ion concentration is 1.0×10^{-4} M for a [nucleotide]:[metal ion] ratio of 8×10^3. The remaining carbon nuclei are unaffected.

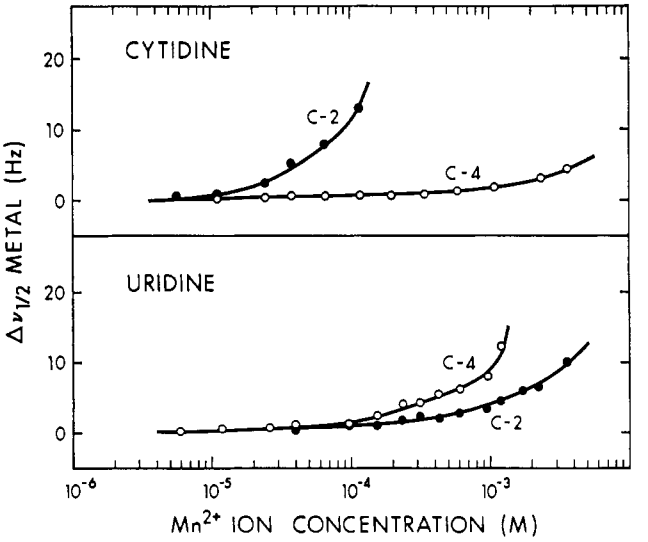

FIGURE 6: The dependence of $\Delta\nu_{1/2}$(metal) on the Mn^{2+} ion concentration for two base carbon nuclei C-2 and C-4 of cytidine and uridine.

the ^{13}C resonances with the addition of the paramagnetic ions. The results of these experiments on the pyrimidine nucleotides are summarized in Figure 5 and may be compared with the Mn^{2+} ion effect on the ^{13}C spectra of the pyrimidine nucleosides (Figure 6) and the purine nucleotides (Figure 7).

The ^{31}P nmr line widths increase from 14 ± 3 Hz for the pure nucleotides to about 200 ± 40 Hz for solutions with a metal concentration about 2×10^{-4} M.

Discussion

As seen from the broadening of the ^{31}P nmr resonances, Mn^{2+} ions bind to the phosphate group of 5′-AMP (Shulman *et al.*, 1965) and to the phosphate groups of ATP (Cohn and Hughes, 1962). In the present study, the ^{31}P resonances are

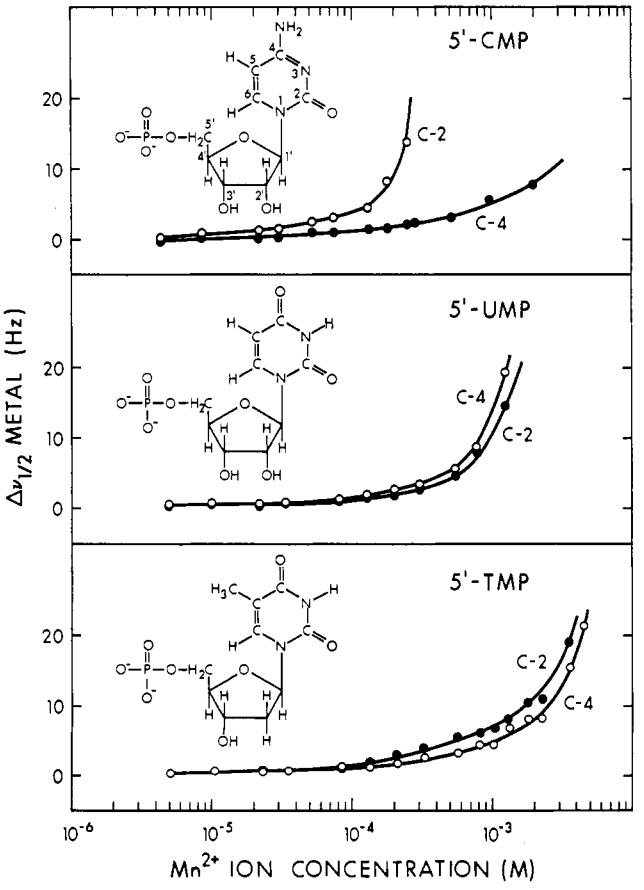

FIGURE 5: The dependence of $\Delta\nu_{1/2}$(metal) on the Mn^{2+} ion concentration for the two base carbon nuclei C-2 and C-4 of 5′-CMP, 5′-UMP, and 5′-TMP.

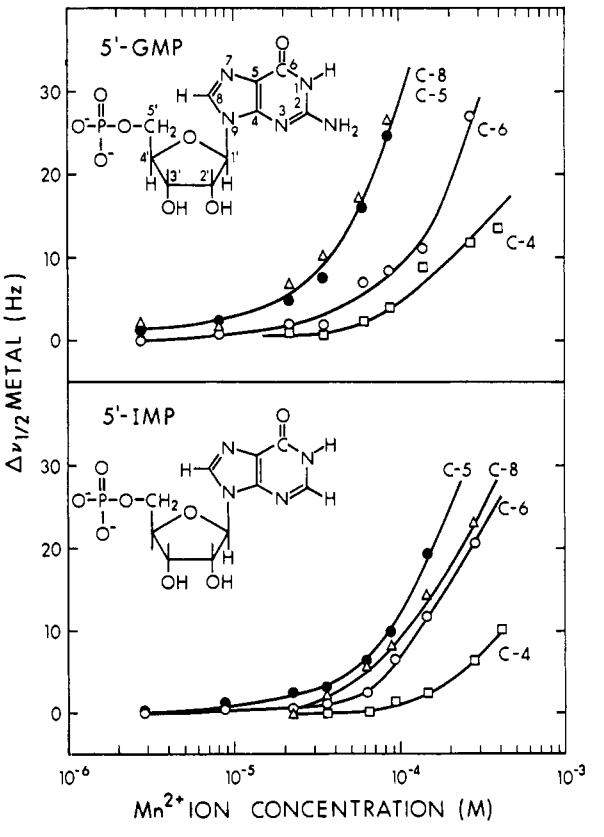

FIGURE 7: The dependence of $\Delta\nu_{1/2}$(metal) on the Mn^{2+} ion concentration for four base carbon nuclei. The carbon nuclei are represented as follows: (□) C-4, (●) C-5, (○) C-6, (△) C-8.

also broadened by the Mn^{2+} ions indicating metal binding to the phosphate groups of all nucleotides in the present study.

In addition, the metal interaction with the ring must be considered. The effect of paramagnetic species on ^{13}C nmr spectra has been studied from the viewpoint of quenching the nuclear Overhauser effect (Gutowsky and Natusch, 1972; LaMar, 1971a,b; Natusch, 1971; Freeman *et al.*, 1971). As has been previously observed (Kotowycz and Hayamizu, 1973), ^{13}C nmr spectra are very sensitive to the presence of paramagnetic Mn^{2+} ions in solution and specific line-broadening effects are observed, depending on the nature of the Mn^{2+} ion binding site. For example, as can be seen from the spectra shown in Figure 1 and the data plotted in Figure 5 for 5′-CMP, the C-2 carbon resonance broadens first and most rapidly on the addition of Mn^{2+} ions. The C-4 resonance begins to be slightly affected only at much higher Mn^{2+} concentrations whereas the C-5 and C-6 base carbon resonances, the ribose carbon resonances as well as the dioxane reference signal are unaffected. Hence the line width at half-height arising from the presence of the metal ion, $\Delta\nu_{1/2}$(metal), may be expressed as

$$\Delta\nu_{1/2}(\text{metal}) = \Delta\nu_{1/2,\text{obsd}} - \Delta\nu_{1/2,0}$$

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 ion.

The temperature at which these expreiments are carried out is important in the analysis of the data. Swift and Connick (1962) have shown that $\Delta\nu_{1/2}$(metal) is determined by the temperature dependence of T_{2M} and τ_M. T_{2M} has been defined previously and τ_M is the average lifetime of a molecule coordinated at any site. From the plot of log $\Delta\nu_{1/2}$(metal) *vs.* $1/T$ two limiting cases of the Swift and Connick equation are realized in different temperature regions, namely the exchange controlled region with a negative slope of the curve and the T_{2M} region at higher temperatures with a positive slope. As seen from the data in Figure 4 obtained for the 5′-CMP–Mn^{2+} solution, measurements carried out at 25° ($1/T = 3.356 \times 10^{-3}$) are definitely in the region where T_{2M} processes dominate line broadening.

In Figure 5, $\Delta\nu_{1/2}$(metal) is plotted for the C-2 and C-4 carbon resonances for 5′-CMP as well as for 5′-UMP and 5′-TMP. For 5′-UMP and 5′-TMP, the C-2 and C-4 base carbon resonances are equally affected by the Mn^{2+} ions. Similar results were obtained for the nucleosides. The C-2 resonance is affected most strongly in cytidine (Figure 2 and Figure 6) whereas the Mn^{2+} ions influence the C-2 and C-4 resonances nearly equally in uridine (Figure 6). As for 5′-CMP, the remaining carbon resonances are unaffected in these molecules. The dioxane reference signal is also unaffected by the presence of Mn^{2+} ions.

The above data indicate that Mn^{2+} ions interact with the base but not the ribose region of the molecules. The metal ion binds near the carbonyl oxygen at C-2 in 5′-CMP and cytidine and near the carbonyl oxygens at C-2 and C-4 in 5′-UMP, 5′-TMP, and uridine. Similar line broadening is observed for the base carbons of 5′-CMP as for cytidine. As well, the line broadening observed for 5′-UMP is similar to that observed for uridine. Hence the interaction with the base appears to be independent of the phosphate group. For solubility reasons, ^{13}C spectra of thymidine were not obtained. However, proton magnetic resonance measurements indicate that Mn^{2+} ions interact with the base portion of the nucleoside thymidine. In measurements carried out on thymidine at a pH of 6.8 at 80° (Anderson *et al.*, 1971), the methyl resonance was broadened by Mn^{2+} ions by 1.5 Hz for a solution with a Mn^{2+}:thymidine ratio of 0.01. Hence for 5′-TMP and thymidine also the interaction of Mn^{2+} ions with the base appears to be independent of the phosphate group. The Mn^{2+} ion may bind to thymidine near the C-2 and C-4 carbonyl oxygens in analogy with the Mn^{2+} ion interaction with 5′-TMP.

In the purine nucleotides, the C-5, C-6, and C-8 resonances are most strongly and nearly equally affected by the presence of Mn^{2+} ions in 5′-IMP (Figures 3 and 7) and 5′-GMP (Figure 7). The C-4 resonance is affected at higher Mn^{2+} ion concentrations whereas the C-2 base carbon resonance and the five ribose resonances are unaffected. Berger and Eichhorn (1971) have shown that the effect of the paramagnetic Cu^{2+} ion bound to the six-membered ring of a purine base is not transmitted to the second ring. Hence the foremost broadening of the C-5, C-6, and C-8 resonances indicates that the metal binds near the C-6 carbonyl oxygen as well as near the N-7 position of the base in 5′-GMP and 5′-IMP. In 5′-AMP, 3′-AMP, and 2′-AMP (Kotowycz and Hayamizu, 1973) the C-5 and C-8 resonances are broadened preferentially to the C-2, C-4, and C-6 resonances indicating that the metal ion binds near the N-7 position of the base.

To show that the interaction of the metal ion with the base portion of the molecule is independent of the phosphate group, the ^{13}C spectra of 5′-GMP should be compared with those for the nucleoside guanosine obtained as a function of the Mn^{2+} ion concentration as well as from a comparison of the spectra obtained for 5′-IMP and the nucleoside inosine. However, due to the low solubility of these nucleosides, ^{13}C spectra were not obtained. But proton magnetic resonance measurements indicate that these nucleosides do interact with Mn^{2+} ions. Significant line broadening of the H-8 proton resonance is observed for guanosine in the presence of Mn^{2+} ions (Anderson *et al.*, 1971). For a Mn^{2+}:guanosine ratio of 0.006, $\Delta\nu_{1/2}$(metal) is 4.5 Hz at 80° ($\Delta\nu_{1/2}$(metal) = 0.5 Hz for H-1′), reflecting the existence of a discrete binding site on the guanine ring (Anderson *et al.*, 1971). Our proton magnetic resonance (pmr) measurements on inosine in D_2O at 31° indicate that for a solution with a Mn^{2+} to inosine ratio of 0.001, $\Delta\nu_{1/2}$(metal) is the largest for H-8 and equal to 1.3 Hz. This result also indicates the presence of a specific interaction between Mn^{2+} ions and inosine. Hence the presence of the phosphate group is not required for the interaction between Mn^{2+} ions with the base region of the nucleosides. The Mn^{2+} ion may bind to inosine and guanosine near the C-6 carbonyl oxygen as well as near the N-7 position of the base in analogy with the interaction with 5′-IMP and 5′-GMP. Finally, in 5′-AMP, 3′-AMP, and 2′-AMP (Kotowycz and Hayamizu, 1973), the C-5 and C-8 resonances are similarly broadened for the three nucleotides irrespective of the position of the phosphate group on the ribose. 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. This result also suggests that Mn^{2+} ions bind independently to the phosphate group and to the base portion of the molecule.

Another possibility for Mn^{2+}–nucleotide interactions must also be considered. Under the present experimental conditions, the nucleotide:Mn^{2+} ion ratio is very large and about 1000:1. Under these conditions, a possible model for a complex between the nucleotides with Mn^{2+} may involve the binding of the metal ion to the phosphate group of one nucleotide and simultaneously to the base portion of a second nucleotide.

The above line-broadening results are in agreement with the observation of Eichhorn and Shin (1968) that low concentrations of Mn^{2+} ions increase the thermal stability of DNA due to an interaction with the phosphate groups. At higher Mn^{2+} ion concentrations, the thermal stability decreases due to an interaction with the bases. Anderson and coworkers (1971) observed similar results and also showed that the decrease in thermal stability is dependent on the base composition of the DNA.

The ^{13}C chemical shifts are not affected by the presence of the paramagnetic ions. Nucleosides and nucleotides stack in aqueous solutions (Ts'o *et al.*, 1969; Ts'o, 1970) but since the [nucleotide]:[Mn^{2+}] ratio is about 1000:1 for our solutions, the proportion of molecules that are destacked by the Mn^{2+} ions is small. Hence the chemical shift is independent of the Mn^{2+} ion concentration. In addition, Smith and coworkers (1972) have investigated the effect of base stacking on the ^{13}C chemical shifts for the carbons in 5'-AMP over the concentration range 1.2–0.04 M in D_2O at 37°. Only very small downfield shifts are observed upon dilution as the monomer–oligomer equilibrium is shifted toward the monomer. Therefore the effects of destacking arising from the presence of Mn^{2+} ions on the ^{13}C chemical shifts are expected to be very small.

The spectra in Figures 1–3 indicate an additional interesting feature. As the Mn^{2+} ion concentration increases, the C-4' and C-5' ribose ^{13}C resonances become sharp and the ^{31}P–^{13}C coupling disappears. As the Mn^{2+} ions bind to the phosphate groups, they shorten the longitudinal relaxation time (T_1) of the phosphorus nuclei and the coupling to the ^{13}C nuclei is removed.

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