A Multinuclear NMR Spin Labeling Investigation of the Solution Structure of the Base Stacked Mn^{2+}-AMP Complex

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Abstract: ^{15}N, ^{13}C, and ^{31}P electron-nuclear relaxation times (T_1^es) are used to determine the solution structure of the base stacked Mn^{2+}-AMP complex. The nitrogen-Mn^{2+} distances that are determined indicate a direct inner-sphere coordination of Mn^{2+} at N(1), N(7), and the amino NH_2 positions. The phosphorus-Mn^{2+} distance also indicates direct coordination and this second site may explain the anomalously short Mn^{2+}-C(8) distance that is found. A structure is proposed consistent with the measured distances. Comparison of Mn^{2+}-carbon distances with those in the literature is made.

Introduction

The solution structure of transition metal-nucleotide complexes has been of intense interest for many years.[2] First, all of the reactions involving nucleic acids in biological systems such as those involving ATP or dinucleotide coenzymes are mediated by metal ions. Second, metal ions are known to have an effect on the stability of both DNA and RNA in vivo. Physiologically, the important complexes are those involving Mg^{2+}. There is, however, considerable discord as to the structure of these metal-nucleotide complexes.

In 1957, Szent-Gyorgyi proposed a back-bound conformation for the binding of divalent metal ions to ATP.[3a] In this 1:1 complex, the metal is bound to the β and γ phosphates and to the adenine ring at N(7) and the amino NH_2. However, Nakajima and Pullman have calculated that the N(1) position is the most basic site in the adenine ring.[3b] Verification for *protonation* at N(1) in acidic solution comes from X-ray crystallography[4] and a ^{15}N NMR study.[5] That the strongest metal binding is with the phosphate group has never been in question. The binding constants clearly show that nucleotide binding decreases in the order ATP > ADP > AMP > adenosine.[2] However, the site of binding to the base, the importance of each phosphate group to the binding in ATP, whether 1:1 or 1:2 metal-nucleotide complexes are involved at specified conditions, and the general features of these complexes are still unclear after nearly 20 years of experimental work.

One of the techniques most heavily used to study binding sites has been NMR. The earliest studies were those of Cohn and Hughes in 1960 using ^{31}P NMR.[6] They assigned the α, β, and γ phosphorus resonances of ATP[6] and on the basis of equal line broadening in the presence of Mn^{2+} concluded that the three phosphates are equally involved in the binding.[7] Sternlicht et al. in 1965 pointed out that the ^{31}P T_2^es were determined by scalar relaxation or by exchange and not by dipolar relaxation.[8] Therefore structural information cannot be obtained from ^{31}P line broadening techniques. They concluded on the basis of CW progressive saturation T_1^e studies that all phosphates were equally involved in metal binding.[8] Richards et al. in 1973 performed more accurate pulsed ^{31}P T_1^e measurements and concluded that the β and γ phosphates bind the metal more tightly than does the α phosphate.[9]

The earliest ^1H NMR study of metal binding to the ring was concerned with the diamagnetic ions Mg^{2+}, Ca^{2+}, and Zn^{2+}. Hammes, Maciel, and Waugh found in 1961 that addition of Mg^{2+} and Ca^{2+} did not change the H(2) and H(8) resonance positions and therefore the metals did not bind to the ring.[10] Later, Hammes and Miller concluded on the basis of temperature-jump kinetics that Mg^{2+} and Ca^{2+} complexes of ATP do not involve significant metal-ring binding; however, ring binding does occur for Ni^{2+}, Co^{2+}, and Mn^{2+}.[11] Finally, Happe and Morales concluded from ^{15}N NMR chemical shifts that Mg^{2+} does not bind to the ring.[12]

Most subsequent investigations of metal-ring binding have involved paramagnetic metal ions. Cohn and Hughes used paramagnetic line broadening of ^1H resonances to study metal binding.[7] This technique has also been used by Sternlicht et al.[13] and Eichhorn.[14] The use of ^1H NMR is limited by the presence of only two nonexchanging protons on the adenine ring. While Sternlicht et al.[13] and Espersen and Martin[15] have shown that proton T_2^e information is largely dipolar for Mn^{2+} binding and therefore can have a distance dependence, there have been a large number of recent ^{13}C investigations[16-20] of paramagnetic line broadening effects where T_2^e is decidedly not dipolar.[21,22] In fact the scalar relaxation mechanism, which dominates under conditions of usual interest, has no simple distance dependence. Therefore line broadenings are not a reliable way to locate metal binding sites.

A major controversial point concerns the nature of metal binding to the ring. Sternlicht et al. first suggested that the metal ion

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(2) A good review of the literature until 1974 is found in A. T. Tu and M. J. Heller in "Metal Ions in Biological Systems", H. Sigel, Ed., Marcel Dekker, New York, 1974, p 1; C. M. Frey and J. Stuehr, *ibid.*, p 51. The literature prior to 1977 is reviewed in L. G. Marzilli, *Prog. Inorg. Chem.*, **23**, 255 (1977); D. K. Hodgson, *ibid.*, **23**, 211 (1977).
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may bind at N(7) through a bridging water molecule.[23] Swift et al. studied the transverse relaxation rates of bulk water protons and, by comparing results for metal–ATP complexes with those for metal–CTP complexes, concluded that binding of Ni^{2+} to N(7) occurs via a slowly exchanging water molecule.[24] Later, using ^{17}O NMR, Swift et al. concluded that Co^{2+} is similarly bound.[25] Hunt et al. reported for Mn^{2+}–ADP that direct binding at N(7) was likely.[26] Kotowycz et al. have recently suggested on the basis of ^{13}C T_1^es that Mn^{+2} binds directly at N(7) of ATP.[21]

Finally, Sternlicht et al.[23] were able to resolve the disagreement between the early NMR studies (generally at high concentration) and the kinetic studies such as those of Hammes and Miller.[11] They proposed that at very low ATP concentration (<0.01 M) the predominant solution species is a 1:1 metal–ATP complex. At higher concentration, especially above 0.1 M, the predominant species is a 1:2 complex.[23] The nature of the 1:2 complex is surrounded by as much controversy as is the structure of the 1:1 complex.

Clearly, the most direct and most sensitive way to probe this binding is to use ^{15}N T_1^es. The metal–ring distances determined in this manner will most accurately determine whether metal–adenine ring binding is inner sphere or outer sphere in nature. Since natural abundance ^{15}N NMR T_1 studies require fairly high concentrations of material and since a thorough investigation of the metal–nucleotide solution structures requires ^{15}N investigation, the multinuclear T_1^e study presented here will explore the nature of the 1:2 Mn^{2+}–AMP structure, leaving the structure of the 1:1 complex to future experiments utilizing ^{15}N-enriched AMP in dilute solutions.

Experimental Section

Adenosine 5′-monophosphate disodium salt was purchased from Aldrich Chemicals and dissolved in distilled and deionized water to a concentration of 0.8 M. Excess Chelex-100 resin was added and stirred on a vortex mixer. The mixture was allowed to settle overnight and the Chelex was filtered off. The resulting solution had a pH of 10.0. No attempt was made to adjust the pH. $Mn(ClO_4)_2 \cdot 6H_2O$ was purchased from Ventron and dissolved in deionized water to form a stock solution. The stock solution for the ^{15}N and ^{31}P studies was 0.005 M in Mn^{2+} while the stock solution for the ^{13}C studies was 0.05 M. Mn^{2+} was added to AMP using a Hamilton microsyringe.

Proton decoupled ^{15}N and ^{13}C NMR spectra were obtained at a field of 6.34 T using a Bruker HX-270 spectrometer with quadrature detection. Samples were contained in a 15-mm tube with a 5-mm coaxial tube containing D_2O lock. About 2200 transients were collected for the ^{15}N spectra and about 100 transients were collected for the ^{13}C spectra. Measurements were made at ambient temperature of about 26 °C. ^{31}P spectra were obtained at a field of 3.52 T on the in-house design multinuclide spectrometer (SEMINOLE) using a tuned 60-MHz 15-mm probe. Spin–lattice relaxation times were measured using the fast inversion–recovery (FIRFT) pulse sequence[27] and evaluated with a nonlinear least-squares fitting procedure having three adjustable parameters.[28]

Theory

Most of the NMR studies of metal–nucleotide structures are based on proton, carbon, or phosphorus differential parmagentic line broadening effects. Inherent to the use of the line broadening for structural studies is the assumption that the electron–nuclear T_2^e relaxation is dominated by the dipolar interaction between the nucleus and the unpaired electrons of the metal ion. However, the scalar contribution to T_2^e, which has no simple distance dependence, may be the dominant relaxation mechanism at the condition of interest. The scalar contribution is given in the equation[29]

$$\frac{1}{T_2^e} = \frac{S(S+1)}{3}\left(\frac{A}{\hbar}\right)^2\left[\tau_{e1} + \frac{\tau_{e2}}{1+\omega_S^2\tau_{e2}^2}\right] \quad (1)$$

$A/\hbar$ is the scalar coupling constant between the nucleus of interest and the paramagnetic ion and τ_e is the correlation time for this process, which may be determined by τ_S, the electron spin relaxation time, or τ_m, the lifetime of the bound state, as shown in the equation

$$\tau_{e1,2}^{-1} = \tau_{S1,2}^{-1} + \tau_M^{-1} \quad (2)$$

The frequency-dependent term in eq 1 is negligible since $\omega_S^2\tau_e^2 \gg 1$, but the overall scalar term can be quite large since $A/\hbar$ has been shown to be on the order of megahertz.[8,9,21]

The expression for the scalar contribution to T_1^e has only the frequency-dependent term and since $\omega_S^2\tau_e^2 \gg 1$ the scalar contribution to T_1^e is negligable. T_1^e is determined solely by the dipolar mechanism and therefore it is possible to obtain structural information as can be seen from the expression given by Solomon[30] and Bloembergen:[31]

$$\frac{1}{T_1^e} = \frac{2S(S+1)\gamma_1^2\gamma_S^2\hbar^2}{15r^6}\left[\frac{3\tau_{C1}}{1+\omega_1^2\tau_{C1}^2} + \frac{7\tau_{C2}}{1+\omega_S^2\tau_{C2}^2}\right] \quad (3)$$

where r is the distance between nucleus I and the metal ion and τ_c is the correlation time for the process as given by the equation.

$$\tau_{C1,2}^{-1} = \tau_R^{-1} + \tau_{S1,2}^{-1} + \tau_M^{-1} \quad (4)$$

τ_R is the rotational correlation time of the metal–nucleotide complex and was determined in this study to be about 2×10^{-10} s using the diamagnetic T_1 and NOE of the two AMP ring carbons which possess directly bonded hydrogens, C(2) and C(8). While it was not anticipated that τ_R should be isotropic, the diamagnetic T_1s and NOEs for the sugar carbons indicate a τ_R in the range $1.7–2.1 \times 10^{-10}$ s. The only nitrogen with directly bonded hydrogens is the amino NH_2 nitrogen. A correlation time of about 1×10^{-10} s is calculated for this nitrogen from the ^{15}N T_1, indicating some degree of internal motion about the N–C bond or some anisotropy of overall motion. Thus the assumption of an isotropic correlation time for the base-stacked complex does not appear to be an appreciable source of error. τ_R for the phosphate may be somewhat shorter than 2×10^{-10} s owing to internal rotation and depending on the size of its hydration sphere.

For Mn^{2+}, τ_S can be calculated to a good approximation at the two magnetic field strengths used in this study, using the Bloembergen and Morgan equation:[32]

$$\frac{1}{\tau_S} = B\left[\frac{\tau_v}{1+\omega_S^2\tau_v^2} + \frac{4\tau_v}{1+4\omega_S^2\tau_v^2}\right] \quad (5)$$

B is a constant containing the spin of the metal ion and the zero-field splitting (ZFS) parameter. τ_v is the correlation time for the modulation of the ZFS interaction. In an EPR analysis of the Mn^{2+}–AMP complex, Reed et al.[33] determined the value of the ZFS and τ_v. The ZFS of the complex was found to be only slightly greater than that of the aquo ion. Using these values and eq 5, τ_S at 6.3 T is 2.1×10^{-7} s and at 3.5 T is 6.7×10^{-8} s.

τ_M has been shown to be in the fast exchange region at room temperature and above for the ATP phosphate–metal[8,9] interaction and for the ATP ring–metal[13,21] interaction. τ_M for the phosphates lies in the range $4–6 \times 10^{-6}$ s,[8,9] and is somewhat shorter (ca. 4

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$\times 10^{-8}$ s) for the ring interaction.[21] Thus, based on the measured, calculated, and anticipated values for τ_R, τ_S, and τ_M, τ_c should be dominated by τ_R.

Another important consideration is the effect of chemical exchange and translational diffusion relaxation on the observed T_1 The expression for this is given by Swift and Connick[34] and by Luz and Meiboom[35] as shown in the equation

$$\frac{1}{T_1^e(\text{obsd})} = \frac{qf}{\tau_M + T_1^e(\text{C})} + \frac{1}{T_1^e(\text{t})} \quad (6)$$

T_1^e(C) is the dipolar contribution from complex formation shown in eq 3, τ_M is the bound state lifetime, T_1^e(t) is the outer-sphere or translation diffusion contribution, q is the number of identical ligand nuclides in the coordination sphere of the metal ion, and f is the fraction of substrate bound to the metal ion. Usually a sufficiently large stability constant is assumed, to approximate f by the ratio of metal ion concentration to nucleotide concentration. In the region of rapid exchange $\tau_M \ll T_1^e$(C); T_1^e(C) and T_1^e(t) can be separated by graphical means.[36] Once T_1^e(C) is known, r can be calculated from eq 3.

T_1^e(obsd) is determined as the difference in relaxation rates for paramagnetic and diamagnetic solutions. Relaxation due to all mechanisms other than the electron–nuclear mechanism is thereby eliminated. This is shown in the equation

$$\frac{1}{T_1^e(\text{obsd})} = \frac{1}{T_1^e(\text{para})} - \frac{1}{T_1^e(\text{dia})} \quad (7)$$

Finally, in addition to the considerations above, there is the distinct possibility that the dipolar equation for T_1^e given in eq 3 may not be applicable. Equation 3 was derived by Solomon[30] and Bloembergen[31] on the basis of a point–dipole approximation. Waysbort and Navon have shown that the electron distribution within the orbitals of the metal has little effect on metal–nucleus distances calculated using the Solom–Bloembergen equation.[37] However, they have also shown that delocalization of electron density into ligand orbitals can have a sizable effect on the electron–nuclear dipolar relaxation, depending on the degree of covalency of the metal–ligand interaction.[37] Doddrell et al. have taken an MO approach to the problem and have demonstrated the importance of ligand-centered unpaired spin density to both ^{13}C and ^{1}H relaxation of ligand nuclei in some cases.[38]

Results

At the high concentrations of AMP necessary for natural abundance ^{15}N NMR, the nucleotides form stacked complexes.[23] In order to minimize any disruption of these stacked complexes and also in order to vary the macroscopic solution viscosity as little as possible, the concentration of AMP was held fixed and was constant for all relaxation studies. Therefore T_1^e(obsd) was determined as a function of Mn^{2+} concentration.

The ^{15}N electron–nuclear relaxtion rates ($1/T_1^e$) are shown in Figure 1 as a function of Mn^{2+} concentration. The strongest interaction judged by the slopes of the lines appears to be at N(7) of the ring, although the interactions with NH_2 and N(1) are similar, compared with the lack of slope for N(3) and N(9). (The relaxation of N(3) and N(9) is unaffected at this level of Mn^{2+} concentration.) ^{15}N spectral assignments of Roberts et al. were used.[5] The intercept of these lines should theoretically be at the origin; however, the least-squares-determined intercepts lie between -0.18 and -0.30 s^{-1}.

The ^{13}C relaxation rates are shown in Figure 2. The interaction at N(7) is reflected in the steep slope of C(8), but this relaxation rate is more sensitive to the presence of Mn^{2+} than proximity to N(7) alone would indicate. Notice that C(2) and C(6), which have similar proximities to the interaction sites N(1) and NH_2, have less steep slopes than C(8). There was considerable scatter in the relaxation rate as a function of Mn^{2+} concentration for C(2) and so it is indicated as a broken line. There is no question, however, that this line lies between that of C(5) and C(6). The relaxation dependence of the ribose carbons is indicated by the shaded line at the bottom of Figure 2. ^{13}C spectral assignments for AMP were those of Dorman and Roberts.[39]

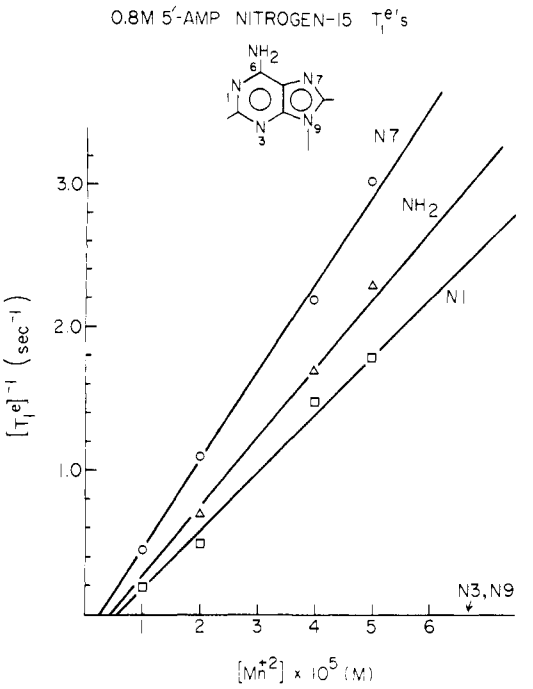

Figure 1. Plot of ^{15}N T_1^es for 0.8 M 5′-AMP in H_2O at pH 10.0 as a function of Mn^{2+} concentration. N(3) and N(9) are along abscissa. Numbering is indicated on inset.

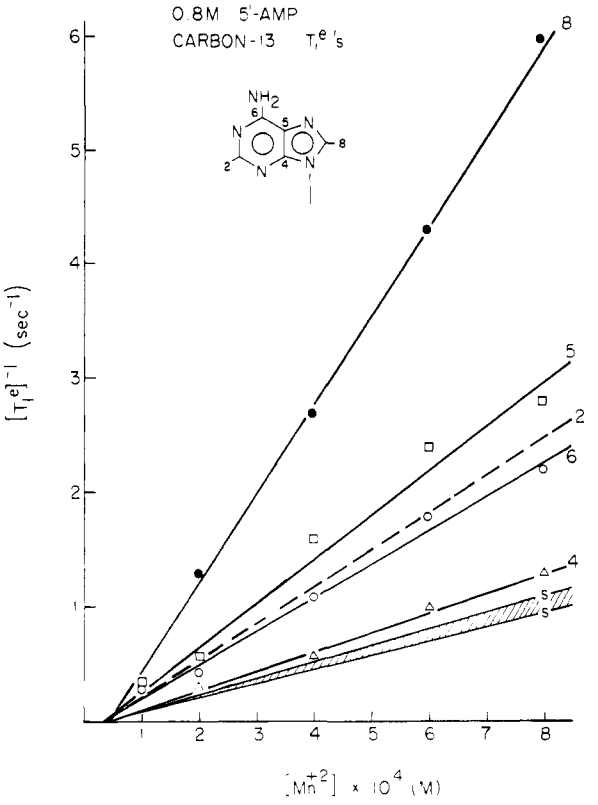

Figure 2. Plot of ^{13}C T_1^es for 0.8 M 5′-AMP in H_2O at pH 10.0 as a function of Mn^{2+} concentration. C(2) is indicated as a dashed line for reasons described in the text. The shaded band indicates the ribose carbons. The numbering is indicated on the inset.

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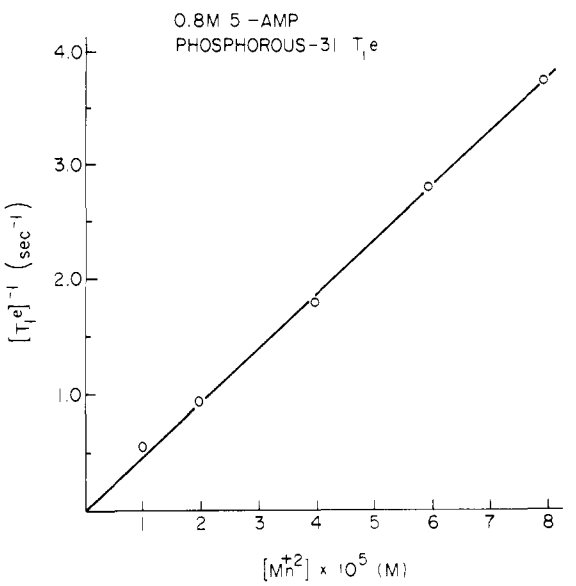

Figure 3. Plot of ^{31}P T_1^e for 0.8 M 5'-AMP in H_2O at pH 10.0 as a function of Mn^{2+} concentration.

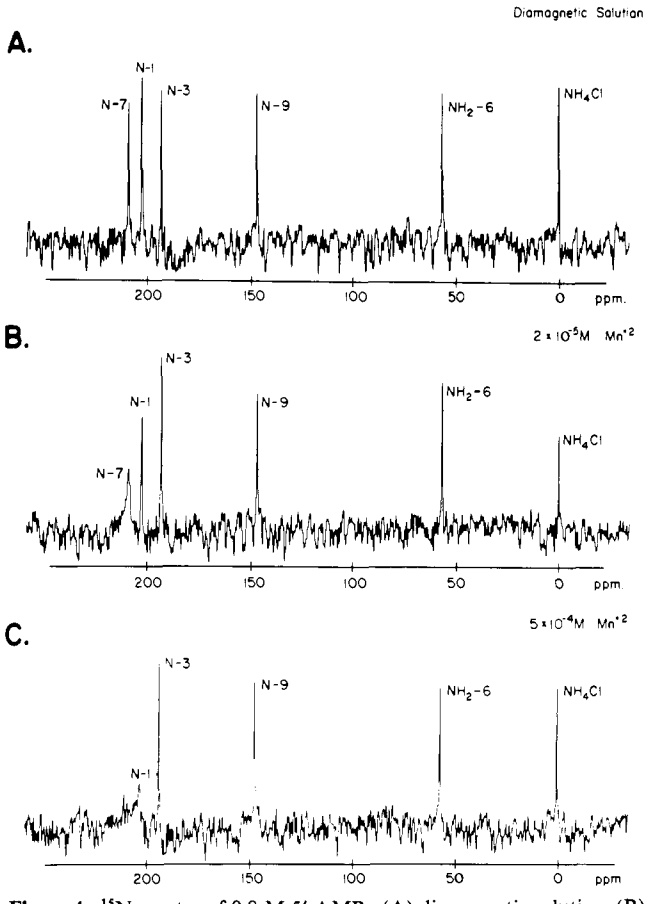

Figure 4. ^{15}N spectra of 0.8 M 5'-AMP: (A) diamagnetic solution; (B) 2×10^{-5} M Mn^{2+}; (C) 5×10^{-4} M Mn^{2+}. Shifts are referenced to an external standard of 1 M ^{15}N-enriched NH_4Cl in 1 M HCl.

The ^{31}P relaxation rate is plotted in Figure 3 as a function of Mn^{2+} concentration. The fit of the data to a straight line is excellent and the intercept is at the origin.

Finally, as an example of the misleading results which can be obtained from differential line broadening experiments, Figure 4 shows the ^{15}N NMR spectrum for a diamagnetic solution of AMP along with spectra at two levels of $[Mn^{2+}]$. On the basis

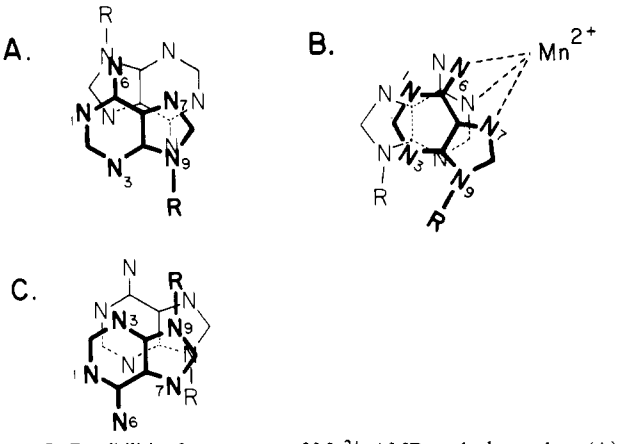

Figure 5. Possibilities for structure of Mn^{2+}–AMP stacked complex: (A) crystal structure of Cu^{2+} oxypurine, ref 41; (B) from crystal structure for Ni^{2+}–AMP, ref 42; inclusion of Mn^{2+} binding is illustrative of a structure consistent with the data in the present study; (C) proposed structure for Cu^{2+}–AMP stacked complex, ref 44.

of differential line broadening, the order of metal–nitrogen interaction is determined to be N(7) ≫ N(1), and no interaction at all is detected with NH_2. On the basis of the slopes of the relaxation rate dependences on Mn^{2+} concentration in Figure 1, the order of interaction is shown to be N(7) > NH_2 > N(1). The reason for the discrepancy is that the ring N(7) and N(1) line broadenings are determined by scalar relaxation and therefore no structural information is obtainable. The ^{15}N T_1^es are determined by dipolar interactions and therefore provide distance information on the structure of the metal–nucleotide complex.

The average change in chemical shift for the nitrogen resonances at all levels of $[Mn^{2+}]$ is never more than 0.05 ppm (or ~1.4 Hz) from the resonance position in the diamagnetic solution. There is no regular increase in shift with increased $[Mn^{2+}]$ and therefore this average shift difference is interpreted as experimental error in locating the resonance position and not due to any significant electron delocalization from Mn^{2+} to the adenine ring. In their analysis of the bishistidine complex of Mn^{2+}, Led and Grant[40] found no evidence of spin delocalization onto the histidine ring. Distances calculated from T_1^e data agreed well with distances determined from an X-ray study of the analogous Ni^{2+} complex.[40]

Discussion

Crystal structures have been determined for three metal complexes of 5'-AMP or AMP analogues. Sletten performed an X-ray analysis of a Cu^{2+}–9-methyl-6-oxypurine complex.[41] The Cu^{2+} was found to be directly bonded to N(7) (bond length = 2.05 Å) and hydrogen bonded via a coordinated water to the carbonyl oxygen at C(6). The base stacking structure of the complex is shown in Figure 5A. Collins et al. determined a crystal structure for Ni^{2+}–AMP.[42] Again, direct metal–nucleotide binding was found at N(7) (bond length of 2.08 Å). Base stacking was found to be quite different (Figure 5B) than for the oxypurine. Also, intramolecular hydrogen bonds were indicated between two of the phosphate oxygen atoms and two coordinated water molecules. Finally, Sternglanz et al. reported a structure of Ba^{2+}–AMP.[43] The Ba^{2+} was completely hydrated and all ligand interactions were found to be outer sphere. They found that the most important binding sites were provided by the phosphate and N(7) of the base. Other outer-sphere interactions were found with N(1) and the C(3') hydroxyl group. The base stacking for the Ba^{2+} complex is similar to that shown in Figure 5A for Cu^{2+}–oxypurine.

There have been several structures proposed for base-stacked metal–adenosyl nucleotide complexes on the basis of NMR studies.

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Table I. Diamagnetic T_1s, Dipolar T_1^es and Calculated Distances for Mn^{2+}–AMP Stacked Complex

nucleus	T_1^{dia}, s	$T_1^e(C) \times 10^4$, s	r, Å
N(1)	4.4	0.30	2.1
C(2)	0.25	5.8	4.8
N(3)	2.8		$\gtrsim 5^a$
C(4)	3.0	28	6.2
C(5)	5.3	4.9	4.6
C(6)	2.1	7.3	4.9
NH_2	1.4	0.26	2.1
N(7)	3.2	0.20	2.0
C(8)	0.25	1.9	3.9
N(9)	7.0		$\gtrsim 5^a$
P	3.0	0.27	3.3

[a] Low limit estimate based on experimental T_1 error of ±10% and the observed lack of an effect of Mn^{2+} on T_1 at the concentrations studied.

Sternlicht et al. proposed a general structure for the Mn^{2+}–ATP stacked complex in which Mn^{2+} binds the phosphate of one nucleotide and the adenine ring of a second.[23] Eichhorn proposed a similar structure for a bis Cu^{2+}–AMP stacked complex.[44] The base stacking is illustrated in Figure 5C. More recently, several solution structures for metal–ATP stacked complexes have been proposed.[45,46] For simplicity, only the structures indicated for AMP complexes will be considered.

The slopes of the lines in Figures 1–3 were analyzed in terms of eq 6 to determined $T_1^e(C)$, or the dipolar relaxation contribution from complex formation. Fast exchange ($\tau_M < T_1^e(C)$) was assumed on the basis of rates of exchange found for Mn^{2+}–ATP complexes.[8,9,21] Since N(3) and N(9) relaxation was uninfluenced by the presence of Mn^{2+} (at 10^{-4} M), outer-sphere contributions to relaxation for the nitrogen resonances can be assumed to be minimal; therefore, no corrections were made. For the carbon resonances, the ribose carbons were judged to represent outer-sphere relaxation contributions at the somewhat higher levels of Mn^{2+}. Correction was made according to eq 6 for this estimate of $T_1^e(t)$. For phosphorus, the level of Mn^{2+} is similar to that used for the ^{15}N experiments; no correction for outer-sphere relaxation is made. The ratio of Mn^{2+} concentration to AMP concentration is used for f; initial calculations assume that $q = 1$.

Table I lists dipolar electron–nuclear relaxation times for the base nuclei and the phosphate. $T_1^e(C)$s are not listed for N(3) and N(9) since these are too large to be measured at the concentration of Mn^{2+} used. The values of $T_1^e(C)$ can be used with eq 3 to obtain the distances between each nucleus and the metal ion; a τ_c of 2×10^{-10} s was used for all nuclei (vide supra).

One possible source of error in the calculated distances of Table I arises from the zero-field splitting of the electronic energy levels of Mn^{2+}. Since Mn^{2+} has $S = {}^5/_2$, both longitudinal and transverse relaxation is characterized by three relaxation times with relative weights that depend on ω and τ, and all of which contribute to τ_c.[47] For the condition $\omega\tau > 1$, one relaxation time usually has a greater weight at most frequencies.[48] For Mn^{2+} bound to a protein, the ZFS can be considerable and the contributions to τ_c of short electronic relaxation times can be significant.[49] As described above, the EPR study of Reed et al. found both the ZFS and τ_v for the Mn^{2+}–ATP complex to be quite comparable to those of the aquo ion.[33] The degree to which short electronic relaxation times contribute to T_1^e for the Mn^{2+}–AMP complex will limit the accuracy of the distances presented in Table I. Fortunately, distance has a sixth-root dependence on τ_c (eq 3) and therefore even considerable error in τ_c results in little error for r.

There are several characteristics of the structure of the Mn^{2+}–AMP stacked complex evident for Table I. First, N(1) NH_2(6), and N(7) must all be *directly* bound to the Mn^{2+} ion. Second, N(3), N(9), and C(4) must all be remote from the metal binding site. Third, the phosphate must bind directly to the Mn^{2+} ion through one of the phosphate oxygens. Finally, the somewhat shorter value for C(8) relative to C(2) and C(6) indicates that its relaxation is probably influenced by a different metal ion, i.e., a second binding site.

A structure which is consistent with these considerations is that shown in Figure 5B. One metal binding site is comprised of equal binding to NH_2 and N(7) of one base and N(1) on the second adenine ring. This would provide equal Mn^{2+}–N distances and somewhat longer Mn^{2+}–C distances for proximal carbons C(6) and C(2). A second binding site at the phosphate could provide an additional relaxation pathway for C(8) not available for C(6) and C(2). This model indicates a 1:2 metal–nucleotide complex for the Mn^{2+} binding to the ring. The implication that nucleotide base stacking only involves pairs of nucleotides is not intended. This model merely represents the local structure about the Mn^{2+} which binds to the ring. The extent of base stacking or the geometry of stacking in the absence of the metal ion cannot be determined from our data. However, it is interesting to note that structure 5B is consistent with the observation that base stacking in the absence of metal ion has a greater chemical-shift effect on H(2) than on H(8).[50]

The metal–phosphorus distance of 3.3 Å reported in Table I compares favorably with the average metal–phosphorus distances reported by Sloan and Mildvan for the 1:1 Mn^{2+}–ATP complex[51] and is identical with the distance found for the $LiMnPO_4$ crystal.[52] In contrast, Sloan and Mildvan found essentially outer-sphere complexation with the phosphates for the ternary Mn^{2+}–ATP–pyrurate kinase complex with an average distance of ca. 5 Å.[51] The metal–C(8) distance of 3.9 Å and the metal–C(2) distance of 4.8 Å are consistent with a Mn^{2+}–H(8) distance of 3.5 Å and a Mn^{2+}–H(2) distance of 4.6 Å found for 0.35 M Mn^{2+}–ATP complexes.[13] The metal–N(7) bond length agrees well with the crystallographic bond lengths of 2.05 and 2.08 Å for the Cu^{2+}–oxypurine[41] and Ni^{2+}–AMP complexes[42] discussed above. A bond length of 2.11 Å was found for the metal–N(7) bond in the Ni^{2+} complex of GMP.[53]

There is some disagreement between the metal–carbon distances listed in Table I and the distances calculated by Kotowycz et al.[21] There are several possible reasons for this disagreement. First, Kotowycz et al.[21] make no attempt to correct their ^{13}C T_1^e data for translational diffusion relaxation effects (vide infra). Second, the Kotowycz et al.[21] study was of 0.3 M ATP at pD 7.0, while this study is of 0.8 M AMP at pH 10.0. Finally, the method of purification of the nucleotide samples was different. Granot et al. have recently shown the sensitivity of ^{31}P relaxation for nucleotides to purification techniques.[54]

In conclusion, it has been shown that *a multinuclear spin-labeling* approach to the determination of metal-bound structures in solution is a complex problem but is also an accurate way to probe these structures. The error in the use of differential line broadening to obtain structural information has been shown for ^{15}N here and elsewhere for other nuclides.[21,22] While no effort has been made to generate an exact structure for the base-stacked Mn^{2+}–AMP complex, a slight variation on the X-ray crystal structure of Ni^{2+}–AMP[42] provides a structure consistent with the data presented here. The most important feature of this study is that strong interaction is observed between the metal ion and

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N(1), NH_2(6), and N(7) of AMP adenine rings. The bond distances determined from the ^{15}N investigation clearly show that the metal-nucleotide ring interaction is an *inner-sphere* coordination effect. Evidence for two separate binding sites is presented and serves to explain an anomalously short Mn^{2+}-C(8) bond distance.

Extension of these multinuclear studies to ^{15}N-enriched nucleotides will allow investigation of 1:1 metal-nucleotide complexes at physiologically significant nucleotide concentrations, <0.01 M; such studies will be initiated in this laboratory.

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Role of Diffusion in Trigger Wave Propagation in the Belousov-Zhabotinskii Reaction

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Abstract: The role of diffusion is investigated in the ferroin-catalyzed Belousov-Zhabotinskii reaction. Trigger wave patterns are formed and the velocity of propagation of the front bands is correlated to the diffusion coefficients of the participating species. The diffusion coefficients are varied by adjusting the viscosity of the solutions. The propagation velocity is proportional to the reciprocal of the square root of the viscosity, hence it depends linearly on the square root of the diffusion coefficient. This behavior is in agreement with theoretical predictions based on the coupling between the kinetic model and diffusion.

Introduction

The Belousov-Zhabotinskii reaction is the only known chemical reaction exhibiting both temporal and spatial oscillations. Belousov[1] first reported temporal oscillations during the cerium ion catalyzed oxidation of citric acid by bromate ion. Zhabotinskii[2,3] demonstrated oscillations in similar systems where the cerium ion can be replaced by Mn^{2+} and $Fe(phen)_3^{2+}$ and the citric acid can be replaced by malonic and maleic acid derivatives.

Busse[4] observed the formation of horizontal bands in solutions subjected to concentration gradient. Zaikin and Zhabotinskii[5] observed oxidation bands propagating through the thin layer of the solution. Winfree[6] observed two types of spatial structures. Phase waves occur under concentration gradient and trigger waves which initiate in local centers and propagate owing to diffusion-reaction coupling.

The mechanism of the Belousov-Zhabotinskii reaction has been investigated extensively by Noyes, Koros, and Field.[7,8] The concentration of the intermediate bromide ion Br^- controls two mechanisms, and the bromous acid concentration switches rapidly between the two limits.

The mechanism of the spatial oscillations in the trigger waves cases has been explained by various authors. Field and Noyes[9,10] have shown that their mechanism for temporal oscillations coupled with diffusion can explain trigger waves. The waves move by destroying Br^- in front of the wave and leaving a high concentration of Br^- behind, which is the domain for the next wave to propagate.

The governing equations are given by[10]

$$\frac{\partial C_A}{\partial t} = D_A\left(\frac{\partial^2 C_A}{\partial x^2}\right) + k_3[H^+][BrO_3^-]C_B - k_2[H^+]C_AC_B + k_5[H^+][BrO_3^-]C_A - 2k_4C_A^2 \quad (1)$$

$$\frac{\partial C_B}{\partial t} = D_B\left(\frac{\partial^2 C_B}{\partial x^2}\right) - k_3[H^+][BrO_3]C_B - k_2[H^+]C_AC_B \quad (2)$$

where $C_A = [HBrO_2]$ and $C_B = [Br^-]$. Experimentally the bands move with a constant velocity, v

$$v = (\partial x/\partial t)_{C_A,C_B} \quad (3)$$

and

$$\partial C_A/\partial t = v(\partial C_A/\partial x) \quad (4)$$

Approximated solution is given for maximum sharpness[10]

$$v = \{4D_Ak_5[H^+][BrO_3^-]\}^{1/2} \quad (5)$$

From the rate constant $k_5 = 1 \times 10^4\ M^{-2}\ s^{-1}$ and the diffusion coefficient $D_A \sim 1.8 \times 10^{-5}\ cm^2/s$, the velocity is given by

$$v\ (mm/min) = 509[H^+]^{1/2}[BrO_2^-]^{1/2}$$

Experimentally the velocity was found to be

$$v\ (mm/min) = 24.75[H^+]^{1/2}[BrO_3^-]^{1/2}$$

obviously representing large numerical discrepancy.

Recently, Reusser and Field[11] numerically solved the partial differential equations describing the dynamics of interaction of reaction and diffusion of the trigger wave propagation. Their calculations reduce the quantitative discrepancy with the experimental velocities measured by Field and Noyes.[10] Reusser and Field[11] attribute the discrepancies to two approximations in the Oregonator model and the possibility of air presence in the thin layer experiments.

Dreitlein and Smoes[12] analyzed the velocity of trigger wave propagation. They claimed that all waves must propagate with at least the velocity

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