out by the g values, which indicate that the unpaired electrons are largely in $d_{x^2-y^2}$ orbitals. A weak δ bond is consistent with the g values, but may not be the most important[17] contribution to J in view of the substantially larger J in $[Cu_2(RCO)_4(NCS)_2]^{-2}$ for R = H than for R = CH_3, in spite of longer Cu–Cu separation for R = H.[30] Thus superexchange through the bridging ligands provides the largest contributions to J. Such contributions are not presently amenable even to semi-quantitative analysis, and in practice J is a purely experimental quantity. Lines[31] has pointed out that exchange striction in a dimer of $S = {}^1/_2$ spins leads, through the distance dependence of J, to a slight temperature dependence of J. Gregson, Martin, and Mitra[17] are satisfied with a constant J for $Cu_2Ac_4 \cdot 2H_2O$ for the 77–300°K susceptibility, but Jotham, Kettle, and Marks[32] favor an additional excited singlet state in many binuclear copper complexes to provide greater

flexibility in fitting susceptibility data. A slight temperature dependence in J would also provide such additional flexibility and wider range susceptibility measurements may thus provide additional evidence for exchange striction. Previous magnetic studies, in which J was necessarily a phenomenological parameter, were therefore quite insensitive to exchange striction in binuclear copper complexes.[33]

(30) D. M. L. Goodgame, N. J. Hill, D. F. Marsham, A. C. Skapski, M. L. Smart, and P. G. H. Troughton, *Chem. Commun.*, 629 (1969).

(31) M. E. Lines, *Solid State Commun.*, 11, 1615 (1972).

(32) R. W. Jotham, S. F. A. Kettle, and J. A. Marks, *J. Chem. Soc., Dalton Trans.*, 428 (1972).

(33) NOTE ADDED IN PROOF. Dr. B. Morosin (private communication) has suggested that a centered $C2/m$ cell with $Z = 2$ is preferable for Cu_2Ac_4pyr and has undertaken both a 125 and 300°K structure determination.

The Manganous–Adenosine Triphosphate System.
A Reinvestigation of Its Proton Magnetic Resonance Spectra[1]

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Abstract: A reinvestigation of the Mn^{2+}-induced broadening of the H_8 and H_2 pmr signals of adenosine triphosphate has been carried out. Contrary to the literature, Mn^{2+} does *not* cause equal broadening of the H_8 signals of ATP and AMP. Rather, for an equimolar ATP and AMP mixture, 0.25 M each, containing 5.0×10^{-5} M Mn^{2+} in D_2O at pD 8 and 27°, the ATP H_8 signal was broadened 3.90 times as much as the AMP signal. When the Mn^{2+} concentration was 1.0×10^{-4} M, as in the literature, the ratio of these broadenings was less certain, but was between 3.1 and 6.6. In conjunction with our studies of the dependence of line broadening on temperature, pD, and concentration, the broadening ratio of 3.90 in the former solution is consistent with an equilibrium between >25% $MnATP^{2-}$ and <75% $Mn(ATP)_2^{6-}$, corresponding to a value of <29 for $K_{eq} = (1:2)/(1:1)(ATP^{4-})$. The results imply that *both* adenine H_8 protons in the 1:2 complex are about equally distant from the Mn^{2+} nucleus and couple with the metal magnetically. When the pD was lowered from 6.4 to 5.4 a drastic increase occurred in the Mn^{2+} broadening of both the H_8 and the H_2 signal of 0.25 M ATP but not of 0.02 or 0.1 M solutions. Consideration of the relative broadening values of the four adenine proton signals of an equimolar ATP–AMP mixture at pD 5.4 and at pD 8 suggested that the fundamental cause for the drastic pD effect in the pD 5.4–6.4 range might be a transition in the triphosphate chelation, from $\alpha\beta\gamma$ to $\beta\gamma$ resulting from phosphate protonation as the pD is lowered, with an accompanying change in the ligand exchange mechanism.

$\mathbf{B}$ecause of the biochemical importance of metal ion–adenosine triphosphate complexes, their structures and stabilities have been extensively studied.[3] In 1968

Sternlicht, Jones, and Kustin[4] claimed to have cleared up apparent discrepancies between previous nuclear magnetic resonance results[5] and earlier ultraviolet spectroscopy[6] and temperature-jump studies.[7] These authors presented pmr evidence to support a suggestion of Brintzinger and Palmer[8] that the Mn(II)–ATP system

(1) (a) This paper is based on work performed partially under contract with the U. S. Atomic Energy Commission at the University of Rochester Atomic Energy Project and has been assigned Report No. UR-3490-308. Presented at the 166th National Meeting of the American Chemical Society, Chicago, Ill., Aug 1973. (b) Part of the Ph.D. Thesis of Victorio Wee, University of Rochester, Rochester, N. Y., 1973.

(2) (a) University of Rochester School of Medicine and Dentistry; (b) Eastman Kodak Research Laboratories.

(3) The literature up to 1966 was thoroughly reviewed by R. Phillips, *Chem. Rev.*, 66, 501 (1966). More recent papers are found among the following references.

(4) H. Sternlicht, D. E. Jones, and K. Kustin, *J. Amer. Chem. Soc.*, 90, 7110 (1968).

(5) H. Sternlicht, R. G. Shulman, and E. W. Anderson, *J. Chem. Phys.*, 43, 3133 (1965).

(6) P. W. Schneider, H. Brintzinger, and H. Erlenmeyer, *Helv. Chim. Acta*, 47, 992 (1964).

(7) G. G. Hammes and S. A. Levison, *Biochemistry*, 3, 1504 (1964).

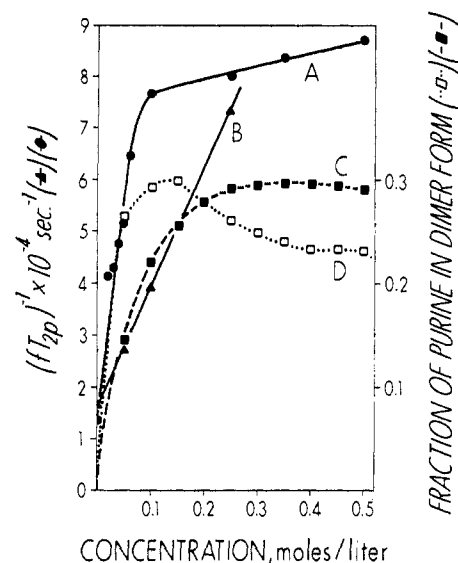(8) Cited as a personal communication in ref 4.

Figure 1. Comparison of the experimental normalized Mn^{2+}-induced broadening of ATP H_8 pmr signal, $(_fT_{2p})^{-1}_{exptl}$, at pD 8.4 and 27° with the *upper limit* calculated for the intrinsic normalized broadening of the 1:1 $Mn(ATP)^{2-}$ complex, $(fT_{2p})^{-1}_{1:1,max}$, and with fractions of aqueous purine and 6-methylpurine in dimer form: (A) $(fT_{2p})^{-1}_{exptl}$; (B) $(fT_{2p})^{-1}_{1:1,max}$ (calculated by eq 18); (C) and (D) fraction of purine and 6-methylpurine, respectively, in dimer form, data taken from Ts'o and Chan.[11] For curve A the factor f is 4×10^{-4}.

contains only 1:1 complexes when the ATP concentration is low (*ca.* 1 × 10^{-3} *M* or less) but that a 1:2 complex predominates when the ATP concentration is high (*ca.* 0.35 *M*). Sternlicht, *et al.*, further claimed to have shown that in this 1:2 complex the Mn^{2+} ion is bound simultaneously to the triphosphate chain of one ATP molecule and to the N_7 atom of the adenine ring of a second ATP molecule, which is juxtaposed, or "stacked" to the adenine ring of the first ATP molecule, with the latter adenine ring *not* being bound to the metal.

We have reinvestigated the effect of Mn^{2+} ions on the pmr spectra of ATP and ATP–AMP mixtures and have shown that the structure proposed by Sternlicht, *et al.*, for the 1:2 complex is highly unlikely. Rather, our data indicate that at pH 8 the two adenine H_8 protons in the 1:2 complex interact magnetically with Mn^{2+} to about the same extent, which, in view of the evidence of Glassman, *et al.*,[9] for a water bridge between Mn^{2+} and N_7 in the 1:1 complex, implies a 1:2 structure containing two water bridges, as shown in structure **1**.

O = OXYGEN
⊖ = NITROGEN
● = CARBON

1
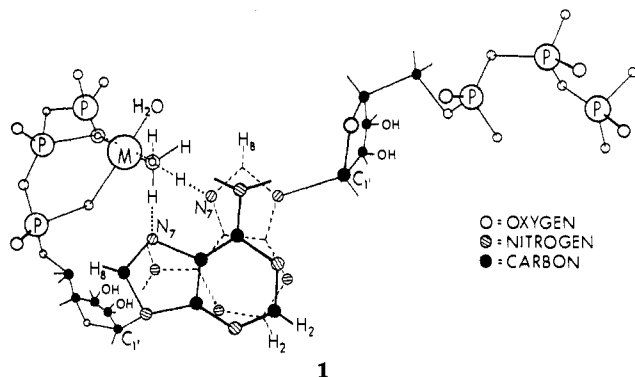
(9) T. A. Glassman, C. Cooper, L. W. Harrison, and T. J. Swift, *Biochemistry*, **10**, 843 (1971).

Experimental Section

For solutions containing 0.01 *M*, or more, ATP the pmr spectra were recorded with a JEOL 4H-100 (100 MHz) nmr spectrometer kept in a 24° constant-temperature room, using highest quality grade (Wilmad Glass Co.) nmr tubes. A JEOL JESVT2 temperature controller enabled us to vary the sample temperature, which was determined by measuring the chemical shift of a calibration compound (ethylene glycol for high temperatures and methanol for low temperatures) before and after each spectrum was taken. A JEOL JRA-1 spectrum accumulator was used to increase signal-to-noise ratio, whenever desired.

For low-concentration samples (0.001 *M* ATP) a Varian HA-100 nmr spectrometer modified for Fourier transform analysis was employed.

The solutions studied were prepared with nucleotides (Sigma Chemical Co.) which had been freed of paramagnetic metal impurities by treatment with Chelex 100 (Bio Rad) resin and then dried by lyophilization. Samples of this residue were weighed and diluted to the desired concentration with deionized water. pH's were adjusted with tetramethylammonium hydroxide or HCl for the high ATP solutions and with NaOH and HCl for the low ATP solutions. For the high ATP solutions tetramethylammonium chloride was added to provide sufficient internal reference for the nmr lock signal. For low ATP solutions *p*-dioxane was used as the internal reference. The desired amount of aqueous manganous chloride (Fisher ACS Analytical Grade) was then added, the pH was readjusted, and the resulting solution was lyophilized for about 24 hr. The residue was taken up in 99.8 % D_2O (Diaprep Inc.) and relyophilized. This process was repeated, and the residue was stored in a desiccator at $-5°$. Just before the spectrum was taken, the desiccated residue was dissolved in 100% D_2O (Diaprep Inc.) to a 1.0-ml final volume while under N_2 atmosphere. This solution was then transferred to a 5-mm nmr tube, which was tightly stoppered immediately thereafter. After each spectrum was taken, the pH of the sample was again measured, and the nucleotide concentration was determined from its absorbance measured at 260 nm with a Beckman DU spectrophotometer.

pD values reported in this paper were obtained by adding 0.4 to the pH measured with a Beckman Research pH meter, Model 1019.[10]

Results

Addition of a very small concentration of Mn^{2+} to an ATP solution broadens both the H_8 and the H_2 magnetic resonance signals to extents strongly dependent on both the ATP concentration and the pH without detectable change in the resonance frequencies. It is frequently convenient and customary to express this metal-induced broadening as the normalized broadening, $\pi W_P/f$, where W_P is the difference between the line widths (*i.e.*, width at half-height) in hertz of a given proton signal in presence and in absence of Mn^{2+} and f is the ratio of the total Mn^{2+} concentration, $[Mn]_t$, to total ATP concentration, $[ATP]_t$. The normalized broadening is related to the Mn^{2+}-induced change in the proton relaxation rate, T_{2p}^{-1}, by the expression

$$\pi W_P/f = (T_2^{-1} - T_{2a}^{-1})/f = (fT_{2p})^{-1} \quad (1)$$

where T_2 and T_{2a} are the transverse relaxation times in presence and in absence of metal.

The concentration dependence of the normalized broadening of the H_8 signal when pD = 8.4 and f = 4 × 10^{-4} is demonstrated by curve A in Figure 1. The H_2 broadening at this pD is insignificant. After an initial very steep increase (*ca.* fivefold) up to the 0.1 *M* ATP point, curve A shows a drastic decrease in slope, which appears to be leading toward either a plateau or a very broad maximum. It is significant that a somewhat similar type of curve (Figure 1, curve C) describes the extent of dimerization of purine in aqueous solution and also that the dimerization curve of 6-methylpurine

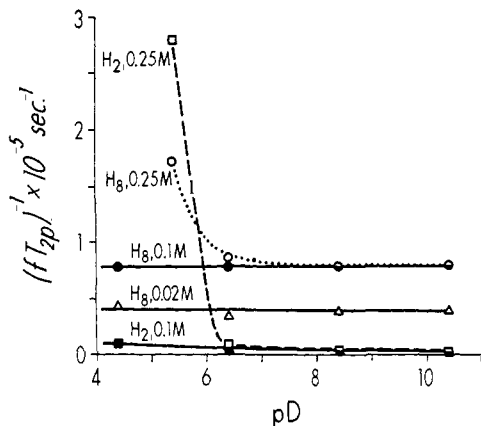(10) P. K. Glasoe and F. A. Long, *J. Phys. Chem.*, **64**, 188 (1960).

Figure 2. pD dependence of $(fT_{2p})^{-1}_{exptl}$ of ATP H_8 and H_2 pmr signals at various concentrations, temperature 27°. Identities of proton and ATP concentration corresponding to each curve are shown in the figure.

(curve D) shows a relatively sharp maximum at an early stage, i.e., near 0.15 M.[11] The maxima in these dimerization curves are due to the formation of higher order polymers (i.e., $(ATP)_n$ with $n > 2$), which increases with concentration and occurs to a greater extent for 6-methylpurine than for purine.

The pD dependence of the normalized broadening is shown in Figure 2 for both the H_8 and the H_2 signals at three ATP concentrations, 0.02, 0.1, and 0.25 M, with varying f values. For 0.02 M ATP and 0.1 M ATP the Mn^{2+} ion affects the H_8 signal primarily, this broadening being relatively constant in magnitude over the pD range 4.4–10.4. The H_2 broadening is quite small for these two solutions. For 0.25 M ATP both the H_8 and the H_2 broadening undergo drastic change between pD 5.4 and 6.4. In fact, at pD 5.4 the H_2 signal is broadened even more than the H_8 signal, $(fT_{2p})^{-1}$ being 28×10^4 and 17.5×10^4 sec^{-1}, respectively. Raising the pD from 5.4 to 6.4 decreases the H_2 broadening precipitously to a relatively insignificant value, <1 sec^{-1}, while the H_8 broadening decreases twofold, almost to the 0.1 M ATP value.

In Figure 3 are presented the results of an "equimolar ATP–AMP competition study" performed at pD 8. Figure 3A shows the H_8 signals of ATP and AMP in the pmr spectrum of an equimolar mixture of the two nucleotides (0.25 M in each). The difference between the chemical shifts of the two signals at this pD is 5.5 Hz. Figures 3B and 3C show the H_8 spectra obtained when 5×10^{-5} M Mn^{2+} and 1×10^{-4} M Mn^{2+}, respectively, were present in this equimolar nucleotide mixture. The outer solid-line curves in Figure 3 are the observed spectra. The H_2 signals were not broadened significantly. Curve analysis was carried out by computer using the assumption that the component signals, i.e., ATP and AMP, are Lorentzian and additive. Very close agreement between the observed spectra and the sums of the resolved components (indicated by the arrows in Figure 3) is evident from the locations of the dashed segments, which represent the sums of the resolved components where the sums deviate from the observed spectra.

It is obvious from Figure 3 that the ATP and AMP

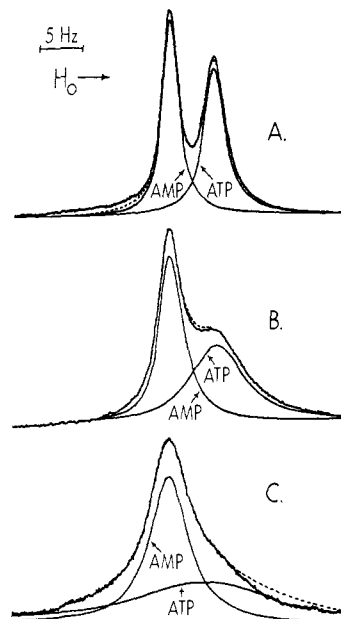(11) P. O. P. Ts'o and S. I. Chan, J. Amer. Chem. Soc., **86**, 4176 (1964).

Figure 3. Equimolar ATP–AMP competition study of Mn^{2+}-induced broadening of the H_8 signal at pD 8 and 27°. Each solution was 0.25 M in both ATP and AMP: (A) no Mn^{2+} present; (b) 5.0×10^{-5} M present; (C) 1.0×10^{-4} M Mn^{2+} present. Outer curves are observed spectra. Resolved ATP and AMP components indicated in the figure. Dashed segments indicate deviation of the sum of the components from the observed spectrum.

H_8 signals were not broadened by Mn^{2+} to an equal extent at pD 8, even approximately, as had been reported previously by Sternlicht, et al.,[4] for pD 7.6. The line widths in absence of Mn^{2+} (Figure 3A) were 3.22 ± 0.12 and 2.38 ± 0.12 Hz, respectively. In Figure 3B the line widths are 8.05 ± 0.25 and 3.62 ± 0.12 Hz, respectively. The broadening of the ATP and AMP signals at pD 8 when $[ATP]_t = [AMP]_t = 0.25$ M and $[Mn]_t = 5 \times 10^{-5}$ M was, then, 4.83 and 1.24 Hz, respectively, i.e., in the ratio 3.90:1.

Because of the large overlap of the much broader component signals at higher $[Mn]_t$, i.e., 1×10^{-4} M, there is only one peak in the observed spectrum shown in Figure 3C. Even without curve analysis, i.e., visually, it is evident that this curve is skewed to the right. The results of curve analysis of this spectrum are much less accurate than those in Figure 3B. The line widths of the ATP and AMP component curves shown in Figure 3C are 22.5 and 6.0 Hz, respectively, corresponding to a value of 5.3 for the ratio of the ATP and AMP line broadenings. Although there is considerable uncertainty in this latter ratio, the actual value lying between 3.1 and 6.6, it is definitely at least as great as three.

Our Figure 3C may be compared with the one which was presented by Sternlicht, et al.,[4] (i.e., Figure 1(b) of ref 4) as evidence for equal Mn^{2+}-induced broadening of the ATP and AMP H_8 signals. The experimental conditions under which these two spectra were obtained were the same except for a small difference in the pD. Sternlicht, et al., employed pD 7.6; we employed pD 8.0. Despite the poor quality of their spectrum, it can be seen that their H_8 signal is skewed to the right at least as much as, if not more than, our own in Figure 3C. Furthermore, it seems obvious that their conclusion of equal broadening was made without benefit of curve analysis.
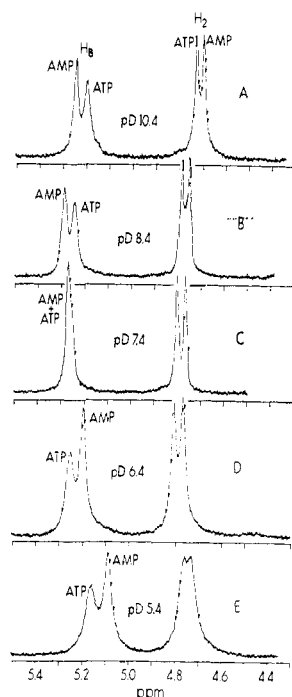
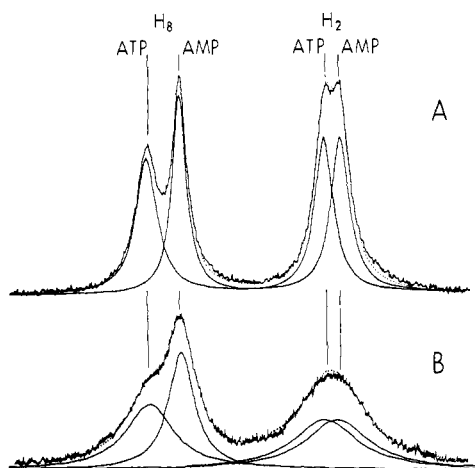Figure 4. Effect of pD on chemical shifts of ATP and AMP H_8 and H_2 pmr signals of equimolar mixture at 27°.

Figure 5. Equimolar ATP–AMP competition study of Mn^{2+}-induced broadening of H_8 and H_2 signals at pD 5.4 and 27°; each solution was 0.25 M in both ATP and AMP: (A) no Mn^{2+} present; (B) 5 × 10^{-5} M Mn^{2+} present. Outer curves are observed spectra. Resolved ATP and AMP components indicated in the figure. Dotted segments indicate deviation of the sum of the components from the observed spectrum.

We employed pD 8 rather than pD 7.6 in order to optimize the resolution of the ATP and AMP H_8 signals. Nonmonotonic changes in the chemical shifts of both of these signals, especially the latter, first to low field then to high field, as pD is decreased make their spacing quite pD-dependent and, in fact, produce a reversal in their order near pD 7.4, where the two signals have approximately the same chemical shift. Hence, at pD 8 the spacing, 5.5 Hz, is almost twice as high as the 3 Hz value at pD 7.6, with a consequently much better resolution of the two signals at pD 8. These pD effects can be seen in Figure 4. At each pD, distinction between the H_8 and H_2 signals was accomplished by noting
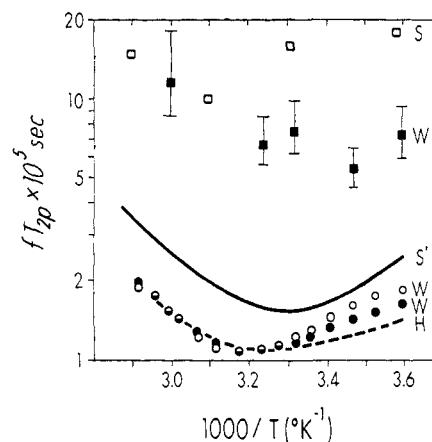

Figure 6. Temperature dependence of $(fT_{2p})_{exptl}$ of ATP H_8 signal at pD 8.4 at various concentrations. S and S' data taken from Sternlicht, et al., references 4 and 5, respectively. Curve H taken from Heller, et al.[12] W refers to data of Wee, et al., in this paper: □ and ■, 0.001 M ATP; O and —, 0.35 M ATP; ● and - - -, 0.50 M ATP.

the change in the relative areas of the signals when the ratio of the ATP and AMP concentrations was changed from 1:1 to 2:3.

Figure 5 shows the results of an equimolar competition study at pD 5.4. It is seen that at this pD the H_2 signals of ATP and AMP, as well as the H_8 signals, were broadened. In absence of Mn^{2+} (Figure 5A) the line widths were 5.85 and 3.95 Hz for the ATP and AMP H_8 signals, respectively, and 5.05 Hz for each H_2 signal. In presence of Mn^{2+} (Figure 5B) the line widths were 12.75 and 7.00 Hz for the ATP and AMP H_8 signals, respectively, and 17.00 Hz for each H_2 signal. Thus, in this study the broadenings of the four signals—ATP H_8, AMP H_8, ATP H_2, and AMP H_2—were 6.90, 3.05, 11.95, and 11.95 Hz, respectively, i.e., in the ratio 2.26 : 1 : 3.92 : 3.92.

The temperature-dependence of the normalized Mn^{2+}-induced broadening of the ATP H_8 signal at pD 8.4 is shown in Figure 6 for the three ATP concentrations which have been studied previously by others; namely, 0.001 M ATP with f = 4.0 × 10^{-4}, 0.35 M with f = 2.8 × 10^{-4}, and 0.5 M ATP with f = 2.8 × 10^{-4}. Also presented in Figure 6 are the results reported by previous authors for approximately the same pD. There is excellent agreement between our 0.35 M, our 0.50 M results, and the 0.5 M results of Heller, et al.,[12] over the range 27–70°, but the three curves diverge with decreasing temperature below 27°. These results indicate that the line broadening below 27° has both a ligand-concentration dependence and a frequency dependence. There is considerable discrepancy between our results and those of Sternlicht and his colleagues, at both 0.001 and 0.35 M ATP.

Theoretical Considerations

Swift and Connick[13] have derived equations to describe the broadening effect of a paramagnetic metal ion on the line width of the magnetic resonance signal of a proton in its vicinity in a solution in which the proton can exchange between three different environ-

(12) M. J. Heller, A. J. Jones, and A. T. Tu, Biochemistry, 9, 4981 (1970).
(13) T. J. Swift and R. E. Connick, J. Chem. Phys., 37, 307 (1962).

ments, a, b, and c, with the concentration conditions [a] $\gg$ [b] or [c]. For the special case where the resonance frequency of the proton is not detectably shifted and (i) when only a $\rightleftarrows$ b and a $\rightleftarrows$ c exchanges can occur, they obtained

$$\frac{1}{T_{2p}} = \sum_{j=b}^{c} \frac{\tau_{aj}^{-1}\tau_{ja}}{T_{2j} + \tau_{ja}} \tag{2}$$

where, as defined in eq 1, $T_{2p} = (T_2^{-1} - T_{2a}^{-1})^{-1}$, τ_{aj} and τ_{ja} are the lifetimes of a $\rightarrow$ j and j $\rightarrow$ a exchanges, respectively, and T_{2j} is the transverse relaxation time of the proton while it is in the j environment, and (ii) when only a $\rightleftarrows$ b and b $\rightleftarrows$ c exchanges can occur, they obtained

$$\frac{1}{T_{2p}} = \frac{1}{\tau_{ab}}\left[\frac{\dfrac{1}{T_{2b}} + \dfrac{1}{\tau_{bc}(1 + T_{2c}\tau_{cb}^{-1})}}{\dfrac{1}{T_{2b}} + \dfrac{1}{\tau_{bc}(1 + T_{2c}\tau_{cb}^{-1})} + \dfrac{1}{\tau_{ba}}}\right] \tag{3}$$

For a two-component case, a $\rightleftarrows$ b, with [a] $\gg$ [b], these equations reduce to

$$\frac{1}{T_{2p}} = \frac{\tau_{ab}^{-1}\tau_{ba}}{T_{2b} + \tau_{ba}} = \frac{[b]/[a]}{T_{2b} + \tau_{ba}} = \frac{fq}{T_{2M} + \tau_{M}} \tag{4}$$

in which the symbols T_{2M} and τ_M are substituted for T_{2b} and τ_{ba} to emphasize the fact that T_{2M} refers to the relaxing effect of the metal ion, M, on the excited proton during the time, τ_M, when the metal and proton are in close proximity, and q is the number of protons of the given type magnetically coupled to one metal ion. If the proton were part of a particular ligand coordinated to M, τ_M would be the lifetime of the complex and q would be the number of these ligands in one complex. However, as Glassman, et al.,[9] have pointed out, the metal-induced line broadening does not require actual complexation of the metal by a proton-containing ligand but only requires that the metal and proton be sufficiently close together for magnetic coupling. In fact, these authors have shown that Mn^{2+}-induced broadening of the H_8 signal in the pmr spectrum of a Mn(II)-ATP solution is not the result of direct attachment of Mn^{2+} to the adenine ring but that it is due to indirect binding via an intervening H_2O bridge, i.e., the N_7 is hydrogen bonded to the proton of a water molecule which is coordinated to Mn^{2+}.

For a system containing only the 1:1 complex, ML, and a large excess of free ligand, L, with magnetic coupling between M and a proton, H_L, in the ligand, eq 4 would reduce to

$$\frac{1}{fT_{2p}} = \frac{1}{T_{2M} + \tau_M} \tag{5}$$

i.e., negligible concentration of free metal ion is assumed.

However, if there were also to exist simultaneously a second 1:1 complex, ML', differing from ML in that H_L and M are too far apart to couple, eq 4 would reduce to

$$\frac{1}{fT_{2p}} = \frac{1 - x}{T_{2M} + \tau_M} \tag{6}$$

where x represents the fraction of the complex concentration in the ML' form. In this case the ML' complex

is considered as part of the bulk nucleotide concentration insofar as τ_M is concerned.

The polymerization tendency of adenine nucleotides must be taken into account in the present study. If only 1:1 complexes were present the ratio [b]/[a] in eq 4 would be equal to $(1 - x)[M]_t/[A]$, where $[M]_t$ is the total metal concentration and [A] is the equilibrium concentration of ATP in the monomer form. Substituting this expression into eq 4 leads to

$$\frac{1}{fT_{2p}} = \frac{(1 - x)[A]_t/[A]}{T_{2M} + \tau_M} \tag{7}$$

for a metal-ATP mixture having all of the metal in a 1:1 complex.

A much more complicated expression than eq 7 would prevail, however, if 1:2 complexes were to predominate in the Mn(II)-ATP system with high ATP concentration. The following cases would then have to be considered.

Case I. The MA_PA_R Case. The metal is bound to the phosphate moiety of one ATP molecule, A_P, and, either directly or indirectly, to the adenine ring of a second ATP molecule, A_R, which is stacked to the adenine group of the A_P molecule. This is the case which Sternlicht, et al.,[4] claimed to have demonstrated. For such a case one can assume negligible broadening of the H_8 signal of A_P. Dissociation of A_R would be the two-component case, i.e., a $\rightleftarrows$ b, for which eq 4 holds. However, the A_R exchange might be either (i) $MA_PA_R \rightleftarrows MA_P + A$ or (ii) $MA_PA_R \rightleftarrows M + A_2$.

Case I(i) would be governed by an equation differing from eq 7 only in that the quantity x is replaced by x', which is the fraction of the 1:2 complexes in which the two adenine groups are stacked without either being close enough to the metal to experience observable magnetic effect.

For case I(ii), eq 4 leads to

$$\frac{1}{fT_{2p}} = \frac{(1 - x')[A]_t/[A_2]}{T_{2M} + \tau_M} \tag{8}$$

For either case I(i) or I(ii) one would expect equal broadening of the ATP and AMP H_8 signals in an equimolar ATP-AMP competition study, since ATP and AMP seem to dimerize to about the same extent.[4,14] That is, ATP and AMP should function equally well as the stacked A_R ligand in the MA_PA_R complex. True, the A_P ligand should be exclusively ATP, because of the much greater affinity of the triphosphate group for M^{2+} compared to that of the monophosphate group,[15] but in case I A_P is assumed not to couple significantly with the metal ion.

Case II. Simultaneous Exchange Case. $MA_iA_o \rightleftarrows M + A_2$. In this case, illustrated by structure **1**, both adenine groups are considered to be bound to the metal ion, presumably via water bridges as in the 1:1 complex, and they are assumed to exchange simultaneously as a stacked dimer. The two adenine groups in this 1:2 complex will be differentiated by the symbols i and o, which refer, respectively, to the *inner* adenine group, the one in the nucleotide whose phosphate moiety is attached to the metal, and the *outer* adenine group, the one which is stacked to the inner nucleotide and has its phosphate moiety free.

(14) I. Feldman and R. P. Agarwal, J. Amer. Chem. Soc., 90, 7329 (1968).

(15) M. M. Khan and A. E. Martell, J. Phys. Chem., 66, 10 (1962).

The broadening effect of the metal ion on the two H_8 signals of structure 1 should be additive, i.e., $(T_{2p})^{-1}_{M(ATP)2}$ $= (T_{2p})_i^{-1} + (T_{2p})_o^{-1}$. Molecular models (CPK) show that there should be no steric hindrance to linearity of either of the two N_7–H hydrogen bonds, so that the two H_8 protons should be about the same distance from M. Hence,[16] one may assume $T_{2M,i} \approx T_{2M,o} = T_{2M}$. For simultaneous dissociation of the two adenine groups from the vicinity of M, $\tau_{M,i} = \tau_{M,o} = \tau_M$. Hence, $T_{2p,i} \approx T_{2p,o}$, for this case.

Since ATP and AMP dimerize to about the same extent, an equimolar mixture should contain three dimer species—$(ATP)_2$, $(AMP)_2$, and $(ATP–AMP)$—in approximately equal concentration, i.e., $[(ATP)_2] \approx [(ATP–AMP)] \approx [A_2]/3$. The total concentration of 1:2 complexes in which two H_8 atoms are close to the metal, $[MA_2]$, should be comprised almost equally of the two species $M(ATP)_i(ATP)_o$ and $M(ATP)_i(AMP)_o$, since the monophosphate group cannot compete with the triphosphate group for M.

The broadening of the ATP H_8 signal in an equimolar competition study should, therefore, be given by the following equation, if only 1:2 complexes are present and if the two adenine groups bind to Mn^{2+} (albeit via H_2O bridges) and dissociate from it simultaneously, i.e., as a dimer.

$$\left(\frac{1}{1-x'}\right)\left(\frac{1}{T_{2p}}\right)_{ATP} = \frac{[M(ATP)_2]/[(ATP)_2]}{T_{2M,i} + \tau_{M,i}} +$$
$$\frac{[M(ATP_2)]/[(ATP_2)]}{T_{2M,o} + \tau_{M,o}} +$$
$$\frac{[M(ATP)_i(AMP)_o]/[(ATP–AMP)]}{T_{2M,i} + \tau_{M,i}} \quad (9)$$

$$= \frac{3[M(ATP)_2]/[(ATP)_2]}{T_{2M} + \tau_M} =$$

$$\frac{3 \times {}^1\!/_2[MA_2]/{}^1\!/_3[A_2]}{T_{2M} + \tau_M} = \frac{4.5[MA_2]/[A_2]}{T_{2M} + \tau_M} \quad (10)$$

The broadening of the AMP signal in this mixture should be given by

$$\left(\frac{1}{1-x'}\right)\left(\frac{1}{T_{2p}}\right)_{AMP} =$$
$$\frac{[M(ATP)_i(AMP)_o]/[(ATP–AMP)]}{T_{2M,o} + \tau_{M,o}} = \frac{1.5[MA_2]/[A_2]}{T_{2M} + \tau_M} \quad (11)$$

The ratio of the ATP to AMP line broadening in the equimolar competition study for case II should, then, be 3:1.

If no AMP were present eq 9 would reduce to

$$\left(\frac{1}{1-x'}\right)\left(\frac{1}{T_{2p}}\right)_{ATP} = \frac{2[M(ATP)_2]/[(ATP)_2]}{T_{2M} + \tau_M} \quad (12)$$

from which

$$(fT_{2p})^{-1} = \frac{2(1-x')[A]_t/[A_2]}{T_{2M} + \tau_M} \quad (13)$$

It should be noted that eq 13 for case II differs from eq 8 for case I(ii) by the factor two in the numerator of the former.

Case III. Stepwise Exchange. (i) A_o Dissociates

(16) Since for this system it is dipolar determined,[5] T_{2M} is proportional to $r^6\tau_c^{-1}$, where r is the distance between metal and proton and τ_c is the rotational correlation time; $\tau_c \propto$ viscosity.

First. $MA_iA_o \rightleftarrows MA_i + A \rightleftarrows M + 2A$. As in case II, case III(i) assumes structure 1 for the 1:2 complex. If A_o dissociates before A_i, the contribution of the broadening of the H_8 signal of A_o to the total broadening may be considered to be mathematically similar to the simple two-component case, I(i), discussed above with eq 7 applying, since A_o is exchanging with bulk monomeric nucleotide. Thus, for an equimolar competition study

$$\left(\frac{1}{T_{2p}}\right)_{o,AMP} = \left(\frac{1}{T_{2p}}\right)_{o,ATP} =$$
$$\frac{(1-x')[M(ATP)_2]/[ATP]}{T_{2M,o} + \tau_{M,o}} \quad (14)$$

The contribution of the broadening of the H_8 signal of A_i in this case would be governed by the three-component exchange described by eq 3, in which the symbols a, b, and c would represent A, MA_i, and MA_iA_o, respectively. The data cannot be obtained to solve eq 3, because the different rotational correlation times of the MA_i and MA_iA_o complexes produce different $T_{M,i}$ values for these two environments and, of the four τ values, only τ_{ab} and τ_{ba} can even be estimated.[7] However, one can arrive at a qualitative conclusion.

One can assume that before dissociation of A_o, i.e., during the lifetime of the MA_iA_o complex, $T_{2M,i} \approx T_{2M,o}$. However, since T_{2M}^{-1} increases with the particle radius and since the radius of MA_iA_o is greater than that of MA_i, then $T_{2M,i}$ in MA_i (i.e., after dissociation of A_o) $> T_{2M,o}$ in MA_iA_o. Also, by definition $\tau_{M,i} > \tau_{M,o}$. Consequently, the contribution of $(T_{2p})_i^{-1}$ to the total H_8 broadening should be smaller than the contribution of $(T_{2p})_o^{-1}$. Hence, since

$$(T_{2p})_{ATP}^{-1} = 2(T_{2p})_i^{-1} + (T_{2p})_o^{-1} \quad (15)$$

then

$$(T_{2p})_{ATP}^{-1} < 3(T_{2p})_o^{-1} \quad (16)$$

Combining eq 14 and 16 we arrive at the relation

$$(T_{2p})_{ATP}^{-1}/(T_{2p})_{AMP}^{-1} < 3 \quad (17)$$

for an equimolar competition study governed by case III(i).

(ii) A_i Dissociates First. $MA_iA_o \rightleftarrows MA_pA_R \rightleftarrows M + A_2$. This case defies mathematical analysis, even a qualitative one. Both the A_o exchange and the A_i exchange are three-component cases governed by eq 2. Unlike case III(i) in which both T_{2M} and τ_M are smaller for A_o than for A_i, in case III(ii) $T_{2M,o} < T_{2M,i}$, since M would be much further from A_i than from A_o in the intermediate species, while by definition $\tau_{M,o} > \tau_{M,i}$. One cannot predict whether the T_{2M} ratio or the τ_M ratio would have the greater influence on the H_8 broadening in this case without knowing their actual magnitudes.

However, we believe that simple logic rules out this case. The weakest points in structure 1 are the hydrogen bonds from the N_7 atoms to the water bridges and the inter-adenine stacking. Since no steric strain is indicated by space-filling atomic models of this structure, simultaneous exchange of the two adenine groups, i.e., case II, would be expected if breaking of hydrogen bonds is the determining factor. On the other hand, destacking would favor prior dissociation of A_o, since it would remove the beneficial chelate effect from the

A_o binding, while leaving A_i binding relatively unaffected.

Discussion

The steep slope at the beginning of curve A of Figure 1 cannot be attributed to an increasing concentration of the 1:1 complex, $MnATP^{2-}$, since the stability constant, 6×10^4, reported[15] for this complex indicates almost complete complexation of the metal at the first point of the curve, i.e., in 0.001 M ATP. Nor is it likely to be due to an increasing rate of ligand exchange between 1:1 complex and bulk nucleotide, since the rate limiting step of the exchange should be a dissociation step.[17]

In addition, one can apply eq 7 to conclude that the 35% increase in viscosity plus the increase in the $[A]_t/[A]$ ratio between 0.001 and 0.1 M ATP also *cannot* cause the fivefold increase in the experimental normalized broadening, $(fT_{2p})^{-1}_{exptl}$, over this concentration range. Ts'o and Chan[11] have reported values of 2.1 and 6.7 M^{-1} for the dimerization constants, K_D, of aqueous purine and 6-methylpurine, respectively. By comparison of the concentration dependences of the chemical shifts, Sternlicht, *et al.*, concluded[5] that in its polymerization tendency ATP behaves intermediate to these two purines, but closer to purine. However, in a later paper[4] they employed a value of 5 M^{-1} for the K_D of ATP at room temperature, i.e., closer to the 6-methylpurine value.

If one were to ignore any polymerization above the dimer stage for ATP, as Sternlicht, *et al.*, have done, the calculated values of the ratio $[A]_t/[A]$ in 0.1 M ATP at room temperature corresponding to the limiting K_D values, 2 and 6.7 M^{-1}, would be 1.31, and 1.76, respectively. Values of 1.39 and 2.13, respectively, would be calculated if higher polymerization were not ignored, i.e., if the $[A]_t/[A]$ ratio were obtained directly from the experimental data of Ts'o and Chan. An *upper* limit for the increase in $(fT_{2p})^{-1}$ between 0.001 and 0.1 M ATP to be expected *if only 1:1 complexes prevailed throughout*, i.e., in $(fT_{2p})^{-1}_{1:1}$, should be obtainable by employing the highest of the $[A]_t/[A]$ ratios given above, i.e., 2.13, and using minimum τ_M, i.e., zero, in absence of knowledge of an acceptable value.

Since T_{2M} is proportional to the viscosity,[16] η, eq 7 leads to

$$(fT_{2p})^{-1}_{1:1,c,max} \approx$$

$$(fT_{2p})^{-1}_{1:1,0.001M} \frac{([A]_t/[A])_c}{([A]_t/[A])_{0.001M}} \frac{\eta_c}{\eta_{0.001M}} \quad (18)$$

as the upper limit to be expected for $(fT_{2p})^{-1}_{1:1}$ at a nucleotide concentration, c.

Thus, since both $[A]_t/[A]$ and η are close to unity when $[ATP]_t = 0.001$ M, one calculates 1.64×10^4 sec^{-1} $\times 2.13 \times 1.35 = 4.7 \times 10^4$ sec^{-1} for $(fT_{2p})^{-1}_{1:1,0.1M,max}$. This value is 38% below the experimental value, 7.6×10^4 sec^{-1}. In fact, a twofold difference is calculated when polymerization of ATP above the dimer stage is ignored, i.e., if the 1.76 value is used for $[A]_t/[A]$ in 0.1 M ATP, instead of the 2.13 value. As shown below, there is both logic and evidence to support the latter procedure.

(17) F. Basolo and R. G. Pearson, "Mechanisms of Inorganic Reactions," 2nd ed, Wiley, New York, N. Y., 1967. Chapter 3.

Further, the observed normalized line broadening $(fT_{2p})^{-1}_{exptl}$ increases only 14% between 0.1 and 0.5 M ATP, even though *at least* a 50% change could be expected for $(fT_{2p})^{-1}_{1:1}$ due to the polymerization and viscosity factors. In this estimate we have employed $K_D = 2$ with higher polymers being ignored and have assumed $T_{2M,1:1} \ll \tau_{M,1:1}$ in order to minimize the expected change in broadening.

On the other hand, curve A of Figure 1 is consistent with an equilibrium between 1:1 and 1:2 complexes, with the latter having a stacked pair of adenine groups, as, for example, in structure 1, and an intrinsic $(fT_{2p})^{-1}$ i.e., $(fT_{2p})^{-1}_{1:2}$, considerably higher than $(fT_{2p})^{-1}_{1:1}$. Such an equilibrium is suggested by the fact that the dimerization curves of purine and 6-methylpurine (curves C and D of Figure 1) and curve A all show the initial steep slope and a sharp decrease in slope near the 0.1 M point. The possibility does exist that there may also be some complex with a third adenine group stacked in such a way that its H_8 and H_2 protons are too far from the metal to couple. Since such 1:3 complexes cannot be experimentally distinguished from 1:2 complexes of the structure 1 type they will be considered as part of the 1:2 complex concentration in our calculations. Since the fraction of complexes in the 1:3 form should approximate the fraction of $[A]_t$ in the trimer form, there is little doubt that the error thus introduced is very small in the context of our calculations, for there is probably a negligible amount of trimeric ATP. Support for this view may be obtained from the smallness of the slope of curve A in the 0.2–0.5 M range, from which it is obvious that the numerator $[A]_t/[A_2]$ in eq 13 must be a decreasing function of $[ATP]_t$, at least up to 0.5 M ATP.

The original data of Ts'o and Chan does not show such a decreasing function above 0.2 M for either purine or 6-methylpurine because of the increasing amounts of higher polymers. However, one does calculate such a function using either $K_D = 2$ or 6.7 *if higher polymers are ignored*, and it is noteworthy that it also changes slope drastically near the 0.1 M point. This is not an unexpected development, since interphosphate repulsion should decrease the formation of higher polymers.

Curve B of Figure 1 shows the concentration dependence of $(fT_{2p})^{-1}_{1:1,c,max}$ obtained from eq 18 when a K_D of 6.7 is employed, higher polymers than dimers are ignored, and $T_{2M,1:1} \gg \tau_{M,1:1}$ is assumed. The use of space-filling (CPK) atomic models shows clearly that all three members of a trimeric adenine stack cannot bind simultaneously to one Mn^{2+} ion, even *via* H_2O bridges. Also, the possibility of direct binding to the metal of a third unstacked adenine group is ruled out by the inability of Mn^{2+} to bind to adenosine.[12]

For a mixture of 1:1 and 1:2 complexes, between which there is no ligand exchange

$$(T_{2p})^{-1}_{exptl} = (T_{2p})_t^{-1} = (T_{2p})^{-1}_{1:1} + (T_{2p})^{-1}_{1:2} \quad (19)$$

By definition

$$(T_{2p})^{-1}_{1:1} = y_1 f(fT_{2p})^{-1}_{1:1}$$

$$(T_{2p})^{-1}_{1:2} = y_2 f(fT_{2p})^{-1}_{1:2} \quad (20)$$

where y_1 and y_2 are the fractions of $[Mn]_t$ in 1:1 and 1:2 complexes, respectively.

Hence, curve A of Figure 1 should obey the expression

$$(fT_{2p})^{-1}{}_{exptl} = y_1(fT_{2p})^{-1}{}_{1:1} + y_2(fT_{2p})^{-1}{}_{1:2} \quad (21)$$

Since curve A lies above curve B in Figure 1 it is obvious that $(fT_{2p})^{-1}{}_{1:2} > (fT_{2p})^{-1}{}_{1:1}$, so that the *lower* limit for $(fT_{2p})^{-1}{}_{1:2}$ at any ATP concentration is the $(fT_{2p})^{-1}{}_{exptl}$ at that concentration. Thus, curves A and B may be considered to represent the *lower* limits of $(fT_{2p})^{-1}{}_{1:2}$ and the *upper* limits of $(fT_{2p})^{-1}{}_{1:1}$, respectively.

The value, 3.90, obtained from Figure 3B for the ratio, R, of the Mn^{2+}-induced broadenings of the ATP and AMP signals in the pD 8 equimolar competition study is significantly higher than any of the R ratios calculated above, in the Theoretical Considerations section, when only 1:2 complexes were considered, namely, unity for case I, three for case II, and <3 for case III(i).

Two possible reasons for the high experimental R value are apparent. One, the *inner* nucleotide in the 1:2 complex may be significantly closer than the *outer* nucleotide to the metal, so that $T_{2M,i} < T_{2M,o}$. This disparity in distances would not affect the R value predicted for case I, since only the outer nucleotide H_8 signal is considered to be broadened in this case. Of course, one might suggest that $T_{M,o}$ for the ATP < $T_{M,o}$ for AMP because of a possible difference in the two $Mn-H_8$ distances. The probability of this latter inequality seems very small, however, since there is no steric bar to a linear hydrogen bond between N_7 and the H_2O bridge, and therefore the geometry of the 1:2 complex should be independent of the identity of the outer nucleotide. The possibility that $T_{2M,i} < T_{2M,o}$ for structure 1, *i.e.*, in case II, does indeed merit consideration, because of the r^6 dependence of T_{2M}. For instance, one can calculate from eq 9 and 11 that an R of 3.90, instead of 3.0, would be predicted for case II if r_i and r_o differed by only 6.4% and if $T_{2M} \gg \tau_M$. A 17% difference in the two distances would lead to an R of 3.90 if $T_{2M} \approx \tau_M$. A second possible cause for a high R value could be the presence of back-bound (albeit *via* H_2O bridges) 1:1 complexes coexisting with back-bound 1:2 complexes, because the former would be almost exclusively $Mn(ATP)^{2-}$.

It can be shown that case I type 1:2 complexes are extremely unlikely. The broadening of the ATP H_8 signal in the competition study, Figure 3B, may be considered to obey eq 19. For case I type 1:2 complexes, $(T_{2p})^{-1}{}_{ATP,1:2} = (T_{2p})^{-1}{}_{AMP,1:2}$. Making this substitution into eq 19 and then inserting the experimental values 15.17 and 3.89 sec^{-1} for $(T_{2p})^{-1}{}_{ATP,t}$ and $(T_{2p})^{-1}{}_{AMP,1:2}$, respectively, gives 11.28 sec^{-1} for $(T_{2p})^{-1}{}_{ATP,1:1}$ in the competition study. Consequently, for this case $(T_{2p})^{-1}{}_{ATP,1:2}/(T_{2p})^{-1}{}_{ATP,1:1}$ would be 0.345. Combining this relation with the two relations designated (20) yields

$$\frac{(T_{2p})^{-1}{}_{ATP,1:2}}{(T_{2p})^{-1}{}_{ATP,1:1}} = \frac{y_2(fT_{2p})^{-1}{}_{1:2}}{y_1(fT_{2p})^{-1}{}_{1:1}} = 0.345 \quad (22)$$

Inserting the *upper* limit, 0.92, for $(fT_{2p})^{-1}{}_{1:1}/(fT_{2p})^{-1}{}_{1:2}$, *i.e.*, the quotient of the ordinates of curves B and A of Figure 1 at 0.25 M ATP, gives a value of <0.32 for y_2/y_1. Since $y_1 + y_2 \approx 1$, this means that less than 24% of the Mn^{2+} would be in the 1:2 complex at 27° if

it were case I type. This would imply[18] that almost all of the Mn^{2+} would be in the 1:1 complex form at 72°, where almost all uncomplexed ATP is monomeric,[4] since

$$\frac{[M(ATP)_2]}{[M(ATP)]} = \frac{K_2[(ATP)_2]}{K_1[ATP]} \quad (23)$$

K_2 and K_1 being the stability constants of the 1:2 and 1:1 complexes, respectively.

It can be seen, however, in Figure 6 that (fT_{2p}) at 72° is 7.5 times smaller when $[ATP]_t = 0.35-0.50 \ M$ than at very low ATP, 0.001 M. The ratio of the viscosities of the high ATP and low ATP solutions is 2.4 at 72°. T_{2M} for the two solutions should differ by this factor. In addition, $[A]_t/[A]$ is close to unity at 72° in either solution.[4] Because of the chelate effect, one may assume that the factor x in eq 7 is small enough to be neglected. Hence, even if τ_M were ignored, the two (fT_{2p}) values to be expected at 72° when only 1:1 complexes are present at this temperature, *i.e.*, if the 1:2 complexes at room temperature are case I type, would differ by only a factor of ~ 2.4, *i.e.*, *only one-third of the observed difference*.

In addition, the work of Heller, *et al.*,[12] implies that a significant concentration of 1:2 complex exists even at 70°. These authors observed a significant amount of broadening of the AMP H_8 signal in a solution containing 0.1 M ATP, 0.4 M AMP, and 4 × 10^{-5} M Mn^{2+} at pD 7.8 and 70°. Since ATP binds Mn^{2+} much more strongly than does AMP, and since the $[ATP]_t/[Mn]_t$ ratio of 2500 in this case should be sufficient to bind virtually all of the Mn^{2+} as a 1:1 complex, the broadening of the AMP signal implies that some AMP is present as the outer nucleotide of a 1:2 complex even at 70°. We prefer this interpretation of the import of the AMP broadening to that of Heller, *et al.* They assumed that there was a competitive effect of ATP which slowed the exchange of Mn^{2+} through the solution, thereby causing the AMP broadening in their competition study to be smaller than in 0.5 M AMP, *i.e.*, in absence of ATP. In our opinion, this assumption is invalid if only 1:1 complexation occurs, as they also seem to have assumed, because the very large difference in the Mn^{2+}-binding abilities of ATP and AMP would ensure virtually 100% binding of the Mn^{2+} by ATP with no binding by AMP.

No contradiction occurs when the 1:2 complexes are treated as either case II or case III(i) type. For case II eq 10 and 11 give $(T_{2p})^{-1}{}_{ATP,1:2} = 3(T_{2p})^{-1}{}_{AMP,1:2}$. Making this substitution into eq 19 and, as before, inserting the $(T_{2p})^{-1}{}_{ATP,t}$ and $(T_{2p})^{-1}{}_{AMP,1:2}$ values from the competition study now gives (11.67/3.50), or 3.33, for the ratio $(T_{2p})^{-1}{}_{ATP,1:2}/(T_{2p})^{-1}{}_{ATP,1:1}$. In this case eq 22 yields <3.1 for y_2/y_1 at 27°, which corresponds to 75% as the *upper* limit for the percentage of Mn^{2+} in the 1:2 complex form in this competition study. A tenfold decrease[18] in the $[(ATP)_2]/[ATP]$ ratio produced by

(18) Consider, for instance, the following calculation. An uncertainty of 15-20% in the finding of Sternlicht, *et al.*,[4] that [ATP] ≈ [ATP]$_t$ at 72° when [ATP]$_t$ is 0.35 M would lead to a maximum value of *ca.* 0.1 for the [(ATP)$_2$]/[ATP] ratio in eq 23 for this solution. Employing a K_D of 6.7 and ignoring higher polymers, one would estimate about a tenfold higher value for this ratio at 27°. If one assumes that K_2/K_1 does not change radically between 27 and 72°, one would then estimate from eq 23 that y_2/y_1 is about ten times smaller at 72° than at 27° in 0.35 M ATP, *i.e.*, at 72° y_2/y_1 < 0.032, indicating that at least 97% of the Mn^{2+} should be in the 1:1 complex form.

raising the temperature to 72° would then give a value of <0.31 for y_2/y_1 at 72°. This ratio corresponds to a substantial value, 24%, as the upper limit for the percentage of Mn^{2+} in the 1:2 complex form at 72°.

If the 1:2 complexes were treated as the case III(i) type, a lower value would be calculated as this upper limit, since the $(T_{2p})^{-1}_{ATP,1:2}/(T_{2p})^{-1}_{ATP,1:1}$ ratio would then be less than for case II, i.e., <3.33, by an unknown amount.

Clearly, neither case II nor case III(i) for the 1:2 complex necessarily implies that only 1:1 complexes exist at 72°, so that the previous contradiction does not necessarily follow. It does, however, mean that $T_{2M,1:2}$ and $\tau_{M,1:2}$ cannot be extracted from the high ATP temperature-dependence curve in Figure 6, as Sternlicht, et al.,[5] did, by assuming that this curve becomes linear in the wings. In fact, both our 0.35 M ATP curve and our 0.50 M ATP curve in Figure 6 show curvature at both high and low temperature. Further, in obtaining $T_{2M,1:2}$ and $\tau_{M,1:2}$ in their paper III,[4] these authors made use of their earlier conclusion in paper II[5] that the residence times of the phosphate, τ_P, and the adenine group, τ_H, were equal at all temperatures when $[ATP]_t = 0.35$ M, despite the fact that they had concluded in paper III that they had erred previously in paper II in assuming that 1:1 complexes dominated between 0.02 and 0.5 M ATP. Their own data[4] also show that the $[A_2]/[A]$ ratio, which, by eq 23, governs the $[MA_2]/[MA]$ ratio, decreases rapidly above 45°, becoming unity at ∼80°.

Because of the large uncertainty in the $(fT_{2p})^{-1}_{exptl}$ values of the low ATP solutions, seen in Figure 6, we do not believe that $T_{2M,1:1}$ values can be extracted from these data. Sternlicht, et al., did so in their paper III even though their indicated experimental uncertainty was at least as great as ours. However, we believe that their procedure is no longer tenable. First, we have shown above that case I, in which only one adenine is connected to the metal, does not apply to the 1:2 complex, so that one cannot assume, as these authors did, that $\tau_{M,1:1}$ and $\tau_{M,1:2}$ should have approximately the same activation energy. Secondly, their statement that in 0.001 M ATP T_{2p} and τ_M are approximately equal at room temperature is contradicted by their own data. Thirdly, we do not consider the experimentally determined room temperature value of 1.7×10^{-3} sec for $\tau_{M,1:1}$ of Sternlicht, et al., to be in "excellent" agreement with Hammes and Levison's temperature-jump value,[7] <10^{-4} sec, as the former authors assert. In addition, in a later paper Hammes and Miller[19] reported a concentration- and pH-dependent value of ∼10^{-4} sec for the Mn(II)–ATP system, but they found two relaxation times for the Ni(II)–ATP and Co(II)–ATP systems, a concentration-dependent one, ∼10^{-1}–10^{-2} sec, and a concentration-independent one, ∼10^{-4}–10^{-5} sec. Comparing these systems led them to the conclusion that for the Mn(II)–ATP system the relaxation time for intramolecular ring binding which should be concentration independent, is too short to measure by the temperature-jump method and is, therefore, <10^{-5} sec.

The inability to extract T_{2M} and τ_M for the Mn(II)–ATP system from the pmr data prevents an accurate determination of either K_2 or K_2/K_1. However, a max-

imum value of 29 M^{-1} can be estimated for $K' = [MA_2]/[MA][A]$ at 27° by utilizing the upper limit, 3.1, calculated above for y_2/y_1 in the competition study along with the value of [A], 0.104 M, calculated for 0.25 M ATP from $K_D = 6.7$ when higher polymers are ignored. A higher value for [A] and, therefore, a lower value for K' would be obtained if $K_D < 6.7$ were employed. Since $K_2/K_1 = K'/K_D$, these results give an upper limit for K_2/K_1 of 4.3, which indicates that $-\Delta F°$ for the formation of the 1:2 complex is not even 874 cal greater than for the 1:1 complex. Such a small energy difference is consistent with chelation through the weak hydrogen bonding provided by water bridges, as depicted in structure 1.

To recapitulate, the results of our reinvestigation of the Mn^{2+}-induced line broadening of the ATP and AMP pmr signals when these two nucleotides are in equal concentration (0.25 M each) at pD 8 and 27° indicate that (a) each adenine group of the 1:2 complex is ligated to the metal, probably via an intervening water molecule hydrogen bonded to the N_7 atom, and that (b) either (i) such a mixture contains a significant concentration of both 1:1 and 1:2 complexes, with the Mn^{2+}–H_8 distance being approximately the same for both adenine groups, or (ii) 1:2 complexation is complete but H_8 of the inner nucleotide is closer to the metal than is the H_8 atom of the outer nucleotide. The probability that hydrogen bonds are linear between the N_7 atoms and the oxygen of the "trapped" waters favors alternative i. The nonlinearity of the temperature-dependence curve, fT_{2p} vs. $T(°K)^{-1}$, at the high- and low-temperature ends and the concentration dependence at the low-temperature end at high $[ATP]_t$ also argues for alternative i.

The very large changes seen in Figure 2 in the $(fT_{2p})^{-1}$ values for both ATP signals between pD 5.4 and 6.4 when $[ATP]_t = 0.25$ M must be due to drastic alteration in the structures of the complexes and/or in their ligand exchange mechanism. The fact that these changes were not noticeable when $[ATP]_t = 0.1$ M or less suggests that they are closely related to the formation of higher nucleotide polymers, since curve D of Figure 1 also shows a very abrupt change near 0.1 M ATP. This increase in polymerization at pD 5.4 could be due to the decreased repulsion of the phosphate groups of neighboring molecules resulting from increased protonation when the pD is decreased from 6.4 to 5.4.

The large $(fT_{2p})^{-1}$ value for the H_2 signal, 28×10^4 sec^{-1}, as well as for the H_8 signal, 17.3×10^4 sec^{-1}, when pD 5.4 and $[ATP]_t = 0.25$ M is somewhat similar to the results obtained for the Cu(II)–ATP system.[20] Since Cu^{2+} binds to the β and γ phosphate groups of ATP,[21,22] this resemblance hints that β,γ bidentate chelation by the phosphate chain might occur to some extent in the Mn(II)–ATP system at pD 5.4 when $[ATP]_t = 0.25$ M even though chelation is primarily tridentate at higher pD. A change from α,β,γ chelation to β,γ chelation with decreasing pD might be brought about by steric strains and statistical factors connected with higher polymerization.

Use of CPK molecular models shows that, as far as

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steric factors are concerned, in a β,γ chelate containing D_2O-mediated metal-to-N binding either N_1 or N_7 can function with ease either as the ligand atom in a 1:1 complex or as the ligand atom of the inner nucleotide in a 1:2 complex, and any one of the three adenine nitrogens can be the ligand atom of the outer nucleotide in the 1:2 complex. Because of the differences in the bond angles in the two adenine rings of an adenine nucleotide, the H_2 atom would probably be significantly closer to the metal if N_1 were the ligand atom than H_8 would be if N_7 were the ligand atom. Thus, on the average, T_{2M}^{-1} and, consequently, $(fT_{2p})^{-1}$ might be expected to be larger for H_2 than for H_8, as we have found at pD 5.4. However, the competition study at pD 5.4 points to τ_M, rather than T_{2M}, as the factor responsible for the large H_2 broadening.

The four line-broadenings measured in the equimolar competition study at pD 5.4 (Figure 5) are 6.90, 3.05, 11.95, and 11.95 Hz for the ATP H_8, AMP H_8, ATP H_2, and AMP H_2 signals, respectively. Their relative values are 2.3:1.0:3.9:3.9. Since AMP cannot function as the inner nucleotide of a 1:2 complex when an equal concentration of ATP is present, and since the two H_2 signals appear to be equally broadened, we conclude that AMP and the ATP N_1 (and N_3) function primarily as outer ligands while ATP N_7 functions both as inner and outer ligand. As explained earlier, one would expect the Mn^{2+}–H_8 distance to be approximately the same whether the N_7 is inner or outer ligand and whether the H_8 belongs to ATP or AMP. Consequently, one would estimate that the broadening of the inner ATP H_8 signal is only 3.85 Hz (i.e., 6.90–3.05 Hz) in this experiment. One cannot account for such a small value, considering the magnitude of the sum of the broadenings of the other three signals, by invoking the dependence of T_{2M} on r. On the other hand, this large difference between inner and outer broadenings could result if the outer ligand exchanged much faster than the inner ligand, i.e., according to case III(i) with $\tau_{M,o} < \tau_{M,i}$. This suggests that the fundamental cause for the drastic changes in the line broadenings seen in Figure 2 between pD 5.4 and 6.4 when $[ATP]_t = 0.25$ M might possibly be a change in the ligand exchange mechanism from case III(i) to case II accompanying the structural changes as the pD increases above pD 5.4.

The Sense of Cleavage of Substituted Benzenes on Reaction with Solvated Electrons, as Determined by a Product Criterion[1]
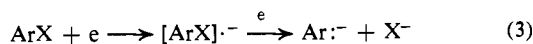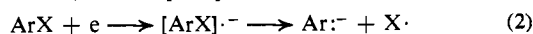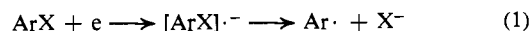
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Abstract: Many compounds of type C_6H_5X, in which the first atom of substituent X represents diverse elements, are cleaved by potassium metal in ammonia. It is possible to tell whether the immediate fragment from cleavage is phenyl radical or phenyl anion by conducting cleavage in the presence of acetone enolate ion. Phenyl radical is trapped by the enolate ion, as recognized by the formation of phenylacetone, 1,1-diphenyl-2-propanone, and/or 1-phenyl-2-propanol, often in high total yield. Phenyl anion does not arylate the enolate ion, and merely takes a proton from the solvent to appear as benzene. The sense of scission of compounds of the type PhY, Ph₂Y, or Ph₃Y, where Y is an element of group VII, VI, or V, correlates with the electronegativities of the elements. Phenyl radical is formed when Y is F, Cl, Br, I, O, or S, and phenyl anion when Y is P, As, Sb, or Bi, and both competitively when Y is Se. Ph_2I^+, $PhNMe_3^+$, $(PhO)_3PO$, and $PhOPO(OEt)_2$ also cleave to phenyl radical, while Ph_2SO, Ph_2SO_2, $PhSO_2Me$, and $PhSO_3^-$ are cleaved to phenyl anion. Cleavage of Ph_3S^+ is complex. Some compounds are unaffected, or cleave in other ways.

M any substituents are cleaved from benzene rings when electrons are supplied, as solvated electrons, from electrodes, or from other donors. There has been interest in the mechanism of cleavage, especially as to whether one or two electrons are required and as to what are the immediate products of bond rupture. Opinions have differed, and some uncertainty still prevails.[3-6]

Insofar as the aryl fragment of cleavage is concerned, the two principal possibilities are the aryl radical and the aryl anion. The former may arise by reaction 1, and the latter by either reaction 2 or reaction 3. The

$$ArX + e \longrightarrow [ArX]\cdot^- \longrightarrow Ar\cdot + X^- \qquad (1)$$

$$ArX + e \longrightarrow [ArX]\cdot^- \longrightarrow Ar\cdot^- + X\cdot \qquad (2)$$

$$ArX + e \longrightarrow [ArX]\cdot^- \overset{e}{\longrightarrow} Ar\cdot^- + X^- \qquad (3)$$

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