Based on X-α scattered wave calculations[11] and the eclipsed structure of $Re_2Cl_4(PEt_3)_4$,[12] the ground-state electronic configuration is thought to be $(\sigma)^2(\pi)^4(\delta)^2(\delta^*)^2$. Reduction of NO^+ to NO requires the addition of an electron to one of the empty π^*_{NO} orbitals. However, if this electron transfer were effected by direct end-on binding of NO^+ to one of the empty, axial positions of the $Re_2X_4(PR_3)_4$ dimer, then a typical HOMO–LUMO electron transfer does not appear possible because the net overlap between the δ^*_{metal} and π^*_{NO} orbitals must, by symmetry, be zero. Therefore, the mechanism of the electron transfer warrants further investigation. It may be that transfer occurs from one of the filled π_{metal} orbitals followed by "relaxation" of an electron from the filled δ^*_{metal} orbital to the π_{metal} orbital.

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Oxygen-17 Nuclear Magnetic Resonance Studies on Bound Water in Manganese(II)–Adenosine Diphosphate

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Abstract: Oxygen-17 line broadening and shift measurements have been made on aqueous solutions containing manganese-ADP complexes. Mono and bis complexes are considered. Log K_2 ($K_2 = [ML_2]/([ML][L])$) is ca. 2.6 at 25 °C and ΔH_2 is ca. -1 kcal/mol. The mono complex appears to have four bound waters which are kinetically equivalent. The bis complex appears to have three or four bound waters suggesting that it is not the usual sort of bis complex but a stacked one. The kinetics of water exchange are not greatly different for $Mn(H_2O)_6^{2+}$, $Mn(ATP)(H_2O)_3^{2-}$, $Mn(ADP)(H_2O)_4^{-}$, and $Mn(ADP)_2(H_2O)_3^{4-}$. Implications of the results are discussed in regard to mechanisms, NMR and EPR studies, and structures. The work supports the validity of using precise measurements of small shifts to determine numbers of bound water molecules.

Many enzymic trans-phosphorylation reactions involving ATP (adenosine triphosphate) and ADP (adenosine diphosphate) require divalent metal ions as cofactors. Consequently, the interactions of such metal ions with nucleotides, particularly ATP, have been the subject of numerous studies, most of which have been discussed in the reviews by Phillips[1] and Izatt et al.[2]

Metal–adenosine diphosphate complexes have received considerably less attention than similar ATP complexes. In this paper we report on Mn(II)–ADP, a complex which is impor-

tant as a leaving group in many in vitro enzyme studies. This complex has been studied by Cohn and Hughes,[3] who found that both the α and β phosphate groups were bound to the manganese. They reported also that manganese interacts with the adenine ring, although the authors recognized that their large $[ADP]_{total}-[Mn]_{total}$ ratio could produce complexes other than $MnADP^-$ which might be responsible for the observed interaction. (In this connection Frey and Stuehr[4] have shown that the stability constant for the $Ni(ADP)_2^{4-}$ complex is of the order of 10^2 M^{-1}.)

Table I. Composition of Solutions at 25 °C

Solution No.	Total Mn $\times 10^3$ M	Total ADP $\times 10^3$ M	pH	$[Mn^{2+}]$ $\times 10^5$ M	$[MnADP^-]$ $\times 10^5$ M	$[Mn(ADP)_2^{4-}]$ $\times 10^5$ M	Ionic strength
1	1.948	2.240	8.16	9.13	171	14.8	0.009
2	2.148	4.771	7.90	1.53	133	80.2	0.023
3	2.102	7.131	7.95	0.657	94.2	115	0.036
4	2.180	10.16	7.96	0.379	73.7	144	0.052
5	1.572	0.857	7.82	(73.0)	(83.9)	(0.353)	0.005
6	4.030	4.740	8.41	15.8	341	46.5	0.018
7	6.033	19.30	8.06	0.966	183	419	0.099
8	3.096		3	309.6			

Oxygen-17 NMR has been used extensively in this laboratory to study paramagnetic ions and their complexes.[5] By using this technique and under favorable conditions, it should be possible to obtain an estimate of the number of waters bound, and therefore the number of ADP binding sites, in the solvated $MnADP^-$ complex and to determine whether or not these waters are kinetically equivalent.[6] Both of these determinations will be relevant to the nature of the Mn-adenine ring interaction. Further, by examining the effect that varying Mn_{total}–ADP_{total} ratios have on the observed ^{17}O line broadening, it may be possible to obtain information on a bis complex, $Mn(ADP)_2^{4-}$, if its formation constant is sufficiently high.

Oxygen-17 NMR also has proven to be an excellent method for determining the rate constant for water exchange (k_{ex}) between waters bound to a paramagnetic complex or ion and bulk water. A comparison of k_{ex} for the $MnADP^-$ complex with the rate constant for the substitution of the 8-hydroxyquinoline anion (Ox^-) into the same complex to form $OxMnADP^{2-}$ should provide adequate information to determine whether water exchange or some other process is rate determining in ternary complex formation. Such comparisons have proved particularly useful in the case of the $MnATP^{2-}$ complex.[7]

Experimental Section

Reagents. ^{17}O water (7 and 18 atom %, normal H content) was obtained from YEDA R & D Co., Ltd., Rehovoth, Israel. It was redistilled in vacuo before each use. Manganese chloride ($MnCl_2 \cdot 4H_2O$; Mallinckrodt AR grade), zinc chloride ($ZnCl_2$; Baker AR grade), and magnesium nitrate ($Mg(NO_3)_2 \cdot 6H_2O$); Mallinckrodt AR grade) were used without further purification. Solutions of these salts, in normal water, were standardized by EDTA titration. ADP was obtained as the lithium salt (Li_3ADP) from Schwarz Bioresearch Inc. Analysis of the ADP by paper chromatography in an isobutyric acid-ammonia-water (66:1:3) mixture gave ~2% AMP content and a negligible ATP content. The inorganic phosphate content, as determined by the method of Rockstein and Herron,[8] was approximately 2%. Solutions of Li_3ADP in normal water were standardized spectrophotometrically at 259 nm and by acid-base titration. These solutions were kept frozen when not in use.

Preparation of $^{17}OH_2$ Solutions. Aliquots of the standard manganese and ADP solutions were mixed in a 10-mm diameter glass tube and normal water was added to give a total volume of ~1.0 mL. The pH of the solution was adjusted to ca. 8 by adding either concentrated NaOH or HCl. The water was evaporated in vacuo at room temperature, 1.0 mL of 18% enriched $^{17}OH_2$ was added to the remaining solid, and the pH was measured on a Beckman Research pH meter equipped with a microcombination electrode. (In some cases the pH was measured on a "dummy" solution prepared in an identical manner to the ^{17}O solution, but with normal water). The sample tube was sealed under ~400 mm of pressure of "prepurified" nitrogen. The compositions of all the solutions used are given in Table I.

Blank solutions for the shift and line broadening measurements contained zinc (solutions 1-4) or magnesium (solution 5) in place of manganese. Otherwise they were identical in composition and pH (to within ±0.1 pH units) to the corresponding sample solution.

NMR Line-Broadening Measurements. Line-broadening mea-

surements were made on solutions 1-5 with the NMR spectrometer in the configuration described previously.[9,10] Low-frequency (15 Hz) square-wave modulation and a locked frequency of 11.5 or 5.75 MHz were used. At these frequencies the ^{17}O resonance signal was observed at ca. 20 or 10 kG, respectively. The spectra were recorded on a X-Y recorder and the full width at half-height was measured by conventional "pen-ruler" techniques. Two upfield and two downfield sweeps were recorded at each temperature. The average standard deviation in line width was 3.6 and 4.3% respectively for the high- and low-field data. The line width measurement was reversible with respect to temperature; this served as a check of the temperature stability of each solution. When hydrolysis of the ADP occurred, it was accompanied by a decrease in line width.

Chemical Shift Measurements. The measurement of useful ^{17}O shifts for manganese(II) systems at temperatures less than 100 °C is difficult since the line broadening to shift ratio is generally very high. However, changes in our NMR instrumentation (made after the line widths were measured) have allowed us to make reasonably precise shift measurements on the Mn(II)-ADP system. Briefly, the magnetic field has been locked by using as an "error" signal the proton resonance of mineral oil, and it is stable to ca. 0.1 ppm. Resonances were obtained by linear frequency sweeping. Frequency reference marks are generated at intervals of 10% of the frequency span. When required, signal averaging was used to increase the signal-to-noise ratio. The starting and finishing frequency of a scan are known precisely, and the frequency of the resonance line can be measured to better than 0.1% of the frequency scan width by using a Lorentzian curve synthesizer. The experimental Lorentzian line, which is stored in a Fabritek signal averager, is displayed on a 20 in. oscilloscope simultaneously with a continuously adjustable synthetic Lorentzian curve produced by an analog device. The height, width, and position of the synthetic curve are adjusted until the "best" visual fit to the experimental curve is obtained. The position (frequency of resonance) and line width can then be read from the previously calibrated synthesizer controls. The advantages of similar comparative measuring techniques, as applied to exponential curves, have been discussed previously.[11] One added advantage of the present system, though, is that the intermediate storing of the experimental trace on the Fabritek allows measurement of the spectra as soon as they are recorded. Such analog systems offer an inexpensive, but still rapid, alternative to on-line digital computer methods. Precision of fitting is better than ±1% on low-noise curves; the trained eye is an extremely good averager.

Paramagnetic shifts for the Mn-ADP system were measured on solutions 6 and 7 at a frequency of 11.5 MHz. The resonance frequency of bulk water in the presence (ν_{obsd}) and absence (ν_0; the zinc or magnesium blank solution) of paramagnetic ions was measured by the method outlined above. Measurements were made at 60 °C, where the shift was almost at its limiting fast exchange value, and at −4.8 °C, where the shift was near zero (slow exchange region). These temperatures were chosen to be as far away from the temperature of maximum broadening as experimentally possible, the high-temperature limit being set by solution stability considerations.

Also, paramagnetic shifts were measured for the aquo manganese(II) ion, solution 8. The concentration of Mn^{2+} and the temperatures at which the shifts were measured were chosen so that the shift and line broadening would be as close as possible to that produced by the Mn-ADP system. In this way a comparison of the measured Mn^{2+} shift with that measured in the same system (see Figure 1) at very high temperatures (160-180 °C) provided a measure of the reliability of the Mn-ADP system shift data. (The shift for Mn^{2+} can be measured
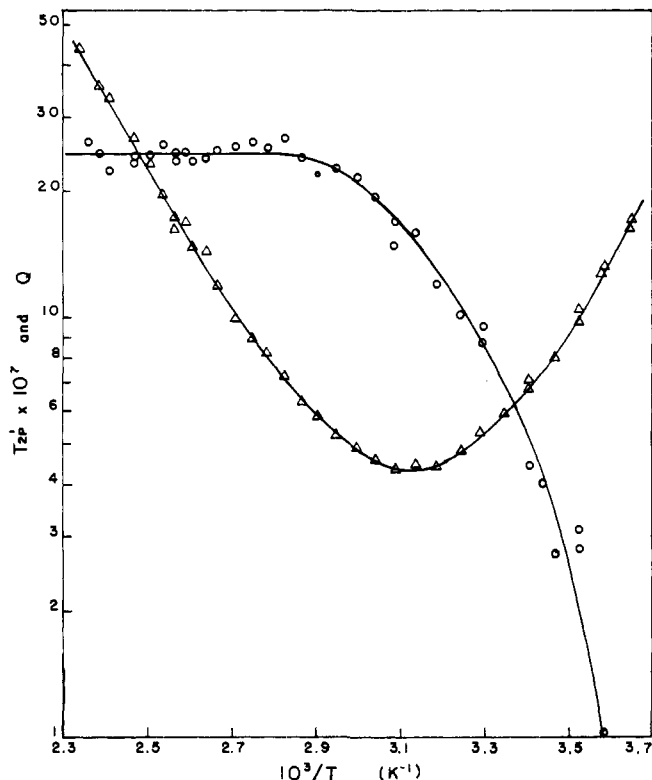
Figure 1. Line broadening and shift data for $Mn(H_2O)_6^{2+}$.

much more accurately at these high temperatures since the line broadening is decreased much more rapidly than the limiting shift as the temperature is increased). The data in Figure 1 were obtained over a wide range of pH and concentrations and using different experimental techniques. They will be discussed in detail in a separate paper.

Results and Data Treatment

Line Broadening. Equilibrium Considerations. The untreated experimental line broadening data of 11.5 and 5.75 MHz are shown in Figure 2. Figure 3 shows the same high-frequency data, but here they are smoothed and plotted as the line broadening produced per mole of total manganese, B_{mol}, vs. temperature. The decrease in B_{mol} as the $[ADP]_{total}-[M]_{total}$ ratio increases is real and indicates that at least two broadening species are present in solution. It can be shown qualitatively that the data cannot be accounted for by assuming that these species are Mn^{2+} and $MnADP^-$, as follows: At low temperatures, B_{mol} is greater than that if all the manganese were present as $Mn(H_2O)_6^{2+}$ on a per mole basis. As the $[ADP]_{total}-[Mn]_{total}$ ratio increases, the $[MnADP^-]-[Mn^{2+}]$ ratio should increase, and so the line broadening should also increase. In fact, B_{mol} decreases and a simple $Mn^{2+}/MnADP^-$ model, therefore, can be discounted. Computer fitting treatments confirm that this model cannot account for the data at low temperatures. However, at high temperatures this model is workable, but the stability constant for the $MnADP^-$ complex would have to be considerably smaller ($\log K = 3.5$) than any of the published values[1,2] (ca. 5) in order to fit the data. If, as expected from reported stability constants, the mono complex is essentially completely formed in solution 1, how can one explain the decrease in B_{mol} with increasing ADP–Mn ratios? The factors which control the line broadening (see later discussion) are T_{1e}, n (number of bound waters), and τ_m (mean life for water exchange). At low temperatures, the line broadening is mainly controlled by n/τ_m. If n decreases, a bis complex is implicated. If τ_m increases with no bis complex formed, one would have to say that excess ligand slows the water exchange rate by some medium effect contrary to any

effects of which we are aware for water exchange; nor is a saturation effect expected.

At the maximum in the B_{mol} curves, T_{1e} makes its largest relative contribution and B_{mol} decreases with decreasing magnetic field at every ADP^{3-} concentration. T_{1e} can be described by the equation[12]

$$\frac{1}{T_{1e}} = \frac{32}{25} \Delta^2 \left[\frac{\tau}{1 + \omega_s^2 \tau^2} + \frac{4\tau}{1 + 4\omega_s^2 \tau^2} \right]$$

where Δ is a parameter measuring the amplitude of modulation of the zero-field splitting, τ is the correlation time for the modulation, and ω_s is the ESR frequency (rad/s). A decrease in B_{mol} with decreasing field requires $\omega_s \tau > 1$ which in turn leads to the conclusion that T_{1e} is proportional to τ. If the only effect of increasing ADP^{3-} concentration is to increase the viscosity of the solutions and not form any complex species beyond the mono complex, τ would increase, T_{1e} would increase, and B_{mol} would increase. This is contrary to what is observed.

A reasonable interpretation of the data can be made assuming that the relevant species are Mn^{2+}, $MnADP^-$, and $Mn(ADP)_2^{4-}$. The nature of the bis complex is not specified at this point except to assume that some water is still bound to manganese. Thus, at the pH's used, the following equilibrium constants were sufficient to define the distribution of species:

$$K_H = \frac{[HADP^{2-}]}{[ADP^{3-}][H^+]} \qquad K_{Li} = \frac{[LiADP^{2-}]}{[ADP^{3-}][Li^+]}$$

$$K_1 = \frac{[MnADP^-]}{[ADP^{3-}][Mn^{2+}]} \qquad K_2 = \frac{[Mn(ADP_2^{4-}]}{[ADP^{3-}][MnADP^-]}$$

The literature values used for these constants are $\log K_H$ (25 °C, $I = 0$) = 7.20;[13] $\log K_{Li}$ (25 °C, $I = 0.2$) = 1.15;[14] $\log K_1$ (25 °C, $I = 0.0085$) = 4.98.[15] The corresponding enthalpies ΔH_H and ΔH_1 were taken as 1.3 kcal[16] and 2.6 kcal,[15] respectively. ΔH_{Li} was assumed to be the same as that for ΔH_H. No literature data are available for K_2, but a rough preliminary treatment of the data showed $\log K_2 = 2.5$ at 25 °C. The ionic strength dependence of K_H, K_{Li}, and K_1 was calculated from the following equations:

$$\log K_H = 7.20 - 2.54(I^{1/2}) + 3.84I \qquad (I)$$

$$\log K_{Li} = 1.52 - 2.54(I^{1/2}) + 3.84I \qquad (II)$$

$$\log K_1 = 5.43 - 4.06(I^{1/2})$$
$$+ 6.36I - 2.04(I^{1/2})/1 + 6.02(I^{1/2}) \quad (III)$$

Equation I was obtained from Phillips et al.[13] The ionic strength dependence of K_{Li} was assumed to be the same as that for K_H. Equation III was also obtained from Phillips et al.[17] They *derived* this equation for $MgADP^-$ from their measured activity coefficient ratios for protonated and deprotonated AMP and ADP species. The thermodynamic constants $K^°_1$ and $K^°_{Li}$ used in equations II and III were calculated from the K_1 and K_{Li} values given above at $I = 0.0085$ and 0.2, respectively. The ionic strength of each solution, as given in Table I, was calculated by a computerized iterative procedure with the above equilibrium constants and equations. Log K_2 was assumed to be 2.5 in these calculations.

Line Broadening. Quantitative Treatment. The line-broadening data are generally described in terms of the Swift–Connick equation.[18] For manganese systems this equation can be simplified to the form[7]

$$T'_{2p} = \frac{[\text{metal species}]}{\Delta \pi} = \frac{55.5}{n} \left[\tau_m + \frac{1}{C} \left[\frac{1}{\tau_m} + \frac{1}{T_{1e}} \right] \right] \quad (IV)$$

Here Δ is the observed paramagnetic contribution to the line broadening (measured from full width at half-height) in Hz;
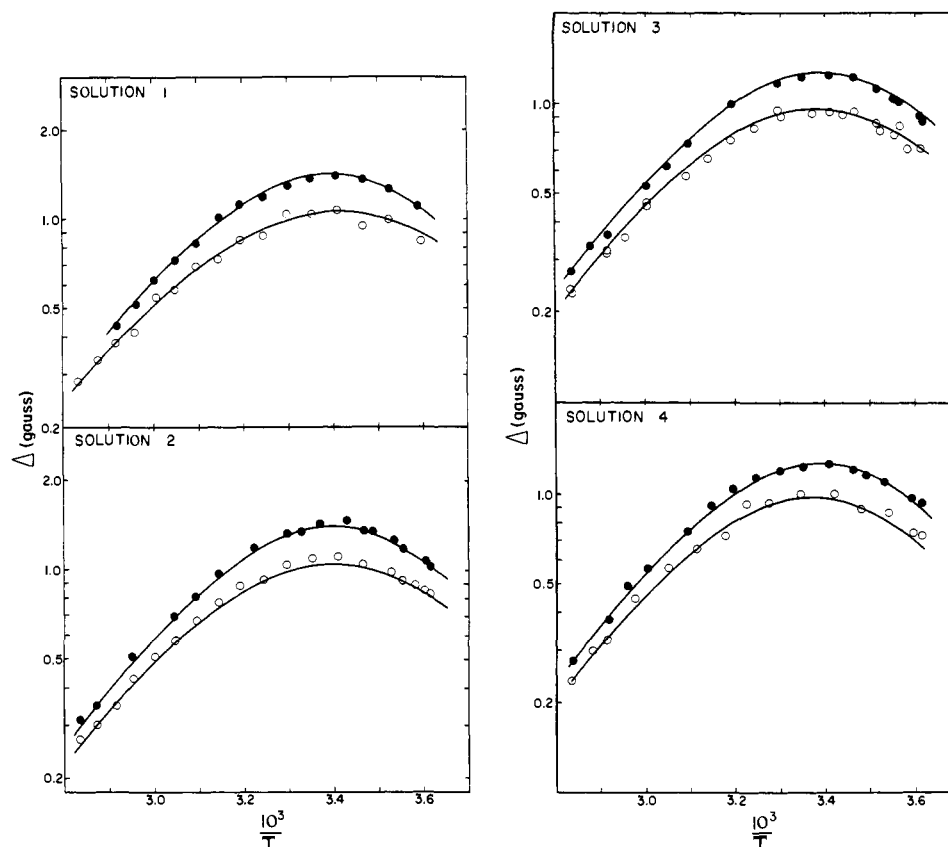
Figure 2. Semilogarithmic plot of the observed paramagnetic line broadening, Δ, vs. $1/T$ for solutions 1–4. ● and ○ represent the data at 11.5 MHz and 5.75 MHz, respectively. The full lines are the computed best fits to the data (see text), as calculated from the results in Table II.

$1/\tau_m$ is the water exchange rate constant, k_{ex}, in s^{-1}; T_{1e} is the longitudinal electronic relation time in s; n is the number of (equivalent) waters bound in the metal's inner coordination sphere; $C = \frac{1}{3}(S)(S + 1)(A/\hbar)^2$, where S is the electron spin for manganese(II) (5/2) and $A/\hbar$ is the scalar coupling constant in rad s^{-1}. k_{ex} can be expressed by the transition state theory equation as

$$k_{ex} = kT/h \exp(-\Delta H^*/RT + \Delta S^*/R) \qquad (V)$$

T_{1e} is the only field-dependent term in eq IV, and it was taken to be independent of temperature because of the short range over which it is important, leaving τ_m as the only temperature-dependent term. If n is unknown a more convenient form of eq IV is

$$T'_{2p} = 55.5 \left[\frac{\tau_m}{n} + \frac{1}{n^2 C} \left(\frac{n}{\tau_m} + \frac{n}{T_{1e}} \right) \right] \qquad (VI)$$

Thus, T'_{2p} can be defined by four variables, nA/h, T_{1e}/n, ΔH^*, and ΔS^* for n/τ_m.

Now the broadening produced by each solution at any given temperature, Δ, can be expressed as (assuming no coupled exchange)

$$\Delta = \Delta_{Mn}[Mn] + \Delta_{MnADP}[MnADP^-]$$
$$+ \Delta_{Mn(ADP)_2}[Mn(ADP)_2^{4-}] \qquad (VII)$$

where the $\Delta_{metal\ species}$ terms are the broadening produced per mole. Since from eq IV $1/T'_{2p} \pi = \Delta/[\text{metal species}]$

$$\Delta = \frac{1}{\pi} \sum_{z=0}^{z=2} \left[[Mn(ADP)_z] \frac{1}{T_{2p(Mn(ADP)_z)}} \right] \qquad (VIII)$$

This is the equation used to treat all the line-broadening data. $T'_{2p}(Mn)$ can be calculated from the previous work on the $Mn(H_2O)_6^{2+}$ system (see Figure 1). K_H, K_{Li}, and K_1 can be calculated at any ionic strength and temperature from the
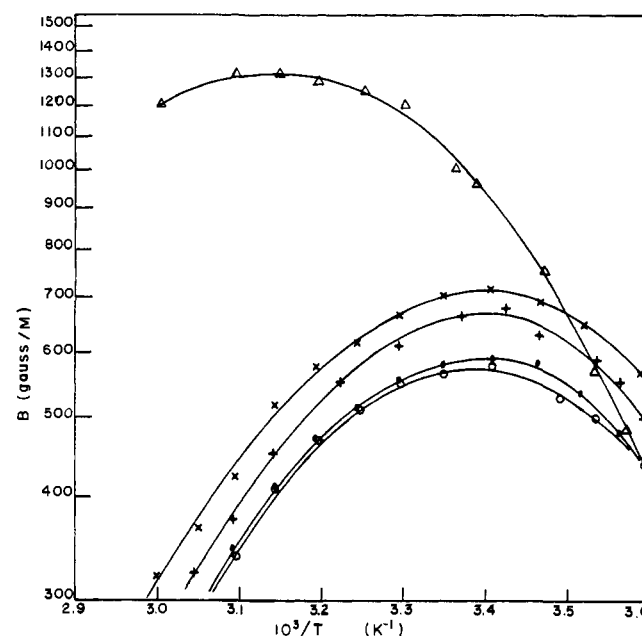

Figure 3. Semilogarithmic plot of the paramagnetic line broadening per mole of total manganese, B_{mol}, vs. $1/T$. From top to bottom the lines represent hand-smoothed data for $Mn(H_2O)_6^{2+}$ and solutions 1–4, respectively.

information given above, and these constants together with assumed values for K_2 and ΔH_2 can be used to calculate the species concentrations. Thus there are ten unknowns involved in eqVII: ΔH^*, ΔS^*, T_{1e}/n, and nA/h for both $MnADP^-$ and $Mn(ADP)_2^{4-}$, and K_2 and ΔH_2. No account could be taken of the possible ionic strength dependences of K_2 or τ_m.
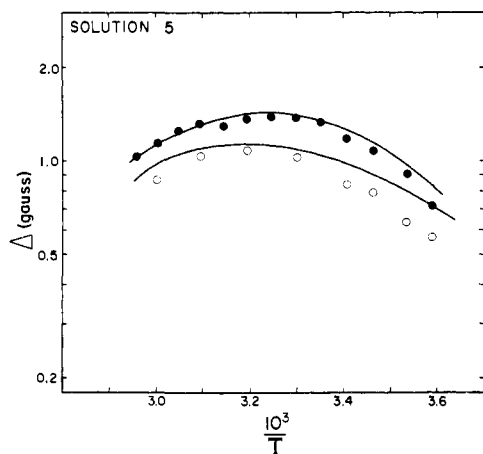
Figure 4. Semilogarithmic plot of the observed paramagnetic line broadening, Δ, vs. $1/T$ for solution 5. ● and ○ represent the data at 11.5 and 5.75 MHz, respectively. The full lines are calculated from results in Table II.

Table II. Computed NMR, Equilibrium, and Kinetic Parameters[a] for Mn–ADP System

| | At 11.5 MHz | | At 5.75 MHz | |
	For MnADP⁻	For Mn-(ADP)₂⁴⁻	For MnADP⁻	For Mn-(ADP)₂⁴⁻
ΔH^* (for n/τ_m), kcal/mol	8.6 ± 0.6	9.1 ± 1.3	8.5 ± 1.3	9.4 ± 2.5
ΔS^* (for n/τ_m), eu	9.1 ± 2.3	9.9 ± 5.0	9.2 ± 5.4	9.9 ± 9.6
T_{1e}/n, s × 10⁹ (25 °C)	3.9 ± 1.9	3.8 ± 2.6	1.9 ± 2.1	5.8 ± 8.9
nA/h, MHz	21.6 ± 3.0	16.3 ± 3.9	22.1 ± 9.2	12.3 ± 6.8
ΔH_2, kcal/mol	-1.5 ± 2.9		-0.8 ± 4.5	
Log K_2 (25 °C)	2.64 ± 0.16		2.64 ± 0.34	

[a] Errors are linear estimates of the standard deviation.

All the data from solutions 1–4 at one frequency were treated simultaneously with a slightly modified form of the nonlinear least-squares program of Dye and Nicely.[19] The modification allowed the program to "recognize" the chemical solution from which each data point came. Then using the appropriate enthalpies and ionic strength corrections to calculate K_1, K_H, and K_{Li}, it calculated Mn^{2+}, $MnADP^-$, and $Mn(ADP)_2^{4-}$ from the pH, total species concentrations, and its current best estimates for K_2 and ΔH_2. The sum of the residuals was then calculated in the usual manner. Very good initial estimates were required for convergence, not surprising with ten unknowns. The data at both frequencies were fitted completely independently in this manner, and the results are summarized in Table II. Generally, the agreement between the two sets of data is excellent. The solid lines in Figure 2 represent the computer fits to the data. It is worth reemphasizing that *all* the data for solutions 1–4 for a *given* frequency were fitted "simultaneously" to give the best overall fit to the data; this accounts for the fact that a fit for a given solution might not appear the best possible. The fits for the low-frequency data are satisfactory, while those for the more precise high-frequency data are excellent. The $Mn^{2+}/MnADP^-/Mn(ADP)_2^{4-}$ model therefore is consistent with these data and assumptions.

The data for solution 5 are plotted in Figure 4. The full lines, computed by using the results in Table II, do not fit the data as well. Possible explanations are given later in the discussion section.

Shift Data. The Swift–Connick equation for the paramagnetic shift may be expressed as

$$Q \equiv \frac{TS[H_2O]}{[\text{metal species}]} = \frac{nT\Delta\omega_m}{\omega_0}$$
$$\times \left[\frac{1}{(1 + \tau_m/T_{2m})^2 + (\tau_m\Delta\omega_m)^2} \right] \quad (IX)$$

Here ω_0 and ω_m ($\omega = 2\pi\nu$) are the resonance frequencies of bulk water and metal-bound water, respectively, $\Delta\omega_m = (\omega_m - \omega_0) \simeq (\omega_m - \omega_{obsd})$ and S is the fractional observed inner-sphere shift $(\omega_{obsd} - \omega_0)/\omega_0$, where ω_{obsd} is the observed resonance frequency of bulk water in the paramagnetic solution. T_{2m} equals $(1/C)(1/\tau_m + 1/T_{1e})$ for manganese. Also, for Mn $(\tau_m\Delta\omega_m)^2 \ll 1$, and the term can be dropped from eq IX.

In the fast exchange region where $T_{2m} \gg \tau_m$ eq IX can be reduced to the limiting form:

$$Q_{\lim} = \frac{nT\Delta\omega_m}{\omega_0} \quad (X)$$

Therefore, eq IX can be written as

$$Q = Q_{\lim} \cdot P \quad (XI)$$

where

$$P = 1/(1 + 2\tau_m/T_{2m} + \tau_m^2/T_{2m}^2) \quad (XII)$$

The scalar coupling constant, A/h, may be defined as

$$A/h = \frac{Q_{\lim}3k\gamma_N}{n2\pi S(S+1)g_{eff}\beta} \quad (XIII)$$

Here, k is the Boltzmann constant; γ_N is the nuclear magnetogyric ratio; S is the electronic spin; β is the Bohr magneton in cgs units; and g_{eff} is the electron g factor (taken to be the "spin-only" value of 2.0). For manganese(II) this equation reduces to:

$$A/h = \frac{Q_{\lim}}{n} \times 1.47 \ (\text{MHz}) \quad (XIV)$$

The total measured fractional shift, S, can be expressed as

$$S' = S^{Mn} + S^m + S^b + S^{bulk} + S^{outer}$$

where S^{Mn}, S^m, and S^b are the shifts attributable to $Mn(H_2O)_6^{2+}$, $MnADP^-$, and $Mn(ADP)_2^{4-}$, respectively. S^{bulk} and S^{outer} are shifts arising from bulk susceptibility effects and second-coordination or outer-sphere effects, respectively.

With the subscripts h and l representing respectively the higher and lower temperatures used to measure the shifts, the following equation holds:

$$(T_hS_h - T_lS_l) = T_h(S_h^{Mn} + S_h^m + S_h^b)$$
$$- T_l(S_l^{Mn} + S_l^m + S_l^b) + (T_hS_h^{bulk}$$
$$- T_lS_l^{bulk}) + (T_hS_h^{outer} - T_lS_l^{outer}) \quad (XV)$$

If the exchange of the outer-sphere waters is assumed to be fast, then Q(outer) should be at its limiting value throughout the entire temperature range used in this study. Further, the bulk susceptibility shift follows a Curie law type dependence with temperature. Thus,

$$T_hS_h^{outer} = T_lS_l^{outer} \text{ and } T_hS_h^{bulk} = T_lS_l^{bulk}$$

and since from eq IX

$$TS = Q[\text{metal species}]/[H_2O]$$

equation XV can be reduced to

$$T_hH_h - T_lH_l = \frac{\nu_0}{[H_2O]}\left[Q_h^m[MnADP^-]_h \right.$$
$$+ Q_h^b[Mn(ADP)_2^{4-}]_h + Q_h^{Mn}[Mn]_h - Q_l^m[MnADP^-]_l$$
$$\left. - Q_l^b[Mn(ADP)_2^{4-}]_l - Q_l^{Mn}[Mn]_l \right] \quad (XVI)$$

Table III. Shift Data

t^0	P^m	P^b	P^{Mn}	$H(\nu_{obsd} - \nu_0)$, Hz		
				Soln 6[a]	Soln 7[a]	Soln 8
−4.8	0.053	0.053	0.002	11.1 ± 1.0	12.3 ± 1.0	
60.0	0.963	0.961	0.644	44.1 ± 1.0	60.1 ± 1.5	
−5.1			0.002			5.4 ± 1.9[a]
119.8			0.988			46.5
129.5			0.993			44.7
138.5			0.996			42.9

[a] Average of at least three independent measurements. Errors are estimates.

where H represents the measured shift in Hz ($\nu_{obsd} - \nu_0$).

The Q values are related to the Q_{lim} values by eq XI, thus eq XVI can be rewritten as

$$T_h H_h - T_l H_l = \frac{\nu_0}{[H_2O]}\left[P_h{}^m Q^m{}_{lim}[MnADP^-]_h \right.$$
$$+ P_h{}^b Q^b{}_{lim}[Mn(ADP)_2{}^{4-}]_h$$
$$+ P_h{}^{Mn} Q^{Mn}{}_{lim}[Mn]_h - P_l{}^m Q^m{}_{lim}[MnADP^-]_l$$
$$\left. - P_l{}^b Q^b{}_{lim}[Mn(ADP)_2{}^{4-}]_l - P_l{}^{Mn} Q^{Mn}{}_{lim}[Mn]_l \right] \quad (XVII)$$

Values of P for MnADP$^-$ and Mn(ADP)$_2{}^{4-}$ were calculated from eq XII by using the results presented in Table II. Similar values for Mn^{2+} were calculated from the results in Table V. The calculated values are given in Table III. It should be noted that these P values are close to 1 at the higher temperature and 0 at the lower temperature. In other words, slight imprecisions in the line-broadening data and results will have very little effect on the values of P since care has been taken to use temperatures where P is close to its limiting values.

As with the line-broadening data, the species concentrations were calculated from the literature values of the stability constants and enthalpies cited above and from our calculated values of K_2 and ΔH_2. $Q^{Mn}{}_{lim}$ was taken to be 24.5 ± 1.1 (Figure 1).

With the calculated P's, the concentrations, and the shift data presented in Table III, two simultaneous equations can be obtained from eq XVII. They have the form:

$$T_h H_h - T_l H_l = \frac{\nu_0}{[H_2O]}\left(constant^m Q^m{}_{lim} \right.$$
$$\left. + constant^b Q^b{}_{lim} + constant^{Mn} \right) \quad (XVIII)$$

The values of $Q^m{}_{lim}$ and $Q^b{}_{lim}$ obtained from the solution of these equations are given in Table IV.

The shift data for the aquo manganese system (solution 8) were treated in a similar manner. In this case eq XVIII can be further simplified to give

$$T_h H_h - T_l H_l = \frac{\nu_0}{[H_2O]}\left[Q^{Mn}{}_{lim}[Mn^{2+}](P_h{}^{Mn} - P_l{}^{Mn}) \right] \quad (XIX)$$

The value found for $Q^{Mn}{}_{lim}$ is given in Table IV.

Discussion

Considering first the possible equilibria and species in the Mn(II)–ADP system, the line-broadening interpretation (see Table II) at two magnetic fields supports the existence of a bis complex with log K_2 = ca. 2.6 at 25 °C and ΔH_2 = ca. −1 kcal/mol. This result is not unreasonable in the light of Frey and Stuehr's study[4] of the nickel complexes. As can be seen from Table I the amount of bis complex can then be significant and caution must be used in interpreting data at high ligand concentrations (often used in NMR studies).

Table IV. Values of Q_{lim} and n[a] from Shift Data

Species	Q_{lim}	n
Mn(H$_2$O)$_6{}^{2+}$	25.9 ± 1.0	6.3 ± 0.5
MnADP$^-$	15.3 ± 0.9	3.8 ± 0.4
Mn(ADP)$_2{}^{4-}$	14.3 ± 1.1	3.5 ± 0.5

[a] Normalized taking $Q^{Mn}{}_{lim}$ = 24.5 ± 1.1 for 6 waters and assuming A/h to be species independent.

The data on solution 5 (low ligand/metal ratio) shows some deviation from the calculated values based on higher ligand/metal ratios particularly at low field and low temperatures. The deviations do seem to be outside estimated errors, and a possible explanation could be formation of a Mn$_2$(ADP) species analogous to that suggested as Mn$_2$(ATP) by Mohan and Rechnitz.[20] Further work on this could probably decide the issue.

Turning to the question of the number of bound waters in the postulated species, we must draw upon the idea that the A/h value or the NMR shift of a bound water molecule is essentially independent of the number and kinds of other ligands present. The idea is supported at present for Mn(II) by studies we have made on Mn(H$_2$O)$_6{}^{2+}$, Mn(EDTA)(H$_2$O)$^{2-}$, Mn(NTA)(H$_2$O)$_2{}^-$, Mn(Dacoda)(H$_2$O), and Mn(PhDTA)(H$_2$O)$^{2-}$ where a variety of structures are involved. The shift per water is constant to ±10% in these cases. This value corresponds to $Q^{Mn}{}_{lim}$ = 24.5 for 6H$_2$O bound or A/h = 6.0 MHz. In Table IV the n values obtained with the above considerations are given. For the mono Mn–ADP complex, 4H$_2$O/Mn appear to be present. This is consistent with two bound phosphate oxygens or one oxygen and the N-7 from the adenine ring but not simultaneous binding to the two phosphates and N-7. In the case of Mn(ADP)$_2$(H$_2$O)$_n{}^{4-}$ n appears to be 3 or 4 rather than 2 as might have been the naive expectation. Several possible structures can be imagined. Considerable numbers of reports of stacking in the free nucleotides and their complexes exist.[21] Examination of models suggests that a head-to-tail stacking[22] of the ligands in the metal complex would permit two phosphates and one N-7 to bind to the metal leaving 3–4H$_2$O/Mn and perhaps explaining the fair stability apparent for the bis complex. As a check on our postulate of a bis complex we refit the data leaving K_1, ΔH_1, K_2, and ΔH_2 free to vary and obtained the same values within the experimental errors. We found, in addition, that adding a dimerization equilibrium for the free ligand led to the same fit first found with no dimerization (i.e., K dimer was ≪ 0.01). Considering the low free-ligand concentration this is not surprising. It might be mentioned here that we have done similar studies on the Mn–ATP system at high ligand-to-metal ratios and find even more clear-cut evidence for a bis complex again without maximum water replacement. More work using ^{13}C, ^{31}P, and ^{1}H NMR is needed to arrive at a definite structural conclusion. Possibly a phosphate originally bound is displaced. As Mn(II) is known to be 7-coordinate in MnEDTA(H$_2$O)$^{2-}$, this possibility should also be considered.

Table V. Comparisons of Mn(II) System Results Corrected for n^f

	$Mn(ADP)_2(H_2O)_3^{4-}$	$MnADP(H_2O)_4^-$	$MnATP(H_2O)_3^{2-\ a}$	$Mn(H_2O)_6^{2+\ b}$
ΔH^*, kcal/mol	9.1 ± 1.3	8.6 ± 0.6	9.6 ± 0.6	7.7 ± 0.1
ΔS^*, cal/mol deg	7.8 ± 5.0	6.4 ± 2.3	8.8 ± 2.5	0.9 ± 0.4
T_{1e} at 11.5 MHz ($\times 10^8$), s	1.1 ± 0.8	1.6 ± 0.8	1.1 ± 0.7	3.4
T_{1e} at 5.75 MHz ($\times 10^8$), s	1.7 ± 2.7	0.8 ± 0.8	0.7 ± 0.5	1.4
A/h from shift data MHz[e]	7.0 ± 0.5	5.7 ± 0.3		6.0 ± 0.3
A/h from T'_{2p} data MHz[e]	5.4 ± 1.3	5.4 ± 0.8	6.0 ± 1.3	5.4 ± 0.3
$k_{ex}{}^d$ ($\times 10^{-7}$), s^{-1}	6.7	7.6	5.0	2.2

[a] Reference 7. [b] Lo et al., to be published. [c] Errors are linear estimates of the standard deviation. [d] Rate of exchange = nk_{ex}[complex]. [e] n is given by the formulas above for calculating A/h from nA/h. [f] All results apply to data at 11.5 MHz, unless stated otherwise. Temperature 25 °C.

The kinetic parameters of water exchange as shown in Table V are quite similar for all the species listed. Phosphate appears not to significantly donate electron density to the metal, i.e., the interaction is largely ionic. The small rate enhancement compared to the aquo ion occurs, in spite of an activation energy increase, due to a more positive activation entropy. Such a result could be explained by increased dissociative character in the transition state.

As discussed earlier in some detail,[7,23] oxine substitution on Mn–ATP is unusually slow. Since the substitution rate on Mn–ADP can now be directly compared with water exchange in the complex and is found to be "normal" (i.e., both rates are similar), the idea that substitution on Mn–ATP involves a phosphate oxygen or other displacement is strengthened. Apparently the α phosphate oxygen can be removed[24] by lowering the pH, thus indicating that it is the most weakly bound to the Mn. The above discussion of the nature of $Mn(ADP)_2$ also might support this idea as being possible.

Our data do not require postulation of kinetic nonequivalence for the bound water molecules in the Mn–ADP system as was also found in the case of Mn–ATP. There remains, of course, some question as to how sensitive the data are to small differences, but factors of ten should be quite apparent.

The A/h value calculated for $Mn(H_2O)_6^{2+}$ from the low temperature and concentration shift measurement (solution 8 in Table III) is in good agreement with the high-temperature data, 6.4 ± 0.3 compared to 6.0 ± 0.3 (MHz) (see Table V). This lends credence to the use of small shifts in obtaining A/H and should permit study of systems that cannot be done at high temperatures (e.g., in enzymes). The general good agreement (Table V) between A/h determined independently from shifts and line broadenings further suggests we have done a proper treatment of the data in these systems.

The T_{1e} values (Table V) at different fields are quite reasonable though not precisely determined. The relatively large uncertainty in T_{1e} values is due to a strong correlation between A/h and T_{1e}. Nevertheless, it is clear that T_{1e} is field dependent between 10 and 20 kG. We have not tried to treat the ADP results in more detail because of the uncertainties, but the τ and Δ values would be very similar to those derived for ATP.[7] Reed et al.[25] gave τ and Δ values for Mn–ADP similar to those for Mn–ATP. It might be mentioned in this connection that

one need not expect ESR line width and our oxygen-17 NMR data to lead to the same τ and Δ values upon consideration of both static and dynamic zero-field splittings and the different correlation times associated with them. The EPR values concern T_{2e} whereas the oxygen-17 NMR values involve T_{1e} and the static and dynamic zero-field splittings have different contributions to each relaxation time.

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