Measurement of the Water Exchange Rate of Bound Water in the Manganese(II)–Adenosine Triphosphate Complex by Oxygen-17 Nuclear Magnetic Resonance†

Mark S. Zetter, Harold W. Dodgen, and John P. Hunt*

ABSTRACT: Oxygen-17 line broadening measurements have been made on the $MnATP^{2-}$ complex in aqueous solution. The results are interpreted in terms of three kinetically equivalent waters being present in the inner sphere of the complex. The kinetic parameters for water exchange (per water molecule) are: $k_{ex}(25°) = 5.0 \times 10^7\ sec^{-1}$; $\Delta H^* = 9.6$ kcal mol^{-1}; $\Delta S^* = 8.8$ cal mol^{-1} deg^{-1}. These parameters are compared with published kinetic results for the substitution of 8-hydroxyquinoline into the inner sphere of $MnATP^{2-}$ and it is concluded that the generally considered mechanism for metal complex formation does not apply in this case. The discrepancy between these two sets of kinetic data is discussed in terms of possible binding differences of ATP in $MnATP^{2-}$ and 8-hydroxyquinoline–$MnATP^{3-}$. It is suggested that these proposed differences can rationalize, in part, the similar behavior of Mn^{2+} and Mg^{2+} in the enzymatic activation of transphosphorylation reactions involving ATP.

Recent temperature-jump studies on the kinetics of manganese(II) (Hague and Zetter, 1970; Hague *et al.*, 1972) and magnesium (Hague and Eigen, 1966; Hague *et al.*, 1972) ternary complex formation and dissociation revealed several interesting results which may be significant in the understanding of Mg^{2+} and Mn^{2+} activation of enzymes. Of particular interest were the reactions of $MnATP^{2-}$ and $MgATP^{2-}$ with the anion of 8-hydroxyquinoline (Ox^-), *i.e.*

$$M\text{-}ATP^{2-} + Ox^- \underset{k_b}{\overset{k_f}{\rightleftharpoons}} Ox\text{-}M\text{-}ATP^{3-} \qquad (1)$$

where M is Mg^{2+} or Mn^{2+}. The rate of formation in this type of reaction is generally described (Hewkin and Prince, 1970) in terms of

$$k_f = K_{os}k_{ex} \qquad (2)$$

where K_{os} is an outer sphere formation constant, dependent, in part, on the charge product of the reacting species, and k_{ex} is the rate constant for the exchange of a solvent molecule between a metal ion (or complex) and the bulk solvent. For reaction 1, k_f and the associated thermodynamic activation parameters for $MgATP^{2-}$ were consistent with eq 2 if it was assumed that k_{ex} for $MgATP^{2-}$ was similar to that measured for $Mg(H_2O)_6{}^{2+}$ (Neely and Connick, 1970). This was not the case for $MnATP^{2-}$; k_f was lower and the activation enthalpy higher than expected, suggesting that either k_{ex} was lower than that measured for $Mn(H_2O)_6{}^{2+}$ (Grant *et al.*, 1971) or some other process—possibly a change in the type of coordination of the ATP to the Mn^{2+}—was rate determining.

The structure of the $MnATP^{2-}$ complex in aqueous solution has been the subject of much discussion. The picture that has emerged from numerous nmr studies indicates (1) that the α-, β-, and γ-phosphate groups (Cohn and Hughes, 1962; Sternlicht *et al.*, 1965) and the adenine ring are bound to manganese, the latter being separated from the metal by an inner-sphere water molecule (Glassman *et al.*, 1971), and (2) that the complex contains three rapidly exchanging waters (Glassman *et al.*, 1971).

An ^{17}O nmr study can further our knowledge of the properties of the $MnATP^{2-}$ complex in aqueous solution. Firstly, this method can sometimes give an indication of the number of water molecules in the complex. Secondly, it is sometimes possible to distinguish between kinetically nonequivalent waters, as has been found for the Ni(II)–2,2′,2′′-triaminotriethylamine complex (Hunt, 1971), and thirdly, k_{ex} can be measured, thus enabling us to determine whether water exchange or some other process is rate determining in reaction 1.

Experimental Section

^{17}O water (7 atom %; normal H content) was obtained from Yeda R&D Co., Ltd., Rehovoth, Israel. It was redistilled *in vacuo* before each use. The nuclear magnetic resonance (nmr) spectrometer and equipment have been described elsewhere (Desai *et al.*, 1969, 1970). Lorentzian-shaped absorption curves were observed by using a locked frequency of 11.5 MHz and a field of *ca.* 20 kg (or, in one case, 5.75 MHz and *ca.* 10 kg). Static field drift has been reduced to a negligible factor by thermostating the Hall probe used in field regulation. Samples were thermostated with a small constant temperature bath containing silicone oil. Temperatures are known to ±0.1°.

pH measurements were made with a Beckman Zeromatic II meter. Immediately before each sample measurement, the meter was calibrated with standard buffer solutions of 0.05 M potassium hydrogen phthalate and 0.01 M sodium tetraborate at the same temperature as the sample.

Manganese(II) and zinc perchlorates were prepared by the action of perchloric acid on the metal carbonates. The salts were recrystallized from water and analyzed by EDTA complexometric titration, each salt giving a satisfactory result. Na_2ATP was obtained from Schwarz Biochemicals, Inc. Stock solutions (pH ~7) were prepared by dissolving the ATP in dilute potassium hydroxide. The concentration of ATP was

---

† From the Department of Chemistry, Washington State University, Pullman, Washington 99163. *Received June 12, 1972.* This work was supported by U. S. Atomic Energy Commission Contract AT(45-1)-2221-T4.

TABLE I: Solution Statistics.

Solution[a]	pH (25°)	pH (85°)	Total Mn, × 10^3 M	Total ATP, × 10^3 M	[$MnATP^{2-}$], × 10^3 M 25°	[$MnATP^{2-}$], × 10^3 M 85°	[Mn^{2+}], × 10^5 M 25°	[Mn^{2+}], × 10^5 M 85°
A	~7.9	~8.0	2.00	4.68	1.98	1.94	2.52	5.72
B	~7.9	~8.0	2.00	7.01	1.99	1.97	1.24	2.85
C	6.73	6.96	2.00	2.34	1.85	1.74	14.5	25.7

[a] Ionic strength made up to 0.1 with KNO_3 for all the solutions.

determined by quantitatively diluting the stock solution to *ca.* 4 × 10^{-5} M and measuring the absorbance at 259 nm (ϵ (pH ~7) 15.4 × 10^3). The AMP and ADP content of the stock solutions was found to be negligible by paper electrophoresis at pH 5.8 (0.05 M citrate buffer) and paper chromatography in isobutyric acid–NH_4OH–H_2O (66 : 1 : 33).

To prepare the $^{17}OH_2$ solutions, aliquots of standard metal–ATP solutions (in normal water) were evaporated *in vacuo* at room temperature. Sufficient solid potassium nitrate was then added to give the final solution an ionic strength of 0.1. Known quantities of $^{17}OH_2$ (*ca.* 2 ml) and concentrated potassium hydroxide (<10^{-2} ml) were added, the latter to give a solution of the desired pH. The sample tubes were purged with nitrogen gas and stoppered. Line-broadening measurements on each solution were completed within seven hours of solution preparation and were reversible with increasing and decreasing temperature, indicating that within the time span of the measurements, no significant hydrolysis of ATP had occurred.

Reference solutions contained zinc in place of manganese. Otherwise the solutions were identical in composition. The pH of each zinc solution was within 0.2 of the corresponding sample solution over the temperature range studied. (pH's were measured on solutions which were prepared in an identical manner to the ^{17}O solutions, but with ordinary distilled water.) The solution compositions and pH's are given in Table I.

At least four line-broadening measurements (an equal number of upfield and downfield sweeps) were made at each temperature for each blank and sample solution. The line broadenings are given in Figure 1 and are accurate to *ca.* ±4–6%. No chemical shift could be measured for the Mn-ATP^{2-} solutions because of the instability of the solutions at the high temperatures needed.

Treatment of Data and Results

Equilibrium Considerations. Before kinetic information can be obtained from the line-broadening measurements, it is necessary to know the solution compositions in terms of the major species present. For the pH's used, the following equilibrium constants define the Mn–ATP system

$$K_H = \frac{[HATP^{3-}]}{[H^+][ATP^{4-}]} \qquad K_2 = \frac{[Mn(ATP)_2^{6-}]}{[MnATP^{2-}][ATP^{4-}]}$$

$$K_1 = \frac{[MnATP^{2-}]}{[Mn^{2+}][ATP^{4-}]} \qquad K_3 = \frac{[KATP^{3-}]}{[K^+][ATP^{4-}]}$$

The literature values for these constants are: log $K_H(25°, \mu = 0.1) = 7.04$ (Phillips *et al.*, 1963); log $K_1(25°, \mu = 0.1) = 4.78$ (Martell and Taqui Khan, 1966); log $K_2(\sim 20°, \mu \sim 0.3) \simeq 1.6$ (Sternlicht *et al.*, 1968); log $K_3(30°, \mu = 0.1) = 1.15$ (O'Sullivan and Perrin, 1964). The corresponding enthalpies for K_H and K_1 are: $\Delta H_H = 1.2$ kcal/mol (Christensen and Izatt, 1962); $\Delta H_1 = -3.0$ kcal/mol (Martell and Taqui Khan, 1966).

The species which might contribute to the observed line broadening are Mn^{2+}, $MnATP^{2-}$ and $Mn(ATP)_2^{6-}$. For solutions A and B, the values of K_H and K_1 are such that the [Mn^{2+}] is sufficiently low to produce only a very small percentage of the broadening. However, despite the different $[Mn]_{total}$: $[ATP]_{total}$ ratios for these solutions, the broadening produced by them at 11.5 MHz is identical, within experimental error, at any temperature within the range studied. Thus, it may be concluded that either $MnATP^{3-}$ and Mn-$(ATP)_2^{6-}$ produce the same amount of broadening per mole or $Mn(ATP)_2^{6-}$ is not present in significant amounts. The former conclusion is unlikely to be correct since $Mn(ATP)_2^{6-}$ is probably a fully substituted species (no coordinated waters in the complex) and, therefore, would not produce any line broadening. (The literature value of K_2 above would give a [$Mn(ATP)_2^{6-}$] about 10% that of the [$MnATP^{2-}$] in solution A. However, this value of K_2 is only thought to be of the right order of magnitude.) The [$MnATP^{2-}$] in solutions A and B is practically independent of the value of K_3 and thus

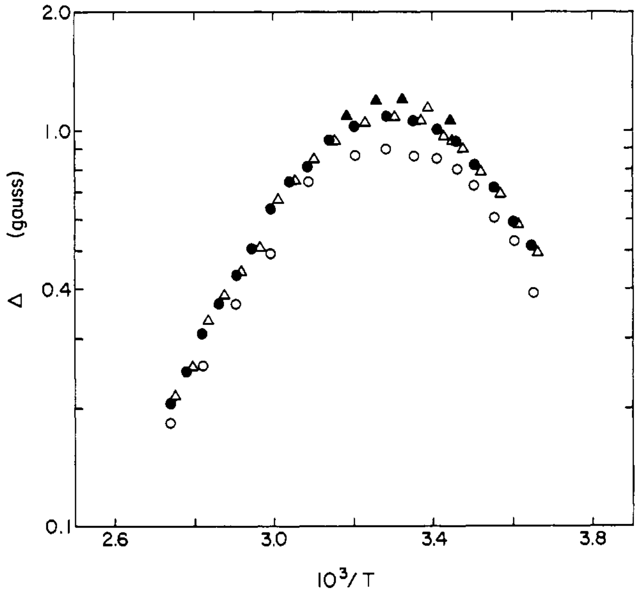

FIGURE 1: Semilogarithmic plot of the observed line broadening, Δ, against 1/*T* for solutions A (△), B (●) and C (▲) at 11.5 MHz and solution B at 5.75 MHz (○).

TABLE II: Kinetic and Nuclear Magnetic Resonance Parameters.[a]

n	ΔH^* (kcal/mol)	ΔS^* (cal/mol deg)	k_{ex}[b] at 25° (× 10^{-7}) (sec^{-1})	A/h (× 10^{-6}) (Hz)	T_{1e} (× 10^8) at 11.5 MHz (sec)	T_{1e} (× 10^8) at 5.75 MHz (sec)	Δ (G)	τ (× 10^{12}) (sec)
1	9.5 ± 0.6	10.5 ± 2.5	15.0	17.2 ● 3.9	0.40 ± 0.27	0.25 ± 0.17	394	1.7
2	9.5 ± 0.6	9.3 ± 2.5	7.4	8.8 ± 2.0	0.76 ± 0.53	0.48 ± 0.34	286	1.6
3	9.6 ± 0.6	8.8 ± 2.5	5.0	6.0 ± 1.3	1.06 ± 0.71	0.67 ± 0.45	242	1.6
4	9.8 ± 0.6	9.1 ± 2.5	3.9	4.8 ± 1.0	1.15 ± 0.73	0.76 ± 0.48	234	1.5
5	9.9 ± 0.5	9.2 ± 2.4	3.3	3.9 ± 0.8	1.30 ± 0.74	0.86 ± 0.49	220	1.5

[a] Errors tabulated are linear estimates of the standard deviation. [b] k_{ex} is per water.

a knowledge of ΔH_3 is not vital. Hence, K_H, ΔH_H, K_1, ΔH_2, and K_3 at 30° are sufficient to define the system.

In solution C the composition does depend significantly on K_3. Thus, the unavailability of a ΔH_3 value restricted the temperature range that could be studied meaningfully for this solution. It can be seen in Figure 1 that at 30° solution C produced more broadening than either solution A or B. The difference, attributed to the presence of significant amounts of Mn^{2+}, may be used to evaluate our own value of K_1 as follows. From a knowledge of the broadening produced per mole of $MnATP^{2-}$ (calculated from solution A or B) and per mole of Mn^{2+} (Grant *et al.*, 1971), the $[Mn^{2+}]$ required to produce the extra broadening in solution C may be calculated. Thus, from the total ligand and metal concentrations, the pH, K_H and K_3, K_1 may be evaluated. Log K_1 (30°) was found to be 4.85, a value which compares moderately well with the literature. (Rechnitz and Mohan (1970) have reported a K_3 value (25°) of 218 M^{-1}; if this value is used in the calculation of K_1, then log $K_1(25°) = 5.40$.)

Evaluation of Kinetic Parameters from the Line-Broadening Data of Solutions A and B. This may be carried out by using the Swift and Connick (1962) approach. The line broadening may be expressed in terms of $T_{2p}' = 2[MnATP^{2-}]/(\gamma_N\Delta)$, where $\gamma_N = 3628\ G^{-1}\ sec^{-1}$ and Δ is the full line width increase (in gauss at half peak height), which may be attributed to the $MnATP^{2-}$ complex (*i.e.*, the broadening arising from Mn^{2+} is subtracted from the observed broadening). The $[MnATP^{2-}]$ and $[Mn^{2+}]$ were calculated from the literature values of K_H, ΔH_H, K_1, ΔH_1, and K_3 (at 30°) given above. The T_{2p}' data are plotted in Figure 2.

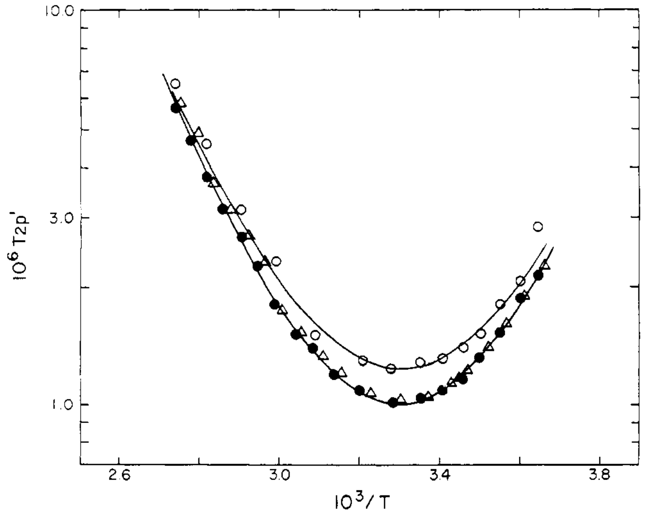

FIGURE 2: Semilogarithmic plot of T_{2p}' *vs.* $1/T$ for solutions A (△) and B (●) at 11.5 MHz and solution B at 5.75 MHz (○). The lower curve represents the fit for the 11.5-MHz data and the upper curve that for the 5.75-MHz data. Both fits are for the case where $n = 3$.

For Mn(II) and its complexes, the relatively long T_{1e} values make T_{2m} contributions, in the full Swift and Connick equation for T_{2p}', considerably greater than those arising from $\Delta\omega_m$. Thus, their equation may be simplified to $T_{2p}' = (55.5/n)(\tau_m + T_{2m})$, where τ_m is the mean lifetime of a water molecule in the inner sphere of the manganese complex, n is the number of water molecules in the complex, and $T_{2m} = (1/C)(1/T_{1e} + 1/\tau_m)$. Here C equals $(^1/_3)S(S+1)(A/\hbar)^2$, S being the spin $(^5/_2)$ and $A/\hbar$ the scalar coupling constant in radians per second. (It should be noted that in the expression for T_{2m} above the dipolar term has been omitted since, for Mn(II), it is negligible compared to the hyperfine term.) Thus, the equation to describe our data is

$$T_{2p}' = (55.5/n)(\tau_m + (1/C)(1/T_{1e} + 1/\tau_m)) \qquad (3)$$

The temperature dependence of $1/\tau_m$, the water exchange rate constant k_{ex}, may then be expressed in terms of the activation parameters, ΔH^* and ΔS^*, by using the transition-state theory equation

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

Equation 3 has two limiting forms. At low temperatures $T_{2p}' = 55.5\tau_m/n$ and at high temperatures $T_{2p}' = 55.5/(nC\tau_m)$. Hence, theoretically, τ_m, ΔH^*, and ΔS^* may be obtained from the limiting low-temperature region by assuming a value of n, and from these parameters, C may be calculated from the limiting high-temperature region. For the $MnATP^{2-}$ system these limiting regions have not quite been reached, and we have chosen to fit our T_{2p}' data for the parameters, ΔH^*, ΔS^*, A/h, and T_{1e} with the nonlinear least squares program of Dye and Niceley (1971). We assumed T_{1e} to be independent of temperature. Table II shows the values of these parameters, obtained from the high field data, for different assumed values of n.

The only term dependent on the field in eq 3 is T_{1e}. Thus, T_{1e} at 5.75 MHz was determined by fitting the low-field data with the values of ΔH^*, ΔS^*, and A/h obtained from the more precise and abundant high field data. The values of Δ and τ

in Table II were calculated from the values of T_{1e} at the two fields from the equation (McLachlan, 1964)

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

Here Δ is the parameter measuring the modulation of the zero-field splitting produced by solvent impacts at a mean rate of $1/\tau$ and ω_S is the electron spin resonance (esr) frequency (rads/sec) at each field. The values of τ and Δ may be compared with those measured by Reed *et al.* (1971), *viz.*, $\tau = 8.5 \times 10^{-12}$ sec and $\Delta = 195$ G.

Discussion

Equivalence and Number of Bound Waters in the MnATP^{2-} Complex. The shape of the T_{2p}' *vs.* $1/T$ plot (Figure 2) shows that within experimental error, the kinetic parameters of the measured waters are equivalent. We estimate that the rate constants of the waters must be within a factor of three to give this shaped curve. If this were not the case, an asymmetrical curve, or a curve with a "bump" at the minimum, would have resulted. However, if an extremely fast or slow water, in comparison to those measured, exists in the complex, then it would remain undetected in our experiments. In other words, the line broadening produced by such a water would be insignificantly small *in the temperature range* we have examined.

The number of bound waters in the MnATP^{2-} complex cannot be determined directly from our data. Nevertheless, an estimate of this number can be made as follows. The scalar coupling constants (A/h) per bound water for paramagnetic ion–^{17}O hyperfine interactions in a large number of Co(II) and Ni(II) complexes are remarkably constant for each metal, regardless of the nature and number of other ligands in the octahedral species (Hunt, 1971). A similar constancy is also found for manganese. From ^{17}O shift measurements, values for A/h per bound water of 5.6, 5.3, and 6.0 ($\times 10^6$) Hz have been obtained for $Mn(H_2O)_6^{2+}$ (Lo *et al.*, to be published), $Mn(NTA)(H_2O)_2^-$, and $Mn(EDTA)(H_2O)^{2-}$ (Zetter *et al.*, 1972), respectively. Thus, if we assume that A/h for MnATP^{2-} is $5.6 \pm 0.4 \times 10^6$ Hz, the following conclusions can be drawn from our data. By inspection of Table II and consideration of errors in A/h for various values of n, it can be seen that when $n = 3$ the most reasonable value of A/h is obtained. A value of $n = 4$ is less reasonable and $n = 2$ even less so. Previous work has also shown that $n = 4$ is an unlikely result (Glassman *et al.*, 1971; Cohn and Hughes, 1962).

We are left, therefore, with the conclusion that the measurements reported probably relate to three rapidly exchanging waters which are, within a factor of approximately three, kinetically equivalent. However, we cannot entirely rule out the possibility that we have measured the effects of only two kinetically equivalent waters and that the third water is very much slower or faster than the measured ones. In this respect Kuntz *et al.* (1972) have shown that Co(ATP)$^{2-}$ contains two rapidly exchanging waters and one slower one.

Comparison of the Water Exchange and Substitution Rate Constants for MnATP^{2-}. Table III summarizes some of the data obtained by Hague *et al.* (1972) for the kinetics of substitution in numerous Mg(II) and Mn(II) complexes. For the magnesium reactions the overall charge on the reacting metal complex has little effect on the forward substitution rate constant in the complexation with the anion of 8-hydroxyquinoline, Ox$^-$ (or little effect on K_{os} in eq 2). This is not unexpected since the local charge density on the central metal

TABLE III: Rate Constants for the Reactions of Mn(II) and Mg(II) Species with the Anion of 8-Hydroxyquinoline.[f]

$$ML^{n-} + Ox^- \underset{k_b}{\overset{k_f}{\rightleftarrows}} OxML^{(n+1)-}$$

ML	$M(H_2O)_6^{2+}$		MNTA$^-$ [a]		MUDA$^-$ [b]		MADP$^-$ [c]		MATP^{2-} [d]		MTP^{3-} [e]	
M	Mg	Mn	Mg	Mn	Mg	Mn	Mg	Mn	Mg	Mn	Mg	Mn
k_f (M^{-1} sec^{-1})	6.0×10^5	3.3×10^8	1.5×10^5	2.3×10^7	5.5×10^4	2.2×10^7	2.5×10^5	8.5×10^7	1.2×10^5	2.5×10^6	5.6×10^4	9.1×10^5
ΔH_f^* (kcal/mol)	12.3	8.8	12.3	6.6	11.2	6.4	10.3	4.4	10.0	12.0	14.7	9.8
k_b (sec^{-1})	20	480	3.7	180	150	130	73	1400	48	90	21	350

[a] NTA, nitrilotriacetate. [b] UDA, uramil *N*,*N*-diacetate. [c] ADP, adenosine 5′-diphosphate. [d] ATP, adenosine 5′-triphosphate. [e] TP, tripolyphosphate. [f] Data taken from Hague *et al.* (1972). Errors for each value are given in the original paper.

ion is probably more important in determining K_{os} than the overall complex charge. Further, the Mg species forward rate constants are consistent with those predicted by eq 2, insofar as current results for other metal ions allow us to predict the effect of ligands on k_{ex} for Mg complexes. The same cannot be said for the Mn(II) complexes; the formation rate constants for OxMnATP^{3-} and OxMnTP^{4-} are considerably lower than expected. Taken by themselves, these results could be a reflection of the high overall complex charge on the binary metal complexes, but the results for the Mg systems suggest this is not the case. The apparently normal result for Ox$^-$ substitution in MnADP$^-$ rules out that phosphate groups are themselves responsible. The water exchange rate constant for MnATP^{2-} (Table II) is similar to that for the aquomanganese ion, thus, in terms of k_{ex} and K_{os} (charge) it is not possible to account for the difference of more than 10^2 in the formation rate constants of OxMn$^+$ and OxMnATP^{3-}. In other words, it is probable that some process other than water exchange is rate determining in the formation of OxMnATP^{3-} and OxMnTP^{4-}.

A Structural Interpretation of the Kinetic Results for MnATP^{2-} and Its Relevance to the Mg^{2+}–Mn^{2+} Activation of Enzymes. We believe that the normality of the MgATP^{2-} substitution rate constant and the abnormality of the similar MnATP^{2-} rate constant lies in the structural difference between the binary complexes, MnATP^{2-} and MgATP^{2-}. The structure of the former complex has been discussed earlier in this paper. Unlike MnATP^{2-}, MgATP^{2-} does not have adenine ring or α-phosphate binding; only the β- and γ-phosphate groups are bound to the metal (Cohn and Hughes, 1962).

The Ox$^-$ ion could substitute normally into the MgATP^{2-} complex and the rate determining step would be the rate of water loss. For the more highly substituted MnATP^{2-}, we propose that a change in the ATP binding to Mn has to take place before the Ox$^-$ ion can substitute into the inner sphere (thus the 2.3 kcal difference between ΔH for water exchange and oxine substitution) and that this change is rate determining. Hence, in the ternary complex, OxMnATP^{2-}, we believe that either the α-phosphate group or the adenine ring or both are no longer bound to the metal. If our speculation is correct then the structures of the metal–oxine–ATP ternary complexes could be almost identical. The interchangeability of Mn and Mg in enzymatic transphosphorylation can be more easily rationalized, therefore, in terms of the (possibly) similar structures of the ternary complexes than in the dissimilar structures of the binary complexes.

Kinetic similarities in the rates of formation and dissociation of OxMnATP^{3-} and OxMgATP^{3-} have also been observed and have been discussed previously in terms of Mg–Mn enzyme activation (Hague and Zetter, 1970; Hague *et al.*, 1972). These similarities are particularly striking when compared to the normal but dissimilar kinetic behavior of OxMgADP^{2-} and OxMnADP^{2-}.

In conclusion, then, ATP appears to impart similar kinetic and (possibly) structural properties on ternary complexes involving MgOx$^+$ and MnOx$^+$. This is considered to be highly relevant to the comparable behavior of Mg^{2+} and Mn^{2+} in the enzymatic activation of reactions involving ATP. As is well known, results from model systems can never be strictly applied to enzyme systems; however, we hope that this study will promote further research on other ternary systems, since we believe it is in these systems, and not in binary systems, that many of the unanswered questions relating to Mg–Mn activation lie.

Acknowledgments

We thank Dr. Ralph G. Yount for his helpful comments and advice throughout the course of this work.

References

Christensen, J. J., and Izatt, R. M. (1962), *J. Phys. Chem. 66*, 1030.

Cohn, M., and Hughes, T. (1962), *J. Biol. Chem. 237*, 176.

Desai, A. G., Dodgen, H. W., and Hunt, J. P. (1969), *J. Amer. Chem. Soc. 91*, 5001.

Desai, A. G., Dodgen, H. W., and Hunt, J. P. (1970), *J. Amer. Chem. Soc. 92*, 798.

Dye, J. L., and Niceley, V. H. (1971), *J. Chem. Educ. 48*, 443.

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

Grant, M., Dodgen, H. W., and Hunt, J. P. (1971), *Inorg. Chem. 10*, 71.

Hague, D. N., and Eigen, M. (1966), *Trans. Faraday Soc. 62*, 1236.

Hague, D. N., Martin, S. R., and Zetter, M. S. (1972), *J. Chem. Soc., Faraday Trans. 1, 68*, 37.

Hague, D. N., and Zetter, M. S. (1970), *Trans. Faraday Soc. 66*, 1176.

Hewkin, D., and Prince, R. H. (1970), *Coord. Chem. Rev. 5*, 45.

Hunt, J. P. (1971), *Coord. Chem. Rev. 7*, 1.

Kuntz, G. P. P., Glassman, T. A., Cooper, C., and Swift, T. J. (1972), *Biochemistry 11*, 538.

Martell, A. E., and Taqui Khan, M. M. (1966), *J. Amer. Chem. Soc. 88*, 668.

McLachlan, A. D. (1964), *Proc. Roy. Soc., Ser. A 280*, 271.

Neely, J., and Connick, R. E. (1970), *J. Amer. Chem. Soc. 92*, 3476.

O'Sullivan, W. J., and Perrin, D. D. (1964), *Biochemistry 3*, 18.

Phillips, R. C., George, S., and Rutman, R. (1963), *Biochemistry 2*, 501.

Rechnitz, G. A., and Mohan, M. S. (1970), *Science 168*, 1460.

Reed, G. H., Leigh, J. S., Jr., and Pearson, J. E. (1971), *J. Chem. Phys. 55*, 3311.

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

Sternlicht, H., Schulman, R. G., and Anderson, E. W. (1965), *J. Chem. Phys. 43*, 3123.

Swift, T. J., and Connick, R. E. (1962), *J. Chem. Phys. 37*, 307.

Zetter, M. S., Dodgen, H. W., and Hunt, J. P. (1972), *Inorg. Chem. 11*, 2701.