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Infrared Spectra of Adenosine Triphosphate Complexes in Deuterium Oxide Solution[1]

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Infrared spectra have been obtained for 0.1 M Na_2H_2ATP in D_2O, alone and in the presence of 0.1 M divalent metal ions. Acid dissociation constants for H_2ATP^{2-} which are in good agreement with those determined by titrimetric methods have been calculated from the pD dependence of the spectrum. The effect of metal ions is seen in the shift in pD values at which ionizations of protons occur. On the basis of the shifts in pD values, the apparent formation constants of divalent metal ions with ATP are in the order $Cu^{2+} > Zn^{2+} > Ni^{2+} > Mg^{2+} > Ca^{2+}$. The formation constants for 1:1 complex formation with Mg^{2+} and Ca^{2+} have been calculated from the infrared data. Agreement with "best estimates" of these quantities based on published results is obtained only when the increased acidity of the proton bound to the adenine moiety is considered. This result is not interpreted to mean that metal binding necessarily occurs at the adenine moiety. It is proposed rather that complex formation occurs at the triphosphate group in advance of proton loss from the terminal phosphate. This view of the equilibria is in accord with other published evidence. Neither infrared nor pH titrimetric methods can provide a reliable measure of the formation constants for binding of metal ions under these circumstances, since there is not a necessary correspondence between proton displacement and metal binding.

The formation of complexes between divalent metal ions and adenosine triphosphate (ATP) has been the subject of numerous studies. A number of authors have dealt with the determination of formation constants for complex formation,[3-8] while others have aimed toward identification of the sites in ATP which are involved in coordination.[9-13] The suggestion that the adenine portion of ATP may be involved in binding of metal ions[14] has occasioned a number of these studies. Although it has been established that interaction of metal ions with adenosine is very weak,[4,8] the influence of the adenine moiety upon metal coordination in ATP is not presently well defined. The background in formation necessary for appreciation of the biological implications of metal ion coordination to ATP is therefore not complete at the present time.

Infrared spectral studies of species in aqueous medium can reveal considerable detail about the interaction of metal ions with ligands. We report here a study of ATP interaction with divalent metal ions in D_2O solution. Some of the quantitative results require modification of views based upon earlier work.

Experimental

Materials.—Adenosine triphosphate, as $Na_2H_2ATP \cdot 4H_2O$, was obtained from the Sigma Chemical Co. and used without further purification.

Deuterium oxide, 99.8% isotopic purity, was obtained from Volk Chemical Co.

The metal ions were introduced as the chloride salts, except that potassium was employed in the form of the nitrate salt, and copper(II) nitrate was also used.

Solution pD values were adjusted using a concentrated NaOD solution prepared by dissolving clean sodium metal in D_2O. A concentrated DCl solution was prepared as described elsewhere[15]; in general, it was not necessary to use acid in pD adjustment. The pD values of the solutions were measured using a Beckman Zeromatic pH meter, with a one-drop electrode assembly, calibrated with standard H_2O buffer solutions. The pD values were in all cases taken to be[16] pD = meter reading + 0.40.

All spectra of ATP solutions were obtained immediately after preparation of the solution, to avoid hydrolysis of the ATP. The temperature of the solutions during the course of spectral measurements was about 32°.

Infrared Spectra.—Spectra were obtained using a Beckman IR-7 spectrophotometer. Eastman Kodak IRTRAN-2 plates, as well as fixed-thickness cells (0.025 mm.) with BaF_2 windows, were employed. The instrument was operated in double-beam operation with a variable transmittance shutter in the reference beam.

The absorbances of each of the conjugate absorptions in the 1630–1670-cm.$^{-1}$ pair were studied for ATP alone over the concentration range from 0 to 0.20 M, using base-line techniques. Series of measurements were carried out at pD values of 3.5 and 12. At the higher pD value only the 1630-cm.$^{-1}$ band is present, at the lower pD value only the 1670-cm.$^{-1}$ band. Graphs of absorbance *vs.* molarity showed that the 1630-cm.$^{-1}$ band is somewhat more intense. The ratio of extinction coefficients in the concentration range of interest (about 0.05 M) is 1.20. The experiments were repeated in the presence of 0.05, 0.1, and 0.15 M $MgCl_2$. No change in the extinction coefficient ratio was observed.

In studying the pD dependence of the ATP spectrum, it is of interest to know the pD value at which the forms which give rise to the 1630- and 1670-cm.$^{-1}$ bands are present in equal concentrations. This pD value, which we refer to as the half-reaction point, was determined by measuring the absorbance of the two bands as a function of pD (Fig. 1). When the ratio of absorbances is 1.20, equal concentrations of the two forms are present. The pD values of the half-reaction point can be determined in this way to within ±0.05 pD unit.

The 965–995-cm.$^{-1}$ pair of conjugate absorptions were dealt with similarly. In this instance, the bands are not very intense, and overlapping with adjacent bands occurs. Experiments with ATP alone showed that the two absorptions possess about equal extinction coefficients. The pD value corresponding to equal absorbances could be estimated to within only about 0.2 pD unit, since a certain amount of subjective judgment is involved in selection of the condition which is to represent the half-reaction point. Once a given ratio of the two absorptions is chosen, however, this same condition can be determined for other solutions to within 0.1 pD unit. Thus, having decided upon a spectral condition to represent half-reaction for solutions of ATP alone, the same point can be determined for the solutions of ATP with metal ions to good precision. That the point chosen for ATP alone is correct is demonstrated by the calculation of an acid dissociation constant for ATP on this basis which is in reasonable agreement with other work (*vide infra*).

The spectra reported upon were obtained in fixed-thickness

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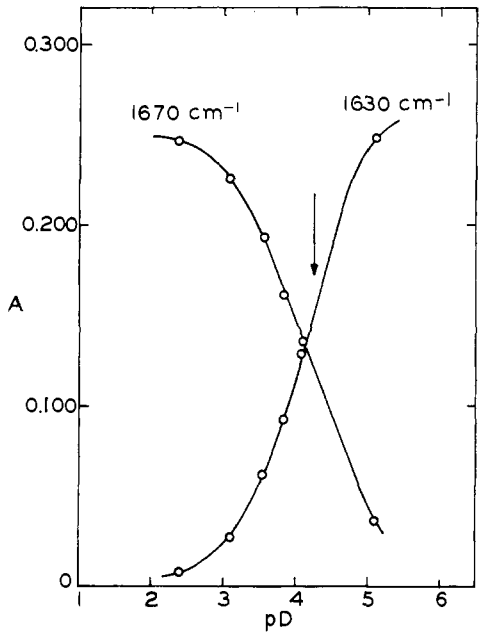


Fig. 1.—Absorbances of the 1670 and 1630 cm.$^{-1}$ absorptions as a function of pD for 0.1 M Na_2H_2ATP + 0.1 M $ZnCl_2$ solution; the arrow indicates the pD corresponding to equal concentrations of the protonated and nonprotonated forms.

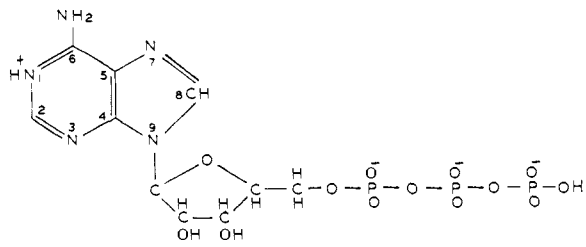
cells. It has been observed, however, that for purposes of comparing two bonds in the same spectrum, it is sufficient to use merely a pair of IRTRAN-2 plates with a drop of solution between them. With no spacer between the plates, a path length of about 0.02 mm. results.

Experiments were also performed in H_2O solution; pure water in a nearly matched cell was employed in the reference beam. Only the lower frequency region can be examined for H_2O solutions, because of solvent absorption in the 1600–1700-cm.$^{-1}$ region. A very steeply changing solvent absorption as a function of frequency in the 925–1000-cm.$^{-1}$ region makes it difficult to obtain satisfactory spectra. It was not found possible to make reliable absorbance measurements on the 965-cm.$^{-1}$ band, but the 995-cm.$^{-1}$ band could be measured easily. The half-reaction points for the H_2O solutions, corresponding to loss of proton from the triphosphate group, were therefore determined by graphing the absorbance of the 995-cm.$^{-1}$ band as a function of pH and choosing the pH value at which the absorbance is half-way between the two extremes. The uncertainty in this measurement is estimated to be about ±0.2 pH unit.

Results

The spectra of 0.1 M Na_2H_2ATP solutions at various pD values is shown in Fig. 2.[17] The pair of absorptions at 1630 and 1670 cm.$^{-1}$ correspond, respectively, to the nonprotonated and protonated form of the adenine moiety.[18] The band at 1585 cm.$^{-1}$, due to a purine ring

(17) The notation employed in this paper is as follows: (a) the symbol ATP refers to adenosine triphosphate without specification as to the overall charge; the symbol H_2ATP^{2-} refers to the species with the structure



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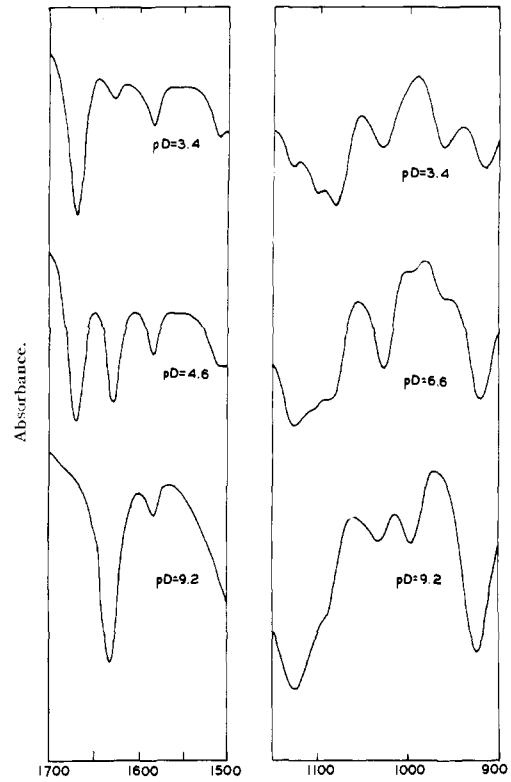

Fig. 2.—Infrared spectra of 0.1 M Na_2H_2ATP solutions in D_2O at various pD values; frequencies are given in cm.$^{-1}$.

mode, is not affected by pD changes. The absorptions attributable to the triphosphate group, occurring in the 900–1150-cm.$^{-1}$ region,[19] exhibit a pD dependence, as shown. The band associated with P–O–P stretching shifts from 915 cm.$^{-1}$ at pD 3.4 to 925 cm.$^{-1}$ in basic solutions. Loss of proton from the terminal phosphate group is manifested chiefly by the behavior of the 965- and 995-cm.$^{-1}$ pair, attributable, respectively, to the protonated and nonprotonated terminal phosphate symmetric stretching mode.

The trio of absorptions appearing at 1082, 1105, and 1130 cm.$^{-1}$ in acid solution are due to the triphosphate group, with probably some coupling to the C–O–P stretching mode. The band at 1082 cm.$^{-1}$ appears to decrease in intensity somewhat on loss of the proton from adenine; the 1105-cm.$^{-1}$ band disappears, and an 1125-cm.$^{-1}$ band grows in intensity, in the same pD range which characterizes the disappearance of the 965-cm.$^{-1}$ absorption and appearance of the 995-cm.$^{-1}$ absorption. The absorption at 1125 cm.$^{-1}$ in basic solution is assigned to the asymmetric $-PO_3^{2-}$ stretch.

A comparison of the spectra of adenosine and ribose in D_2O shows that the band at 1032 cm.$^{-1}$, which shows no pD dependence, is due mainly to the ribose moiety.

The infrared spectral results show clearly that ionization of the first proton from H_2ATP^{2-} is from the adenine moiety. We are in agreement with earlier conclusions[18] that protonation occurs at N_1.

Since the relative extinction coefficients of the 1630–1670-cm.$^{-1}$ pair of absorptions, and of the 965-995-cm.$^{-1}$ pair, are known, the pD values corresponding to equal concentrations of the forms giving rise to those absorptions can be determined. The pD values represent the apparent pK_a values for H_2ATP^{2-}. An

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analogous conclusion follows for the 1630–1670-cm.$^{-1}$ absorptions of adenosine.

For 0.1 M Na_2H_2ATP in D_2O, the pD values are 4.65 and 6.5, respectively. In order to compare these with pK_a values obtained by other means, correction must be made for the solvent isotope effect, and for binding of Na^+. Assuming that pK for an N–H (N–D) dissociation is 0.6 higher in D_2O than in H_2O[20] and that pK for a P–O–H (P–O–D) dissociation is 0.2 higher,[21] correction for the isotope effect leaves apparent pK_a values of 4.05 and 6.3. The former is in good agreement with the 4.06 value obtained by Khan and Martell[8] for Na_2H_2ATP at $\mu = 0.1$ (25°). The higher pK_a value compares well with 6.41 obtained by Smith and Alberty[22] for H_PATP^{3-} in 0.2 M NaCl at 25°, conditions similar to those employed in the present work.

The apparent pK_a for the proton bound to triphosphate—the higher value—must be corrected also for binding by Na^+ or K^+. This correction can be made with sufficient accuracy by assuming that the formation constants for complex formation are the same,[22] for Na^+ and K^+, with a value of 12 M^{-1}. We have

$$H_PATP^{3-} \rightleftharpoons ATP^{4-} + H^+$$

$$K_a = \frac{(ATP^{4-})(H^+)}{(H_PATP^{3-})}$$

Since the infrared spectrum distinguishes only between protonated and nonprotonated forms, the apparent dissociation constant, K_a', is

$$K_a' = \frac{(ATP^{4-} + MATP^{3-})(H^+)}{(H_PATP^{3-})}$$

where M represents Na^+ and/or K^+. Combining this with the equilibrium

$$M^+ + ATP^{4-} \rightleftharpoons MATP^{3-}$$

$$K_M = \frac{(MATP^{3-})}{(M^+)(ATP^{4-})}$$

we have

$$K_a' = K_a[1 + K_M(M^+)]$$

The pD value at which equal intensities are observed for the 965–995-cm.$^{-1}$ conjugate absorptions corresponds to the condition $(H_PATP^{3-}) = (ATP^{4-}) + (MATP^{3-}) = (ATP)_0/2$, and $(M^+)_0 = (M^+) + (MATP^{3-})$, where the subscript indicates initial concentration. By a simple iterative procedure, the correct value of K_a is derivable from the known concentrations and observed spectral behavior. After correction for both the solvent isotope effect and alkali metal binding, the pK_a values obtained for 0.1 M Na_2H_2ATP alone in D_2O and in the presence of 0.3 M KNO_3 are 6.7 and 6.9, respectively. Smith and Alberty[22] obtained 6.95 at $\mu = 0.2$; Khan and Martell[8] obtained a value (after correction for K^+ binding) of 6.87 at $\mu = 0.1$. The infrared spectral observations are thus clearly capable of yielding information regarding proton dissociations of ATP which is in reasonable agreement with the more precise titrimetric values.

It will be necessary in subsequent discussion to have pK_a values which are corrected for alkali metal ion binding, but not for the solvent isotope effect. On the basis of the data for 0.1 M Na_2H_2ATP alone and with 0.3 M KNO_3, these values are taken to be 4.65 and 7.0. The latter value is an average of the corrected values obtained for 0.1 M Na_2H_2ATP alone and in the presence of 0.3 M KNO_3 (Table I).

TABLE I

pD VALUES CORRESPONDING TO HALF-REMOVAL OF PROTONS FROM 0.1 M H_2ATP^{2-} AND 0.1 M ADENOSINE IN VARIOUS SOLUTIONS

Added electrolyte	1630–1670 cm.$^{-1}$	965–995 cm.$^{-1}$
Adenosine triphosphate		
None	4.65	6.5 (6.9)[a]
0.3 M KNO_3	4.55	6.3 (7.1)[a]
0.1 M $CaCl_2$	4.40	5.3
0.1 M $MgCl_2$	4.40	4.7
0.1 M $NiCl_2$	4.30	4.3
0.1 M $ZnCl_2$	4.25	3.9
0.1 M $CuCl_2$	4.15	3.1
0.1 M $Cu(NO_3)_2$	4.00	3.0
Adenosine		
None	3.80	
0.3 M KNO_3	3.75	
0.1 M $CuCl_2$	3.60	

[a] pK_a value for $HATP^{3-}$ after correction for binding by Na^+ and/or K^+ (see text).

A comparison of the spectra of 1:1 divalent metal ion–ATP solutions with spectra of solutions of ATP alone reveals two important facts: (a) the pD dependence of the ATP spectrum is markedly affected by metal ions; (b) metal ion coordination does not produce any significant frequency shifts in the ATP spectrum other than those associated with loss or gain of a proton. Thus, the absorption which occurs at 915 cm.$^{-1}$ in ATP solution of pD 3.5 and lower is shifted to 922 cm.$^{-1}$ on formation of the copper complex at higher pD values, whereas upon increase of pD in solutions of ATP alone, the frequency is shifted to 925 cm.$^{-1}$.

Table I lists the pD values at which absorbance ratios corresponding to equal concentrations of the contributing forms are observed for the 1630–1670- and 965–995-cm.$^{-1}$ conjugate pairs of absorptions, for a number of ATP and adenosine solutions.

Discussion

It is a matter of considerable interest that coordination of metal ions to ATP does not produce significant shifts in the absorptions characteristic of the triphosphate group, other than those which accompany loss of proton. In these circumstances, coordination of metal ion must be inferred from the effect which the metal exerts on the acidities of the protons in H_2ATP^{2-}. The infrared spectra are of great value in this context, however, since the individual proton equilibria are clearly distinguishable.

A number of interesting conclusions follow from the pD values listed in Table I for ATP and adenosine in the presence of 0.1 M divalent metal ion.

From the pD values listed in Table I for half-reaction, as measured by changes in the 965–995-cm.$^{-1}$ conjugate pair of absorptions, the equilibrium constants for metal binding at the triphosphate group are in the order $Cu^{2+} > Zn^{2+} > Ni^{2+} > Mg^{2+} > Ca^{2+}$.

Since it is possible to observe separately the influence of metal ions upon the individual proton equilibria of

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H_2ATP^{2-}, it is convenient to distinguish the adenine (H_A) and triphosphate (H_P) protons. In view of their particular biochemical importance, the Mg^{2+} and Ca^{2+} systems have been most extensively investigated. The important equilibria for these two metal ions may be written as

$$M^{2+} + H_AH_PATP^{2-} \rightleftharpoons MH_PATP^- + H^+ \quad (1)$$

$$MH_PATP^- \rightleftharpoons MATP^{2-} + H^+ \quad (2)$$

$$M^{2+} + H_PATP^{3-} \rightleftharpoons MATP^{2-} + H^+ \quad (3)$$

$$H_AH_PATP^{3-} \rightleftharpoons H_PATP^{3-} + H^+ \quad (4)$$

$$H_PATP^{3-} \rightleftharpoons ATP^{4-} + H^+ \quad (5)$$

$$K_1 = \frac{(MH_PATP^-)(H^+)}{(M^{2+})(H_AH_PATP^{2-})} \qquad K_2 = \frac{(MATP^{2-})(H^+)}{(MH_PATP^-)}$$

$$K_3 = \frac{(MATP^{2-})(H^+)}{(M^{2+})(H_PATP^{3-})} \qquad K_4 = K_{a_1} = \frac{(H_PATP^{3-})(H^+)}{(H_AH_PATP^{2-})}$$

$$K_5 = K_{a_2} = \frac{(ATP^{4-})(H^+)}{(H_PATP^{3-})}$$

We begin with the assumption that complex formation occurs only at the triphosphate group and that the terminal triphosphate proton is entirely displaced upon complex formation, *i.e.*, that eq. 3 represents the important equilibrium. K_3 is evaluated by setting $(MATP^{2-}) = (H_PATP^{3-})$ and $(M^{2+}) = (M^{2+})_0 - (MATP^{2-}) = (M^{2+})_0/2$ at the pD value corresponding to equal intensities of the 965–995-cm.$^{-1}$ absorption pair (Table I). The values of log K_3 obtained for Ca^{2+} and Mg^{2+} are -4.0 and -3.4, respectively. The equilibrium

$$M^{2+} + ATP^{4-} \rightleftharpoons MATP^{2-} \quad (6)$$

is evaluated by combining eq. 3 and 5.

$$K_f = \frac{(MATP^{2-})}{(M^{2+})(ATP^{4-})} = \frac{K_3}{K_5}$$

pK_5 is pK_a for H_PATP^{3-}, corrected for alkali metal ion binding, 7.0. The resulting values of log K_f are 3.0 and 3.6 for Ca^{2+} and Mg^{2+}, respectively. It is of interest to compare these values with those reported previously in the literature. A number of values for log K_f, determined by various workers, are listed in Table II.

TABLE II
SURVEY OF FORMATION CONSTANTS FOR THE INTERACTION OF Ca^{2+} AND Mg^{2+} WITH ATP, EQ. 6

Metal ion	Log K_f	Method	Ref.
Ca^{2+}	3.78	Anion resin adsorption, μ = 0.1, 23°	6
	3.97	Resin adsorption	7
	3.92	Titrimetric (pH shift)	7
	3.90	Colorimetric	7
	3.97	Titrimetric, μ = 0.1, 25°	8
	3.29	Titrimetric, μ = 0.1, 25°	3
Mg^{2+}	4.74	Spectrophotometric, μ = 0.11, 37°	5
	4.04	Adsorption, μ = 0.1, 23°	6
	4.35	Resin adsorption	7
	4.43	Titrimetric (pH shift)	7
	4.22	Titrimetric	8
	3.47	Titrimetric	3

Although wide variations in the reported results are evident, "best" values of log K_f for Ca^{2+} and Mg^{2+} would seem to be approximately 3.9 and 4.3, respectively. These are considerably higher than our results. It is extremely unlikely that the differences can be accounted for in terms of ionic strength effects, or in terms of a solvent isotope effect. Experiments were also conducted in equimolar D_2O solutions of Na_2H_2ATP and metal ion at concentrations of 0.2 and 0.05 M. The values calculated for log K_f were the same as those obtained at 0.1 M to within about 0.1 log unit (the data for the 0.05 M solutions is perhaps less certain than this because of experimental difficulties). A study of 0.1 M Na_2H_2ATP in H_2O, and in the presence of 0.3 M KNO_3, 0.1 M $CaCl_2$, or 0.1 M $MgCl_2$ yielded values for log K_f of 3.3 and 3.7 for Ca^{2+} and Mg^{2+}, respectively. Despite the experimental difficulties encountered in H_2O work, the agreement with the infrared D_2O data is not bad. A further indication that the solvent isotope effect is not large is found in the fact that the formation constant of the cadmium-glycylglycine complex is 0.2 log K *larger* in D_2O than in H_2O.[20] The discrepancy cannot be accounted for in terms of a temperature difference. Burton[5] reports a modest *increase* in K_f for Mg^{2+} with increasing temperature, on the basis of measurements at 25 and 64°. We conclude, therefore, that the discrepancy in the values obtained from the infrared work and those obtained by other methods must be accounted for in terms of a difference in the nature of the measured quantity.

The acidity of the proton bound to adenine in adenosine is only slightly increased by the presence of 0.1 M copper(II) ion (Table I). This indication of very low complexing tendency is in agreement with prior titrimetric work.[4,8] There is, however, indication of significant increase in acidity of the adenine proton in ATP–metal ion solutions. We therefore assume complex formation at the adenine moiety and calculate an apparent value for K_1 based upon the assumption that eq. 1 holds. The apparent acid dissociation constant of the adenine proton in the presence of metal ion can be written as

$$K_{a_1}(\text{app.}) = \frac{[(MH_PATP^-) + (H_PATP^{3-})](H^+)}{(H_AH_PATP^{2-})}$$

$$= K_{a_1} + K_1(M)$$

$$K_1 = \frac{K_{a_1}(\text{app.}) - K_{a_1}}{(M)}$$

K_{a_1} (app) is calculated from the pD value corresponding to equal concentrations of the forms contributing to the 1630–1670-cm.$^{-1}$ absorption pair, at which $(MH_PATP^-) + (H_PATP^{3-}) = (H_AH_PATP^{2-}) = 0.05$ M. Log K_{a_1} is similarly defined for solutions of H_2ATP^{2-} alone. The correct value of (M) is calculated by an iterative procedure using the above relationships along with the equilibrium constant expression for eq. 1. To within the relatively low precision of the infrared data, K_1 has the same value for Ca^{2+} and Mg^{2+}, about 2×10^{-4}. An over-all *apparent* formation constant, K_f', of the form

$$M^{2+} + ATP^{4-} \rightleftharpoons MATP^{2-}$$

can be defined as

$$K_f' = K_1K_3/K_{a_1}K_{a_2}$$

Log K_f' values calculated for Ca^{2+} and Mg^{2+} from the infrared data are 3.9 and 4.6, respectively. These values, admittedly not very precise, are nevertheless in reasonable agreement with the "best" values given above, based upon other methods.

In the methods employed by previous investigators, which include titrimetric, colorimetric, and resin adsorption, the apparent formation constants are based

variously on determination of the activity of uncomplexed metal ion (colorimetric), activity of uncomplexed ATP (resin adsorption), or activity of displaced protons (titrimetric).[23] From the infrared results it appears that the agreement between titrimetric and the other methods is fortuitous. If the displacement of proton from the triphosphate group alone is measured, as in the present work, a low value for log K_f results. The assumption that a prior complex formation occurs at the adenine moiety, with consequent increase in the acidity of the proton bound to N_1, serves to remove the discrepancy. There is good reason for believing, however, that the increased acidity of that proton does not result from complex formation there, but is merely a manifestation of complex formation at the triphosphate group, *without loss of terminal proton*. Watanabe, Evenson, and Gulz[12] have shown that Ca^{2+} and Mg^{2+} both cause a marked reduction of fluorescence in ATP at pH values as low as 2. The reduction in fluorescence is ascribed to interference on the part of the metal ions with triphosphate–adenine interaction,[24] as a result of metal ion coordination. Furthermore, Cohn and Hughes[11] have cited n.m.r. evidence for coordination of Mn^{2+} with H_PATP^{3-}. An increase in the acidity of the adenine proton might very well result as an additional consequence of the metal ion coordination; there is n.m.r. evidence for it in the case of Mg^{2+} coordination. It is obvious that if coordination of metal ion occurs at the triphosphate group in acid medium without an equivalent displacement of proton, the titrimetric method cannot be employed for accurate evaluation of formation constants.

The influence of the metal ion on the acidity of the adenine proton is increasingly evident as the over-all strength of metal ion binding to the triphosphate group increases (Table I). This may be ascribed to increasingly larger formation constants for binding of metal ions to the triphosphate group in advance of ionization of the terminal proton. In summary, the equilibria proposed are

$$M^{2+} + H_AH_PATP^{2-} \rightleftharpoons MH_AH_PATP \qquad (7)$$

$$MH_AH_PATP \rightleftharpoons MH_PATP^- + H^+ \qquad (8)$$

$$MH_PATP^- \rightleftharpoons MATP^{2-} + H^+ \qquad (9)$$

In the case of Ca^{2+} and Mg^{2+}, equilibrium 8 occurs at a lower pH than (9); for the other metal ions, the two processes occur in the same pH range or, as in the case of Cu^{2+}, become reversed in order. In any case, it is assumed that the deprotonation effect observed at the N_1 position results from the first equilibrium. It must be said, however, that it is not possible to say, on the basis of information presently available, that the increased acidity at N_1 is not due in some measure to an interaction between the coordinated metal ion and the adenine moiety. The metal ion may be weakly complexed to the 6-amino group or the N_7 nitrogen, or the complex may persist in a folded configuration so that an appreciable field effect is experienced at N_1.[25] The proton resonance shifts and broadenings observed[11] for the zinc, copper, and manganese complexes could also arise from field effects as well as from specific coordination at N_7.

In view of the argument presented above, neither the pD shifts measured in the infrared nor pH titrimetric data[8,25] can be employed in calculating reliable values of the formation constants for the divalent metal ions. The most that can be said is that the changes in acidity of the triphosphate proton establish a lower bound for the formation constants and that the relative magnitudes of the shifts in pD values for half-reaction probably correspond to the relative order of formation constants. In this connection, it should be noted that Ni^{2+} and Zn^{2+} are inverted in order on the basis of the infrared data as compared with the titrimetric[8] results.

The infrared data do not permit any conclusions to be drawn as to whether ATP coordinates to the various metal ions as a bidentate or tridentate ligand. This question has been discussed in connection with ^{31}P n.m.r. studies.[11] Further, the absence of significant frequency shifts on coordination, other than those due to loss of proton, preclude any meaningful conclusions about the hydrolysis of the bound, aquated metal ion.[13] It is possible that the acidity of water molecules bound to the coordinated metal ion is enhanced, and that this might affect the apparent acidity of the proton bound to the terminal phosphate. The infrared results, however, show only that the proton attached to the triphosphate group is made more acidic by coordination; there is therefore no evidence for the "localized hydrolysis" proton transfer suggested by Brintzinger.[13]

(23) There is a discrepancy in the agreement of Nanninga's[7] values for K_f, obtained by the pH shift method, and the other values listed. The pH shift method does not involve displacement of proton from adenine, and should therefore yield a lower value for K_f than the other methods, including a general titrimetric procedure.[8,8]

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Synthesis and Fluorescence of Some Trivalent Lanthanide Complexes

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A variety of new trivalent lanthanide complexes has been prepared. They include tris(β-diketone) chelates complexed with bidentate ligands of the phenanthroline type, and with N- and P-oxide donors. Of particular interest is a broad class of anionic tetrakis(β-diketone) derivatives which we believe to be eight-coordinate. Well-resolved fluorescence emission spectra of several europium complexes are recorded.

The coordination chemistry of the lanthanide elements has been largely characterized by complexes with oxygen-containing donors, particularly β-diketones. These derivatives have been formulated as