AN INVESTIGATION BY RAMAN SPECTROSCOPY OF THE BASE-PROTON DISSOCIATION OF ATP IN AQUEOUS SOLUTION AND THE INTERACTIONS OF ATP WITH Zn^{++} AND Mn^{++}

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SUMMARY

By studying the pH dependent frequency of particular Raman lines in the spectrum of ATP metal ion complexes in 20 mM water solutions, one can determine the dissociation of both the secondary phosphate proton and the base proton. For ATP alone or its complexes with Ca and Mg the phosphate Raman line changes only at the pK_a for phosphate dissociation, while in the Zn and Mn complexes this same line also shifts at the pK_a for dissociation of adenine, indicating that Zn^{++} or Mn^{++} binding involves both moieties. Quantitative stability constants are obtained, indicating weaker binding of Zn and Mn than of Ca and Mg.

A number of investigations have indicated that there is a marked difference in the nature of the complexes of ATP with various divalent metal ions.[1-12] These complexes play a fundamental role in the enzymatic hydrolysis of ATP. Ca^{++} and Mg^{++} are by far the ions most commonly encountered in ATPase reactions of living systems.[13,14] However, a number of other divalent cations, in particular Zn^{++} and Mn^{++}, have been found to take part as activators or inhibitors in ATPase hydrolysis.[15-19] In addition, Zn^{++} and Mn^{++} have been used in many artificial model systems as activators, especially Mn^{++} which, due to its magnetic moment, permits the application of the very sensitive EPR technique.[19-23] Raman studies on the ATP complexes of Ca^{++} and Mg^{++} have shown that these ions bind strongly to the phosphate moiety.[12] This result has already been deduced indirectly from measurements by several other techniques.[6-8] The Raman studies also revealed additional properties of ATP complexes, concerning dimer formation and exchange rates for deprotonation and complex formation.[24] It has been indicated that some divalent transition metal ions bind to the adenine moiety as strongly as to the phosphate.[6,9,10] This conclusion, however, has been the subject of some controversy.[3-5] In this paper we report on features of the Raman spectrum

characteristic of the base moiety of ATP, which illustrate the effects of divalent metal ion binding. In addition, data on the phosphate indicator Raman line, previously used in the study of Ca and Mg ATP complexes, are presented here for Zn and Mn. These curves clearly indicate a Zn^{++} and Mn^{++} mediated interaction between the phosphate chain and the base of the ATP molecule. It has been concluded, therefore, that for Zn and Mn in millimolar solutions with ATP/M^{++} ratio ~ 1, a complex is formed in which the two ends of the molecule are coupled by the metal ion, while such coupling is absent for Ca and Mg. Further, the data indicates that the binding of Zn and Mn to the phosphate moiety is weaker than that of Ca and Mg.

In an earlier work a number of Raman lines have been identified with the adenine moiety of ATP;[25] most of them change in the dissociation region of the base in a fashion corresponding to a slow chemical exchange mechanism, i.e., there is a progressive interchange of intensities between the lines corresponding to protonated base and those corresponding to the deprotonated base, without frequency shifts, as the pH is varied through the pK_a.[12,24] Thus the determination of the pK_a employing such lines involves intensity measurements, which are much less satisfactory than frequency measurements. There is, however, a strong line at 1330-1340 cm^{-1} which shifts in frequency continuously in this region, and can be used as an indicator line just as the 1125 cm^{-1} phosphate line was used in earlier work.[12] Fig. 1 shows spectra in this region at various pH values, illustrating the preceding argument. Fig. 2 shows the frequency vs pH plots of the indicator line of the base for .02M Na_2H_4ATP alone, and with additions of various divalent cations. Table 1 displays the apparent pK_a of the base, obtained from these data (pK_a is the pH for the midpoint in the transition). Fig. 2 also gives the pH dependence of the frequency of the phosphate indicator line of the Zn and Mn complexes. The data for a pure ATP solution and an equimolar Ca ATP complex are also shown for comparison. All data were taken at $(25 \pm 1)^{\circ}C$.

Consideration of the data indicates, first, that the depression of the base pK_a from its value for the pure ATP solution to that in the equimolar complexes

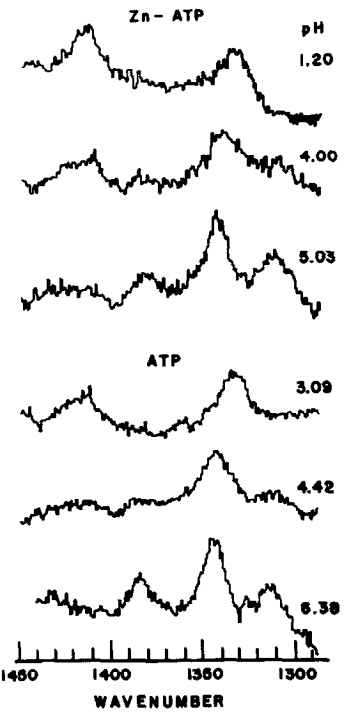

Fig. 1 Typical recorder tracing of the Raman lines characteristic of the adenine moiety of ATP: top .02M ATP + .015M $ZnCl_2$
bottom .02M ATP

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is only by a fraction of a pH unit, unlike the situation for the depression of the phosphate pK'_a. This fact qualitatively indicates that the binding of the metal ion to the base is probably weaker than to the phosphate. Secondly, in Ca and Mg complexes the pK'_a of the phosphate is in the same region as that of the base,[12,24] and, therefore, we expect a strong interference between the two dissociation processes, even without any binding of the metal ion to the adenine. The simultaneous dissociation at two sites in the molecule tends to depress the pK_a values for both sites, and therefore the small depression of the base pK_a in the presence of these ions cannot be taken as proof of binding to the adenine. The situation, however, is different for Zn and Mn. If we consider the phosphate vibration frequency of the Zn and Mn complexes (Fig. 2), we observe two transitions: the one at lower pH corresponds to a change at the phosphate moiety, just in the region where the adenine dissociation is taking place, whereas the

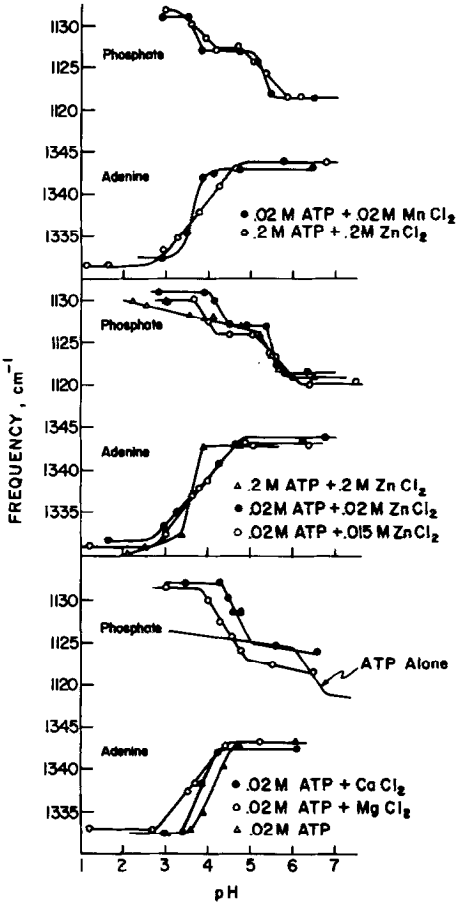

Fig. 2 pH dependence of the center frequency of the Raman triphosphate line (1130-1120 cm^{-1}) and the adenine line (1345-1335 cm^{-1}) in various ATP solutions

higher pH frequency jump occurs when the adenine is completely deprotonated. The latter should correspond to the ionization of the terminal phosphate. The first transition strongly suggests that in the Zn and Mn complexes there is an interaction between the base and the phosphate components of the molecule. The interaction should be looked upon as specifically induced by the complexing of ATP with these two ions, since the effect is not present in pure ATP. This interaction effect is most marked at the lower ATP concentrations, and tends to be washed out as the ATP concentration is increased, as shown by the points for 0.2M Zn ATP, in Fig. 2. Formation of ATP-M-ATP complexes is probably respon-

TABLE I

Sample	Base pK_a from 1330 cm^{-1} line	Base pK_a from phosphate line	Phosphate pK'_a from phosphate line
.02M ATP	4.15 ± .05		6.5 ± .05[24]
.2M ATP	4.15		6.5[12]
.02M ATP, .02M $MgCl_2$	3.68		4.47[24]
.02M ATP, .02M $CaCl_2$	3.82		4.71[24]
.02M ATP, .015M $ZnCl_2$	3.76	3.9 ± .05	5.8
.02M ATP, .02M $ZnCl_2$	3.74	4.2	5.6
0.2M ATP, 0.2M $ZnCl_2$	3.65	3.5	5.42
.02M $MnCl_2$, .02M ATP	3.63	3.66	5.26
.015M $MnCl_2$, .02M ATP	3.70	3.72	5.36

Table I Apparent and dissociation constants of the adenine moiety and the secondary proton on the triphosphate moiety of ATP.

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sible for this washing out effect; we expect a larger variety of such complexes for Zn^{++} and Mn^{++} than for the alkaline earth ions, due to their binding to both the base and phosphate moieties. The fact that the lowering of ATP concentration enhances the lower pH frequency jump supports the contention that Zn and Mn bind to ATP in such a way as to cause an intra-molecular interaction between the adenine and the triphosphate; such an interaction must be much less important in the complexes with Mg and Ca.

The present results do not allow one to distinguish between the following two possibilities for Zn ATP and Mn ATP: a) there is a chemical equilibrium between two complexes, those with the metal bound only to the phosphate and those where it binds to both phosphate and base;[4] b) there is one type of complex involving both moieties.[10] The lack of structure in the phosphate line (at all pH values, even in the nonequimolar solutions) indicates that if possibility a) held, the rate of chemical exchange between the two kinds of complexes would

have to be rather fast ($> 10^{10}$ sec^{-1}).[12] Since in both Mn and Zn complexes the pK'_a for phosphate proton ionization occurs appreciably above that for the base, we can use the observed values of this pK'_a for two different metal concentrations (constant [ATP] = 0.02M) and obtain the stability constants for the complexes with protonated and deprotonated phosphate (both with deprotonated base): $K_{M_1} = [ATPHM^{-1}]/([ATPH^{-3}][M^{+2}])$ and $K_{M_2} = [ATPM^{-2}]/([ATP^{-4}][M^{+2}])$. The assumptions for the calculation are similar to those used in the analysis of titration data.[1] Details and application to the Ca and Mg complexes are to be published elsewhere.[24] For possibility b) this procedure yields the correct stability constant. However, if a) were the case, the correct calculation would have to involve the equilibrium between the two complex forms, while the values, given in Table 2, only measure some average of the two corresponding binding constants. The present results for Zn and Mn are lower than those for Ca and Mg, and lower than earlier values obtained by direct titration, EPR and IR.[1-5,19,25] Some values even smaller than those given in the table have been indicated.[20] Since in all cases similar assumptions have been used in the data reduction, the discrepancies are probably related to the presence of additional components in the solutions, such as buffering molecules and excess concentration of alkali ions.[24] The present samples contained the disodium salt of ATP and the chloride of the metal ion, and the only additive was Na_2O for pH adjustment. We feel that the values in Table II should be considered reliable since they are derived from accurate spectroscopic frequency measurements, which are sensitive to structure.

The fact that Zn and Mn interact more strongly with the base than the biologically more important Ca and Mg tends to support the idea that in living systems it is the metal-phosphate interaction that plays the fundamental role in ATPase mechanisms. The role of the adenine moiety is more probably related to the "modification" of the enzyme,[27] i.e., its interaction with the residues near the active site imposes a conformational change to the enzyme which presumably is important for the catalytic effect. If this is the case, then when Mn or Zn are substituted for Ca and Mg in a model system the activities of the ATPase

TABLE II

	$\log K_{M_1}$	$\log K_{M_2}$
Zn ATP	.92 ± .05	3.40 ± .05
Mn ATP	1.26 ± .04	3.81 ± .09
Ca ATP	1.0 ± .4	3.9 ± .4
Mg ATP	1.7 ± .2	4.6 ± .2

Table II Stability constants for metal ATP complexes. pK_{M_1} below and pK_{M_2} above secondary proton dissociation.

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for specific nucleotide triphosphates should be altered.

In summary, we have extended the "Raman titration" of ATP-metal ion complexes to lower concentrations, within an order of magnitude or less from that encountered in tissue.[13] It was found that, whereas Ca and Mg, which form the more stable complexes, bind only to the phosphate moiety, Zn and Mn bind more weakly to ATP forming a structure in which there is interaction between phosphate and adenine within the same molecule.

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