PN 10064

The structures of adenosine triphosphatemetal ion complexes in aqueous solution

The structures of binary complexes formed between ATP and bivalent metal ions in aqueous solution are far from being understood, as yet. The coordination of metal ions to the heterocyclic moiety of the ATP molecule is still controversial[1-3] and we still have no clear picture of the mode of fixation of these ions to the phosphate chain. By investigation of the changes in the various phosphate absorption bands of ATP in the 900–1300-cm^{-1} region, brought about by complex formation in aqueous solution[4], a further clarification of the latter question was achieved.

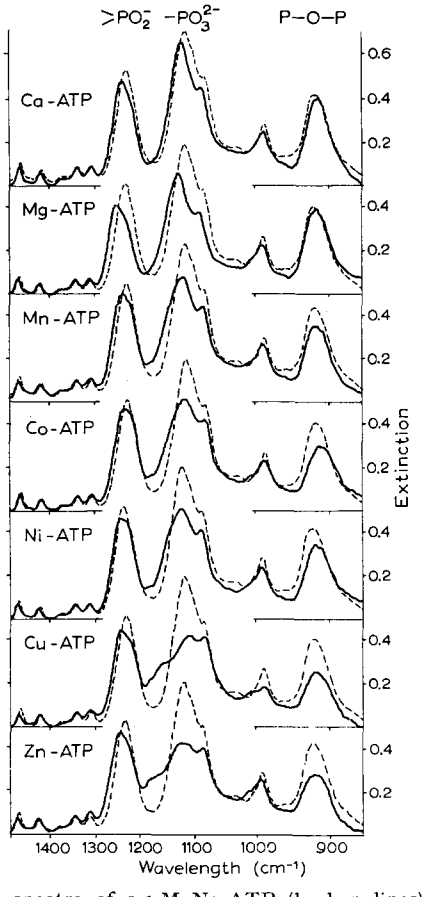


Fig. 1. Infrared absorption spectra of 0.2 M Na_4ATP (broken lines) and of some of its complexes (full lines) measured in 27-μ cells.

The absorption of 0.2 M aqueous solutions of Na_2H_2ATP (Sigma, crystalline grade) at pH 8.9 was measured with a Beckman IR 8 spectrophotometer in Eastman Kodak IRTRAN cells (path length 27 μ), against water as reference. The different metal ions (Ca^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}) were added in form of their

chlorides in amounts equimolar to ATP*, so that only the respective 1:1-complexes were present in the solutions. The following observations were made in the presence of the various metal ions (Fig. 1):

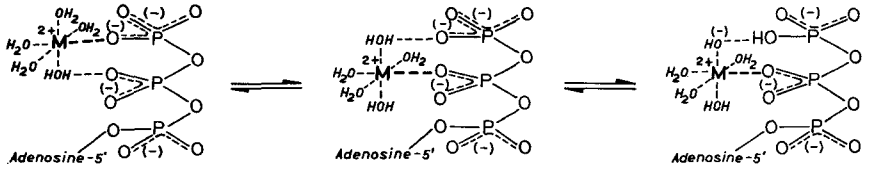
(1) The antisymmetric stretching band of the terminal $-PO_3^{2-}$ group, which has its maximum at 1115 cm^{-1} in ATP, was split to a triplet by complex formation. The extent to which the triplet contributed to the various complex spectra was a direct measure of the percentage to which a conformational species (I) with an immediate coordination between metal ion and terminal $-PO_3^{2-}$ group occurred in the solution of the respective complex[5]. The statistical weight of this species ranged from about 1 with Cu–ATP and Zn–ATP to a few per cent with Ca–ATP. Intermediate values of 40–60% were found for Mg–, Mn–, Co– and Ni–ATP.

(2) The antisymmetric stretching band of the α and $\beta > PO_2^-$ groups at 1230 cm^{-1} was also distorted to higher frequencies. With some complexes (Ca–ATP, Mg–ATP) the band distortion was quite pronounced, but still far from the splitting observable in some model cases[5]. Thus it must be concluded that a coordinative interaction between metal ion and one of the $> PO_2^-$ groups (configuration II) occurred in a weak to moderate percentage in the complexes studied.

These statements ((1) and (2)) are compatible with results of an investigation by COHN AND HUGHES[1] on phosphorus nuclear magnetic resonance in ATP complexes.

(3) The P–O–P stretching band at 920 cm^{-1} decreased together with the $-PO_3^{2-}$ band at 1115 cm^{-1}. Chelate formation cannot account for this feature: in Hg–ATP (not shown in Fig. 1) no interaction of Hg^{2+} with a $> PO_2^-$ group was observed but the P–O–P band still decreased together with the $-PO_3^{2-}$ band.

(4) Another change was observed in the complex spectra at 990–1010 cm^{-1}. The stronger the acidity of the hydrated metal ion, the greater were these changes, which were very similar to those brought about by protonation of the phosphate chain. Thus they could indicate a "localized hydrolysis": the hydrated metal ion (*e.g.* of species II) loses, to an extent depending on its acidity, a proton to the terminal phosphate group to form a hydroxo species fixed by hydrogen bonding to the protonated phosphate group (III).



(I) (II) (III)

Neither the heterocyclic ring nor the sugar influence the features of metal ion–phosphate interaction. Results very similar in all respects have been obtained with methyl triphosphate in place of ATP[5].

Our data suggest that at least the species I, II and III can exist in equilibrium with each other in solution of ATP-complexes. It remains to be investigated with

* $CaCl_2$:ATP was only 0.8 to avoid formation of precipitates.

which of these species ternary enzyme–metal-ion–ATP complexes are formed. Thus *e.g.*, the availability of species corresponding to II or III would be a criterion sufficient to explain, respectively, metal ion specificity of muscle[6] and yeast[7] hexokinase.

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Cortisol induzierter Anstieg der Pyruvatcarboxylaseaktivität in der Rattenleber

Nach Cortisolgabe steigen in der Leber Glykogen[1], Glucose 6-Phosphat und Glucose 1-Phosphat[2] für die Dauer von 4 bis 6 Std. an. Die Geschwindigkeit des Pyruvateinbaus in Glucose erhöht sich innerhalb dieser Periode auf das 3 bis 4 fache[1]. Als Ursache hierfür kamen Änderungen der zwischen Pyruvat und Glucose eingeschalteten Enzyme in Betracht.

Der Mechanismus der Gluconeogenese wurde in verschiedenen Laboratorien untersucht. Als wesentliches Ergebnis dieser Arbeiten werden zwei Wege postuliert, die sich in der Überführung von Pyruvat in Phosphoenolpyruvat unterscheiden: ein direkter Weg über eine Umkehr der durch Pyruvatkinase (ATP: Pyruvat-Phosphotransferase, EC 2.7.1.40) katalysierten Reaktion (Gl. 1)

$$\text{Phosphoenolpyruvat} + \text{ADP} \rightleftharpoons \text{Pyruvat} + \text{ATP} \qquad (1)$$

und ein indirekter Weg über die durch die Malatdehydrogenase (decarboxylierend) (L-Malat: NADP-oxydoreductase (decarboxylierend), EC 1.1.1.40 früher bekannt als malic enzyme), Malatdehydrogenase (L-Malat: NAD-Oxydoreductase, EC 1.1.1.37) und Phosphopyruvat-carboxylase (GTP: Oxalacetat-carboxy-lyase (trans phosphorylierend), EC 4.1.1.32) (Gl. 2–4) katalysierten Schritte.

$$\text{Pyruvat} + CO_2 + \text{TPNH} + H^+ \rightleftharpoons \text{Malat} + \text{TPN} \qquad (2)$$

$$\text{Malat} + \text{DPN}^+ \rightleftharpoons \text{Oxalacetat} + \text{DPNH} + H^+ \qquad (3)$$

$$\text{Oxalacetat} + \text{GTP} \rightleftharpoons \text{Phosphoenolpyruvat} + \text{GDP} + CO_2 \qquad (4)$$