The Proton and Metal Complexes of Adenyl-5'-yl Imidodiphosphate

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(Received 16 July 1976)

The formation constants of the complexes of adenyl-5'-yl imidodiphosphate with H^+ , Mg^{2+} , Ca^{2+} and a number of bivalent transition-metal ions were measured potentiometrically. The complexes are generally a little more stable than the analogous complexes of ATP. By measuring the formation constants at two temperatures, this increase in stability was shown to result from an increased enthalpy change on complex-formation.

The bivalent Mg^{2+} ion is a necessary cofactor in almost every enzymic transphosphorylation reaction involving ATP. However, the role of Mg^{2+} in these reactions is uncertain. As a result, metal-complex-formation constants of ATP, particularly with Mg^{2+} and Ca^{2+} , have been measured by a number of workers. The magnitudes of the constants calculated show a disturbing spread in values. Early determinations were possibly in error as a result of partial hydrolysis of the terminal phosphate group, but, once an understanding of the kinetics of this hydrolysis was achieved, conditions can be selected to minimize it. Mohan & Rechnitz (1972) attribute the major variations in results to a large dependence of activity coefficient on ionic strength, together with possible interference from ionic backgrounds assumed to be inert. The range of results obtained by different workers has been summarized by Phillips *et al.* (1966) and Tu & Heller (1974).

The structure of the Mg^{2+} -ATP complex is still open to question, but available evidence suggests that bonding is, essentially, through the β - and γ -phosphate groups with only weak Mg^{2+} -adenine interactions. With transition-metal ions the metal-adenine bonding is probably more significant (see Tu & Heller, 1974).

In order to elucidate the mode of action of various reactions involving ATP, a number of ATP analogues have been synthesized. One interesting adenyl-5'-yl imido analogue is adenyl-5'-yl imidodiphosphate (H_4L), synthesized by Yount *et al.* (1971a), in which an NH group replaces the terminal bridge oxygen of the phosphate chain:

The compound is comparatively stable if stored at -20°C and can be used in solution at room temperature if solutions are freshly prepared. Adenyl-5'-yl imidodiphosphate is more resistant to hydrolysis by a number of enzymes which hydrolyse ATP and is a potent competitive inhibitor of Ca^{2+} - and Mg^{2+} -moderated heavy-meromyosin adenosine triphosphatase (Yount *et al.*, 1971b). It also inhibits the

action of ATP on nitrogenase (Eady *et al.*, 1975), and the ability of adenyl-5'-yl imidodiphosphate to inhibit many adenosine triphosphatase reactions has been studied by Philo & Selwyn (1974).

The donor properties of adenyl-5'-yl imidodiphosphate would be expected to be slightly greater than those to ATP itself as a result of the lower electronegativity of nitrogen compared with oxygen. This will result in lower acidity of the terminal phosphate group and more stable phosphate-metal bonds. The donor properties of the adenine group should not be affected significantly. Yount *et al.* (1971a) determined some formation constants by the resin competition reaction and found this to be the case.

We report here the results of a potentiometric study of the metal complexes of adenyl-5'-yl imidodiphosphate. Metals studied include Mg^{2+} , Ca^{2+} , Mn^{2+} , Zn^{2+} , Co^{2+} , Ni^{2+} and Cu^{2+} . With the first four metal ions, titrations were carried out at 0°C and 20°C to give an indication of the magnitudes of the enthalpy changes associated with complex-formation. The conditions used did not permit the formation of complexes of formula M_2L , which have been detected at high pH (>8) with ATP and Ca^{2+} and Mg^{2+} (Mohan & Rechnitz, 1972). Because of the uncertain influence of activity effects on the measured constants, some complexes of ATP were studied under identical experimental conditions to make comparisons meaningful.

Experimental

Adenyl-5'-yl imidodiphosphate was obtained from Boehringer Corp. Ltd., Mannheim, Germany, as the tetralithium salt and solutions were all freshly prepared. It was found, by titration, that decomposition in solution at 20°C was insignificant over 8 h. Titrations were carried out on 1 mM solutions in an ionic background of 0.10 M-tetramethylammoniumchloride and pH changes were followed by means of a glass electrode calibrated in terms of H^+ activities (0.05 M-potassium hydrogen phthalate). The titration cell was designed to hold less than 1 ml of solution, and alkali

was added from an automatic burette of total capacity 0.25 ml. Experimental data were processed with the help of the MINQUAD computer program (Gans *et al.*, 1976). Under the experimental conditions used the only significant metal complexes were the species ML^{2-} and MHL^{-} .

Results and Discussion

Calculated proton dissociation constants and metal-complex-formation constants are given in Table 1. Values in parentheses are estimated standard deviations (σ values). Also included in Table 1 are the formation constants for complexes of adenylyl imidodiphosphate with Mg^{2+} , Ca^{2+} and Mn^{2+} measured by Yount *et al.* (1971a) at 25°C. It is noteworthy that, although a totally different experimental technique was used, their results show almost exactly the same relative values as those reported here, being generally smaller by a factor of 4.

Results for the proton-complex-formation constants (pK values) reflect the lower electronegativity of nitrogen compared with oxygen. The first constant ($\log K_{HL}$) refers to association of a proton with the terminal phosphate group. It is therefore influenced most by the change from $=O$ to $=NH$, whereas $\log K_{H_2L}$ is influenced to a smaller extent. The donor power of this terminal phosphate group towards the proton is increased in adenylyl imidodiphosphate by a factor of $\log 1.5$ (i.e. 30), whereas the increase in $\log K_{MgL}$, representing the total donor power towards Mg^{2+} , is $\log 0.33$ (i.e. 2.1). This difference suggests that both adenylyl imidodiphosphate and ATP are multidentate ligands, with metal-ligand interactions

between other centres in addition to the terminal phosphate groups. What is more, the difference for Mn^{2+} ($\Delta \log K_{ML} = 0.32$) is comparable with that with Mg^{2+} and Ca^{2+} , suggesting very similar bonding schemes without markedly greater adenine- Mn^{2+} interaction. The difference between Mn^{2+} on the one hand and Mg^{2+} and Ca^{2+} on the other in complexes of $[H(ATP)]^{3-}$ and $[H(L)]^{3-}$ are, however, greater. This suggests that adenine- Mn^{2+} interaction may increase as the number of donor centres on the phosphate chain decreases. In all these comparisons there is no abnormally different behaviour between ATP and adenylyl imidodiphosphate.

Enthalpy changes calculated from the results at different temperatures are also shown in Table 1. As a result of errors resulting from the experimental technique used the precision of these quantities is necessarily low. Over the 20°C temperature range used, $\Delta H = -76.6 \Delta \log K$. Hence a realistic estimate of the precision of the ΔH values cannot be better than $\pm 4 \text{ kJ} \cdot \text{mol}^{-1}$. Errors in entropy charges will necessarily be greater. However, one consistent difference emerges from a comparison of the enthalpy changes associated with complex-formation with adenylyl imidodiphosphate and ATP. Enthalpy changes play a more important role in complexes of the synthetic nucleotide, and the higher stability constants of complexes of adenylyl imidodiphosphate are due entirely to greater heats of formation, accompanied by somewhat smaller entropy terms. This is particularly important in the Mg^{2+} complexes, where the small positive enthalpy change on formation of the ATP complexes changes sign to give a

Table 1. Thermodynamic quantities for the complexes of adenylyl-5'-yl imidodiphosphate in 0.1 M-tetramethylammonium chloride

$$K_{ML} = [ML]/[M][L]; K_{MHL} = [MHL]/[M][HL].$$

Reaction	log K		ΔH (kJ·mol ⁻¹)	$T\Delta S$ (25°C) (kJ·mol ⁻¹)	log K for ATP (293 K)
	273 K	293 K			
$H^+ + L^{4-} \rightarrow HL^{3-}$	8.58 (2)	8.41 (2)	-13 ± 4	34 ± 5	6.91 (1)
$H^+ + HL^{3-} \rightarrow H_2L^{2-}$	4.52 (3)	4.32 (2)	-15	9	4.08 (1)
$Mg^{2+} K_{ML}$	5.26 (1)	5.05 (2)*	-16	12	4.72 (1)
K_{MHL}	2.91 (3)	2.84 (4)	-5	11	2.72 (2)
$Ca^{2+} K_{ML}$	4.83 (3)	4.58 (2)*	-19	7	4.20 (1)
K_{MHL}	2.86 (4)	2.68 (4)	-14	1	2.38 (4)
$Mn^{2+} K_{ML}$	5.63 (2)	5.44 (3)*	-14	17	5.12 (3)
K_{MHL}	3.14 (4)	3.10 (3)	-3	14	3.14 (4)
$Zn^{2+} K_{ML}$	6.22 (3)	5.87 (2)	-26	7	
K_{MHL}	3.18 (9)	3.10 (2)	-6	11	
$Co^{2+} K_{ML}$		5.63 (1)			
K_{MHL}		3.15 (3)			
$Ni^{2+} K_{ML}$		5.69 (1)			
K_{MHL}		3.22 (1)			
$Cu^{2+} K_{ML}$		6.85 (1)			
K_{MHL}		3.70 (3)			

* Values reported by Yount *et al.* (1971a) were: $\log K_{MgL} = 4.58$; $\log K_{CaL} = 4.07$; $\log K_{MnL} = 4.93$.

favourable negative enthalpy change with adenylyl imidodiphosphate. These results suggest that the metal-ligand interactions have a larger covalent contribution with adenylyl imidodiphosphate compared with ATP, and that the ionic contribution is somewhat less. This is reasonable, since an oxygen atom in ATP is being replaced with the less electro-negative =NH group.

We thank Dr. R. N. F. Thorneley of the ARC Unit of Nitrogen Fixation for help and advice during this work.

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