Inorganica Chimica Acta

LETTER

Rapid hydrolysis of ATP by lanthanum(III) at pH 13

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(Received January 7, 1993; revised March 26, 1993)

Mechanistic elucidation of the enzyme-catalyzed hydrolysis of adenosine 5′-triphosphate (ATP) in cell metabolism has been a goal sought by mechanicians since the discovery of the role of ATP in the late 1930s. Because of the universal requirement of magnesium(II) in the dephosphorylation process, a substantial degree of focus has been directed toward metal ion influence in non-enzymatic, metal ion-catalyzed hydrolysis of ATP [1–4]. In other metal ion related studies a macrocyclic complex of lanthanum(III) was found to catalyze the hydrolysis of 2,4-dinitrophenyl diethyl phosphate [5], and rare earth ions have been observed to assist in the rapid hydrolysis of RNA dinucleoside monophosphates [6]. Our attention was directed toward examining lanthanum(III) as a catalyst in the hydrolytic cleavage of ATP as a result of our finding that the hydrolysis of ATP could be significantly accelerated in the presence of polyammonium macrocycles and $LaCl_3$ [7].

Other early studies of ATP hydrolysis pointed to the efficacy of metal hydroxides and polynuclear hydroxy metal ion gels [2–4], yet while reports have appeared on the catalytic capabilities of lanthanide ions at various pHs [3, 4, 8–12], there have been virtually no studies in recent years at very high pHs, e.g. 13. The striking finding of the study reported herein is the observation of the rapid formation of both AMP and pyrophosphate at pH 13 but not at pH 7.6 for solutions of ATP in the presence of La(III).

Experimental

Aqueous solutions containing nucleotide (0.01 M), $LaCl_3$ (0.005, 0.010 or 0.015 M), and 10% D_2O at 22 °C were adjusted to the appropriate pH with NaOH. As the pH is raised, a gel begins to form and is present at pH 13. A 0.5 ml aliquot was placed in a 5 mm NMR tube, and heated to the desired temperature in the probe. Proton decoupled ^{31}P NMR data were obtained on a Varian XL-300 spectrometer operating at 121.4 MHz (450 scans, 6 min) at appropriate time intervals. Hydrolysis was monitored by the disappearance of the α, β and γ resonances of ATP and the appearance of the signals of the products.

Results and discussion

The rates of hydrolysis of ATP at pH 13 in the presence of La(III) were found to be dependent on the ATP:La(III) ratio and to be almost double the rate observed for ADP hydrolysis under analogous conditions (Table 1). No reaction was observed for a 2:1 ATP:La(III) mixture at 40 °C after 1 h at pHs of 8, 10 and 11, although by raising the temperature to 70 °C, a rate of 0.004 min^{-1} was observed in the presence of La(III) at pH 7.6. At pH 13, however, the reaction accelerated to a first order rate constant, k_{obs}, of 0.017 min^{-1} at 40 °C for a 2:1 ATP:La(III) mixture, compared to approximately 0.002 min^{-1} for ATP at pH 13 in the absence of La(III). While the initial reaction appeared to be first order with respect to ATP, the rate decayed with time, possibly related to the precipitation of lanthanum phosphate salts, which effectively removes the catalyst from the reaction. Two other trivalent metal ion salts, YCl_3 and $LuCl_3$, were also examined at a slightly higher temperature of 50 °C. The results indicated that these 1:1 ATP:metal ion mixtures were less efficient than the analogous La(III) samples, with rates at pH 13 of 0.041 and 0.013 min^{-1}, respectively, compared to 0.054 min^{-1} for La(III).

TABLE 1. First order rate constants ($k_{obs} \times 10^3$) for the hydrolysis of ATP and ADP (0.010 M) in the presence of $LaCl_3$

Nucleotide	La(III):nucleotide	pH	Temp. (°C)	$k_{obs} \times 10^3$ (min^{-1})
ATP	1.0	13	50	54
ATP	0.5	13	50	21
ATP	0.5	13	40	17
ATP	0.5	11	40	<2
ATP	0.5	10	40	<2
ATP	0.5	8	40	<2
ADP	0.5	13	50	14

0020-1693/93/$6.00

The products observed were ADP, AMP, inorganic phosphate and pyrophosphate (Fig. 1). While the precipitation of lanthanide salts made the absolute determination of percentages of each product impossible, ratios of the integrations of ADP and AMP after 11 min indicated approximately a 3:1 ADP:AMP ratio. The formation of pyrophosphate under the influence of metal ion catalysis has been observed previously. It can result from either cleavage between the α and β phosphates [13], or from phosphoryl transfer from ATP to inorganic phosphate [14, 15]. In the lanthanide case, however, the pyrophosphate is presumed to be formed from α–β cleavage, since no evidence of pyrophosphate is observed from the analogous ADP reaction. No indication was seen of cleavage between the ribose and α phosphate, nor other degradation products as reported for the reaction of ATP with $Ba(OH)_2$ at 100 °C [8].

Previous NMR studies of solutions of ATP in the presence of lanthanum(III) indicated that a 2:1 ATP:La(III) complex is formed at pH 8.0 [16]. At pH 13, however, the complex nature of the hydroxide gel precluded a definitive assessment of the stoichiometry. At pH 7.6 significant ^{31}P chemical shifts are observed in the presence of La(III) ion, especially for P_β (+2.64 ppm) (Table 2). At pH 13, however, only slight downfield shifts are observed for all three phosphorus atoms. The catalytic activity of metal ions in ATP hydrolysis has often been attributed to the ability of the positively charged metal ion to 'neutralize' the negative charges on the phosphates of ATP, which would be particularly relevant at the high pHs involved in this study. Nonetheless, the hydrous lanthanide hydroxides and gels comprise a series of complicated compounds, several of which have been structurally characterized by X-ray crystallographic techniques [17, 18]. The metals are usually seven- or nine-coordinate with varying metal–oxygen distances and capped or tricapped trigonal prismatic geometries. In solution, the situation is undoubtedly very complex, and the actual reactive species may not even involve complex formation via direct coordination of ATP to the metal ion. Hence, while coordinative association of ATP with lanthanum(III) may be operable at pH 7.6, it would appear, based on the NMR data, that only weak, if any, associations occur between ATP and the metal ion at pH 13. It may be that the nature of the gel itself is such as to activate hydroxide or metal-bound hydroxide ion attack, rather than the positive metal ion acting to neutralize the negative charge on the phosphates. The slower rates for the two other trivalent metal ions examined may be a reflection of the nature of the gel formed.

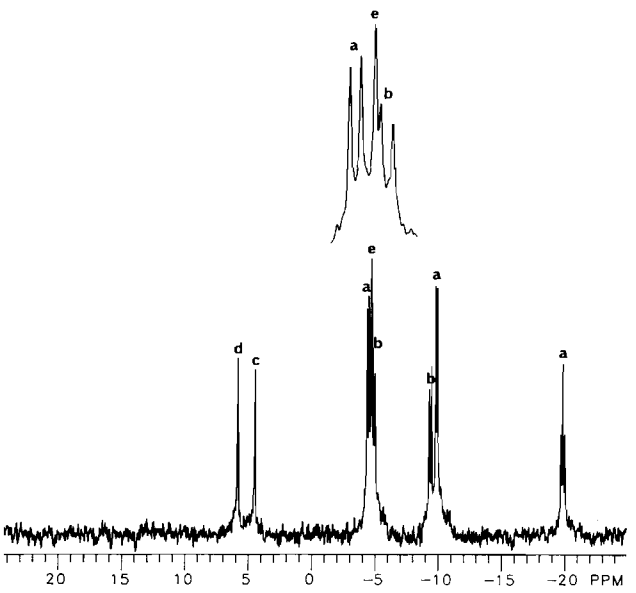

Fig. 1. ^{31}NMR spectrum at pH 13 and 50 °C of ATP (initially 0.010 M) and products in the presence of lanthanum(III) ion (0.005 M) after approximately 70% hydrolysis. Phosphorus atom associations are (a) ATP, (b) ADP, (c) AMP, (d) PO_4^{3-} and (e) $P_2O_7^{4-}$.

TABLE 2. The influence of lanthanum(III) ion (0.005 M) on ^{31}P NMR chemical shifts of ATP (0.010 M) at pH 7.6 and 13

Metal	pH	P_α	$\Delta\delta$	P_β	$\Delta\delta$	P_γ	$\Delta\delta$
−	7.6	−10.60		−21.50		−5.98	
+	7.6	−11.00	−0.40	−18.96	+2.64	−5.60	+0.38
−	13	−9.88		−19.84		−4.47	
+	13	−9.86	+0.02	−19.67	+0.17	−4.34	+0.13

In conclusion, these findings confirm the catalytic effect of metal ion hydroxy gels in the hydrolysis of ATP at high pH. Additionally, compared to the techniques available when earlier work was reported, ^{31}P NMR allows for a more exact assessment of the interaction of the nucleotide with the metal (through ^{31}P chemical shifts) as well as a more definitive analysis of the hydrolysis products.

Acknowledgement

This work was supported by a grant from the Institute of General Medical Sciences (GM 33922) of the National Institutes of Health.

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