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Pyrophosphate Formation via a Phosphoramidate Intermediate in Polyammonium Macrocycle/Metal Ion-Catalyzed Hydrolysis of ATP

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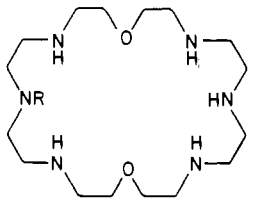
The ATP phosphotransferases, hydrolases, and synthetases, crucial enzymes in the biological energy cycle, often require mono- or divalent metal ions in order to carry out their function.[1] A major portion of the model studies have therefore focused on metal–ATP interactions to elucidate the role of the metal ion.[2] More recently, models for the enzyme substrate reaction were examined employing polyammonium macrocycles and ATP.[3] By melding the two foci, a metal–ATP–polyammonium macrocycle system which even more closely resembles its biological counterpart has been examined in this laboratory. The results of these initial studies, in which not only enhanced rates of ATP hydrolysis but also the formation of pyrophosphate are observed, are described herein.

The hexaazadioxo macrocycle, [24]-N_6O_2 (**1**) catalyzes the



1, R=H
2, R=PO_3^{2-}

hydrolysis of ATP at pH 7.6 via the formation of the intermediate phosphoramidate species **2**, which subsequently hydrolyzes to give inorganic phosphate.[3] This macrocycle has also been found to mediate in the formation of pyrophosphate during the hydrolysis of acetyl phosphate.[4]

The investigation of the effect of the biologically significant metal ions, Ca(II), Mg(II), and Zn(II), added to **1** in this laboratory has revealed a striking metal-dependent influence on ATP hydrolysis and on the formation of the phoshoramidate **2** and pyrophosphate. Partial hydrolysis of ATP in a reaction solution of ATP, [24]-N_6O_2·6HBr (**1**·6HBr),[5] and $CaBr_2$ in a ratio of 0.67:1:1 at pH 7.6 gave a mixture containing approximately 10% AMP, 70% ADP, and 20% ATP after approximately 35 min at 70 °C. At this point 50% of the phosphate derived from the hydrolysis reaction was present as the phosphorylated ligand **2** and no pyrophosphate peak was observed in the ^{31}P NMR spectrum when the pH of the mixture was adjusted to 4.5, in order to dissolve precipitated calcium salts. After standing 24 h at room temperature, the phosphoramide peak was absent, and, in addition to the formation of inorganic phosphate, approximately 10% of this intermediate was converted to pyrophosphate (Figure 1). The possibility that the pyrophosphate results from attack of water on ATP is unlikely, since it is also formed when no ATP is present in the solution.

While the addition of both Ca(II) and Mg(II) increased the observed percentage of the phosphoramidate **2**, only Ca(II) provided a significant rate acceleration in ATP hydrolysis (Figure 2, Table I), almost doubling the first-order rate constant compared to that of the macrocycle alone. This increase cannot be attributed

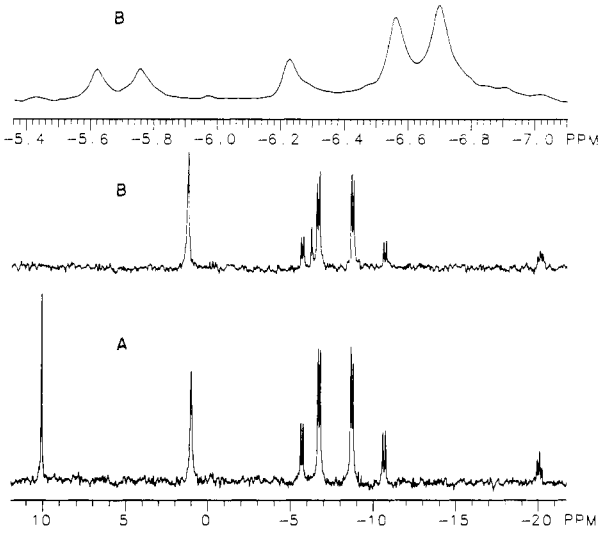


Figure 1. ^{31}P NMR of 0.010 M ATP, 0.015 M [24]-N_6O_2·6HBr (**1**·6HBr), and 0.015 M $CaBr_2$ in 10% D_2O/H_2O at pH 4.5 and 22 °C: (A) at 22 °C with pH adjusted to 4.5 after partial hydrolysis for 38 min at 70 °C at pH 7.6; (B) after 24 h at pH 4.5 at 22 °C. The chemical shift assignments: ATP, α, -10.71, β, -20.12, γ, -5.72; ADP, α, -8.72, β, -6.75; pyrophosphate, -6.22; orthophosphate and AMP +1.0; and phosphoramidate **2**, +10.07 ppm relative to an external standard of 85% H_3PO_4.

Table I. Observed First-Order Rate Constants for the Hydrolysis of ATP and the Maximum Observed Concentrations of the Phosphoramidate **2** in a Solution Containing 0.010 M ATP and 0.015 M [24]-N_6O_2 (**1**) at pH 7.6 and 70°C upon the Addition of 0.015 M Divalent Metal Bromides[a]

MBr_2 addtns	k_{obsd}, min^{-1}	**2**, M (min)[b]
none	0.023	0.0021 (25)
MgBr	0.023	0.0030 (36)
$CaBr_2$	0.043[c]	0.0035 (17)
$ZnBr_2$	0.0039	0.0013 (60)

[a] Solutions (0.5 mL) in a 5-mm NMR tube were heated to 70 °C in the NMR probe (Varian XL 300). ^{31}P NMR integrals were measured from the proton-decoupled spectra programmed for 450 acquisitions (6 min) spaced by 1 min. The integral values and the observed rate constants are accurate to ±10%. [b] The maximum observed concentration of the transient phosphorylated macrocycle **2** as determined from the ^{31}P NMR integral for this species at +10.0 ppm relative to an external standard of 85% H_3PO_4. [c] Calcium phosphate precipitated at this pH.

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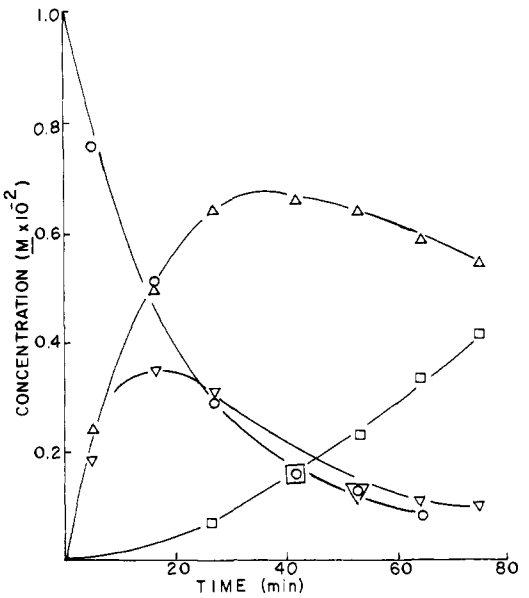

Figure 2. Plot of the concentration of ATP (○), ADP (△), AMP (□), and **2** (▽) vs. time in a solution of 10% D_2O/H_2O at pH 7.6 and at 70 °C containing 0.010 M ATP, 0.015 M [24]-N_6O_2 (**1**), and 0.015 M $CaBr_2$. The concentrations were calculated from the ^{31}P NMR integrals for the various species as described in Table I. Some calcium phosphate precipitated at this pH.

solely to the catalytic effect of the metal ion since the same reaction in the absence of macrocycle has a k_{obsd} of 0.002 min^{-1}.[6] The presence of Mg(II) had no apparent effect on the overall rate of ATP hydrolysis; however, the addition of Zn(II) to the macrocycle–ATP solution decreased the hydrolysis rate to one-sixth of the control value.

The most striking finding for this model system is the appearance of pyrophosphate. The process occurs readily in the presence of **1** and Ca(II) or Mg(II) at pH 4.5. No pyrophosphate formation was observed in the absence of the metal ions under the same conditions. In a study of the reaction of acetyl phosphate with the macrocycle, Hosseini and Lehn observed a greater yield of pyrophosphate (30%) without the necessity of added metal ion. In the case of ATP hydrolysis, however, there is competition in the approach to the phosphorylated macrocycle between the inorganic phosphate as well as ADP and AMP. The net result is a reduced opportunity for the inorganic phosphate to react at the phosphoramidate site. Furthermore, the rate of hydrolysis of the phosphorylated macrocycle in the absence of other phosphate species in the presence and absence of Ca(II) ion was examined and found to be significantly slower in the latter case. Thus, the role of the metal ion is thought to be twofold. It acts to increase the observed percentage of the intermediate **2** via stabilization of the P–N bond, and, additionally, it is capable of complexation with the nucleotides.

Both ^{31}P and ^{13}C NMR data for these ternary complexes indicate significant interactions of both Mg(II) and Ca(II) with ATP. While the terminal phosphate of ATP is normally associated strongly with **1**, the addition of Mg(II) and Ca(II) results in a significant upfield shift of the P_β resonance (~1.7 ppm) of ATP, indicating a substantial interaction at that site. That the Ca(II) interactions with ATP may even be strong enough to disrupt the macrocycle–ATP complex is suggested by the ^{13}C data for **1**, for which the chemical shifts of the ternary Ca(II)–ATP–macrocycle system are almost identical with those of the free macrocycle, while the binary ATP–macrocycle system differs considerably. The net result is that if the nucleotides are complexed with metal ions, they may more readily dissociate from the macrocyclic cavity, allowing for less restricted association of **2** with inorganic phosphate. Such a macrocyclic–phosphate association is in agreement with the proposed mechanism for the formation of pyrophosphate via **2** from the reaction of **1** and acetyl phosphate.[4]

These findings serve to accentuate the analogies between ternary metal–ATP–polyammonium macrocycle systems and their biological counterparts, enzymes utilizing ATP, for which the enzyme function is almost invariably mediated by the presence of certain metal ions.

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Controlled and Catalytic Acylpalladation. A Novel Route to Cyclopentenone and Cyclohexenone Derivatives[1]

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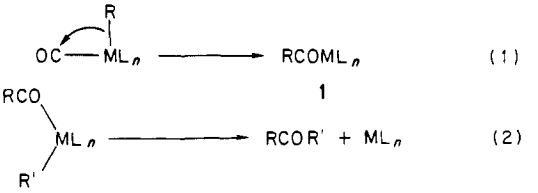
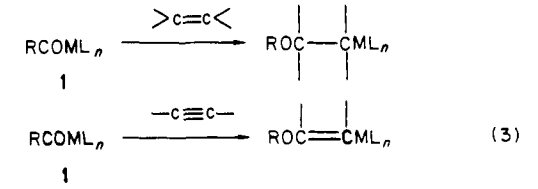
Carbon–carbon bond formation via transition-metal-catalyzed carbonylation with CO almost invariably involves migratory insertion (eq 1) which is often followed by reductive elimination[2] (eq 2). In principle, the product of migratory insertion (**1**) can

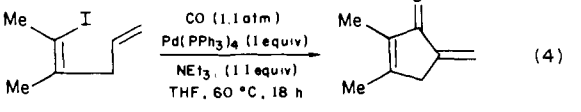


undergo an alternate carbon–carbon bond-forming reaction with alkenes and alkynes as shown in eq 3, i.e., acylmetalation. Al-



though "formal" conjugate addition reactions of acylmetal derivatives to α,β-unsaturated carbonyl compounds,[3] reactions involving addition of $ROOCML_n$ to alkenes and alkynes,[4] and a few examples of the reaction of acylmetalates with ethylene and acetylenes are known,[5] acylmetalation of unactivated alkenes and alkynes shown in eq 3 remains largely unexplored.[6]

We recently reported the conversion of 1-iodo-1,4-dienes into α-methylenecyclopentenones represented by eq 4.[7] This con-



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