Magnesium Ion Catalyzed ATP Hydrolysis

Nicholas H. Williams

Department of Chemistry, Sheffield University
Sheffield, S3 7HF, United Kingdom

Received April 17, 2000
Revised Manuscript Received August 7, 2000

Many enzymes (and ribozymes) require one or more metal ions as cofactors in catalyzing phosphate ester hydrolysis and transesterification. However, despite a large knowledge base of metalloenzyme crystal structures, kinetic and binding data, and extensive studies with model systems, the detailed role the metal ions play is still unclear.[1] In particular, there are several reports concerning metallophosphatases with contrasting proposals on how the metal ions might be used to catalyze phosphate monoester hydrolysis.[2] Most fundamentally, the issue has been raised whether the metal ions stabilize the transition state of the solution reaction (the most energetically efficient way of catalyzing a reaction) or a significantly different transition state (which may be more sensitive to catalysis). In this communication, we report how the combination of multiple metal ions reveals a significant role for Mg(II) ions in promoting ATP hydrolysis which is not otherwise observed in solution.[3]

In previous work, we have shown that phosphate monoesters bound to a dinuclear Co(III) complex are efficiently hydrolyzed through an associative mechanism,[4] so we have made **1**. The γ phosphate bridges the two Co(III) ions and shows a characteristic ~15 ppm downfield shift in the ^{31}P NMR; the α and β phosphates show no significant change in shift.[5] In aqueous solution, ^{31}P NMR shows that this complex is cleanly hydrolyzed to release ADP and leave inorganic phosphate bridging the Co(III) centers; this resembles the reaction catalyzed by purple acid phosphatases and pyrophosphatases.

In basic solution, complex **1** hydrolyzes in a specific base catalyzed reaction, with a second-order rate constant 0.027 ± 0.001 M^{-1} s^{-1} at 25 °C and ionic strength 1.0 M (KCl). At lower pH values, hydrolysis was too slow to measure accurately by continuous UV analysis as expected by extrapolating these data (^{31}P NMR shows that the complex is unchanged after 1 month at pH 8 and ambient temperature). This behavior parallels the reaction observed with similar complexes of aryl phosphates and the analogous mechanism is shown in Scheme 1.

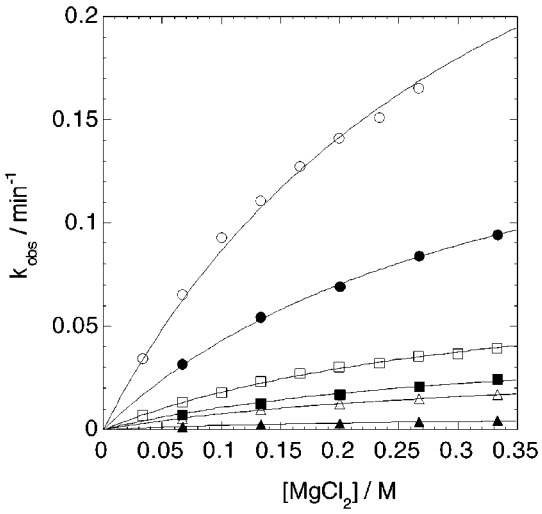
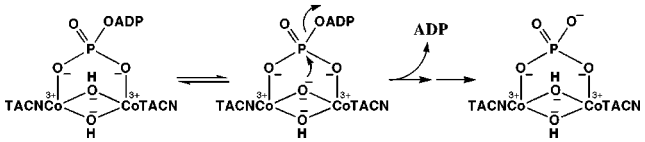


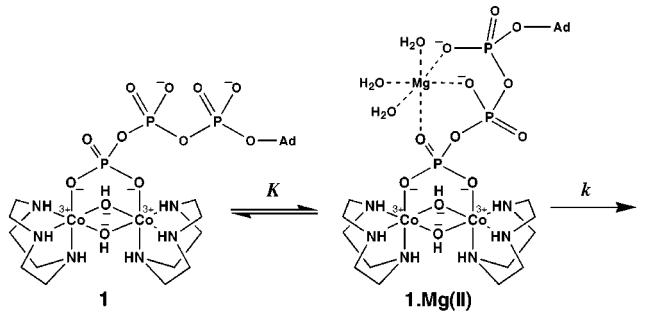
Figure 1. The dependence on the concentration of $MgCl_2$ of the observed rate constants for hydrolysis of **1** (I = 1.0 M (KCl), 25 °C): open circles, pH 9.97; filled circles, pH 9.60; open squares, pH 9.23; filled squares, pH 9.02; open triangles, pH 8.85; filled triangles, pH 8.20 The solid curves are obtained by fitting the data to $k_{obs} = k[Mg]/(K + [Mg])$ with K = 0.35 M.

Scheme 1. Proposed Mechanism of Hydrolysis



However, maintaining the ionic strength at 1.0 M at pH values from 8.1 to 10 with varying ratios of KCl and $MgCl_2$, we observe pseudo-first-order rate constants which increase both with increasing pH and with increasing Mg(II) composition. These data are plotted in Figure 1, and we fit these data to a reaction scheme in which Mg(II) binds to **1** with an equilibrium constant K, and **1**·Mg(II) hydrolyzes with an observed rate constant k at each pH (Scheme 2). We also measured binding between **1** and Mg(II) independently using ^{31}P NMR at pH 7.2. All three signals show shifts of about 0.5 ppm as the Mg(II) ion concentration varies from 0 to 0.33 M and were fit to a single binding event scheme. As neither Mg(II) nor the complex have ionizable groups over this pH range, the equilibrium constant is pH independent under these conditions, so we take the mean of all the kinetic and NMR measurements to obtain K = 0.35 ± 0.1 M. Refitting the kinetic

Scheme 2. Proposed Mechanism for Hydrolysis Catalyzed by Mg(II)



(1) (a) Sträter, N.; Lipscomb, W. N.; Klabunde, T.; Krebs, B. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2024−2055. (b) Wilcox, D. E. *Chem. Rev.* **1996**, *96*, 2435−2458. (c) Hengge, A. C. In *Comprehensive Biological Catalysis: A Mechanistic Reference*; Sinnott, M., Ed.; Academic Press: London, 1998; Vol. 1, pp 517−542. (d) Williams, N. H. In *Comprehensive Biological Catalysis: A Mechanistic Reference*; Sinnott, M., Ed.; Academic Press: London, 1998; Vol. 1, pp 543−562. (e) Narlikar, G. J.; Herschlag, D. *Annu. Rev. Biochem.* **1997**, *66*, 19−59.

(2) (a) For a recent review, see ref 1c. (b) Hollfelder, F.; Herschlag, D. *Biochemistry* **1995**, *34*, 12255−12264. (c) Kim, E. E.; Wyckoff, H. W. *J. Mol. Biol.* **1991**, *218*, 449−464. (d) Breslow, R.; Katz, I. *J. Am. Chem. Soc.* **1968**, *90*, 7376−7377. (e) Klabunde, T.; Sträter, N.; Frohlich, R.; Witzel, H.; Krebs, B. *J. Mol. Biol.* **1996**, *259*, 737−748. (f) Mildvan, A. S. *Proteins* **1997**, *24*, 401−416.

(3) (a) Herschlag, D.; Jencks, W. P. *J. Am. Chem. Soc.* **1987**, *109*, 4665−4674. (b) Herschlag, D.; Jencks, W. P. *Biochemistry* **1990**, *29*, 5172−5179. In both cases, the observed rate of hydrolysis was either retarded by the addition of Mg(II) or provided an observed acceleration of less than 2-fold. This latter acceleration has been interpreted as a large effect by species at very low concentration.

(4) Williams, N. H.; Lebuis, A.-M.; Chin, J. *J. Am. Chem. Soc.* **1999**, *121*, 3341−3348.

(5) ATP was bound to the dinuclear complex following the same methods as previously reported (ref 4). The mixture was purified by passing the aqueous solution through sephadex SP-25 ion-exchange resin with water to remove cationic impurities, then through sephadex QEA-25 resin to remove anionic impurities. The purplish-red solution was lyophilized to give a purple solid which analyzed as **1**·$8H_2O$·$4NaClO_4$. Anal. Calcd for $C_{22}H_{60}Cl_4Co_2N_{11}Na_4O_{39}P_3$: C, 17.08; H, 3.91; N, 9.96; Cl, 9.17. Found: C, 17.16; H, 3.93; N, 9.92; Cl, 9.19. δ_P (D_2O, 101 MHz): 7.01 (d, J 19 Hz), −9.37 (d, J 19 Hz), −19.62 (t, J 19 Hz).

10.1021/ja0013374 CCC: $19.00
Published on Web 11/17/2000

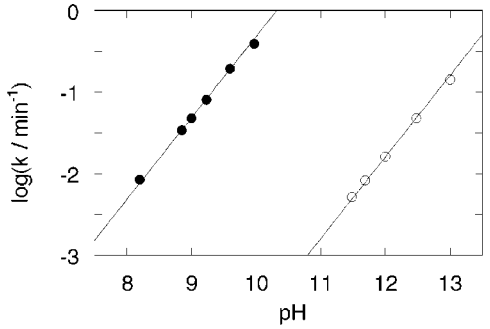


Figure 2. pH−rate profile for the hydrolysis (I = 1.0 M (KCl), 25 °C) of **1** (open circles) and **1**·Mg(II) (closed circles). The solid lines are obtained by fitting the data to a line of slope 1.

data with this parameter fixed still yields good fits (solid curves shown in Figure 1; $r \geq 0.99$), and provides a set of k values which correspond to the rate of hydrolysis of **1**·Mg(II) at each pH. These rate constants are plotted as a pH−rate profile in Figure 2, and fitting the data to a slope of 1 gives the second-order rate constant for hydroxide catalyzed hydrolysis of the **1**·Mg(II) complex as 80 ± 4 M^{-1} s^{-1}. Remarkably, the rate of hydrolysis of the Mg(II) coordinated complex is 3000-fold faster than that of **1** in 1.0 M KCl solution.

Most mechanistic proposals for biological phosphoryl transfer where metal ion cofactors are implicated are based (either explicitly or implicitly) on an associative transition state.[6] Catalysis is rationalized by clustering the metal ions round all the phosphoryl oxygens to stabilize the highly negatively charged transition state. However, in aqueous solution, the hydrolysis of ATP follows a dissociative mechanism, where the bond to the leaving group (ADP) is largely broken before the bond to the incoming water forms to any extent. Maintaining this transition state structure for the catalyzed reaction suggests that metal ions will have the most catalytic effect if they interact primarily with the leaving group: interaction at the nonbridging oxygens would be anticatalytic. Supporting evidence for this hypothesis comes from recent detailed mechanistic studies which show that the hydrolysis of phosphate monoesters[7] and anhydrides[8] is *not* sensitive to the presence of Mg(II) ions in aqueous solution even though the Mg(II) is associated with the γ phosphate (and so is apparently poised to catalyze an associative reaction). The Mg(II) ions preferentially bind between the β and γ phosphates, making the dianionic γ phosphate less effective at expelling the ADP leaving group, but do not facilitate an alternative associative pathway.

In our model, the γ phosphate is coordinated to the two Co(III) ions, making an associative hydrolytic pathway available. Our data show that by providing this environment for ATP to react through, a substantial catalytic role for Mg(II) ions is revealed, an observation not previously directly made in simple aqueous solutions. Clearly, an associative transition state *can* be sensitive to stabilization by Mg(II) ions. A possible role for the Mg(II) is shown in Scheme 2. Coordinating the ADP leaving group decreases the pK_a of ADP by 2 units;[9] a similar sensitivity to the leaving group as for phosphate monoesters would provide 100-fold acceleration. Interaction between the Mg and the γ phosphate, stabilizing developing negative charge in the transition state, could provide the additional acceleration observed.

It has also been proposed that Mg(II) may play a significant role as an enzyme cofactor through an outer-sphere electrostatic effect, instead of through direct coordinate bonds between the ion and substrate. Evidence for this proposal comes from reports that Co(III) hexamine can be used instead of Mg(II) to support nuclease- and ribozyme-catalyzed hydrolysis and transesterification of phosphate diesters.[10] As the ammonia ligands only exchange extremely slowly with water, the Co(III) hexamine cannot form direct coordinate bonds with the substrate. Black and Cowan[11] have reported similar binding affinities to DNA for this ion and Mg(II) so we investigated whether Co(III) hexamine shows similar activity to Mg(II) with our model. The reaction is still hydroxide catalyzed, but 0.165 M Co(III) hexamine only accelerates the reaction by a factor of 2 relative to 1.0 M KCl. At this concentration, Mg(II) accelerates the reaction by about 1000-fold. In this context, which (unusually for ATP hydrolysis in a simple model system) we have shown to be sensitive to catalysis by Mg(II), outer-sphere activation is not a significant factor.

In conclusion, we have demonstrated that ATP hydrolysis promoted by multiple metal ions in an associative mechanism is highly sensitive to catalysis by Mg(II) ions in aqueous solution; this activation is not due to an outer-sphere interaction.

Acknowledgment. We thank the Royal Society and the Nuffield Foundation for financial support, and Dr. B. F. Taylor and S. Bradshaw for recording the ^{31}P NMR data.

Supporting Information Available: Individual graphs of observed rate constants vs $MgCl_2$ concentration at each pH; variation of δ_P with $MgCl_2$ concentration; observed rate constants vs $Co(NH_3)_6Cl_3$ concentration (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA0013374

(6) For example: (a) Hassett, A.; Blattler, W.; Knowles, J. R. *Biochemistry* **1982**, *21*, 6335−6340. (b) Goldberg, J.; Huang, H.-B.; Kwon, Y.-G.; Greenbard, P.; Nairn, A. C.; Kuriyan, J. *Nature* **1995**, *376*, 745−753. (c) Egloff, M.-P.; Cohen, P. T. W.; Reinemer, P.; Barford, D. *J. Mol. Biol.* **1995**, *254*, 942−959. (d) Tari, L. W.; Matte, A.; Goldie, H.; Delbaere, L. T. J. *Nature Struct. Biol.* **1997**, *4*, 990−994. (e) References 1a,b and 2c,d,e.

(7) Catrina, I. E.; Hengge, A. C. *J. Am. Chem. Soc.* **1999**, *121*, 2156−2163

(8) Admiraal, S. J.; Herschlag, D. *Chem. Biol.* **1995**, *2*, 729−739.

(9) Phillips, R. C.; George, S. J. P.; Rutman, R. J. *J. Am. Chem. Soc.* **1966**, *88*, 2631−2640.

(10) (a) Jou, R.; Cowan, J. A. *J. Am. Chem. Soc.* **1991**, *113*, 6685−6686. (b) Hampel, A.; Cowan, J. A. *Chem. Biol.* **1997**, *4*, 513−517. (c) Young, K.; Gill, F.; Grasby, J. A. *Nucleic Acids Res.* **1997**, *25*, 3760−3766

(11) Black, C. B.; Cowan, J. A. *J. Am. Chem. Soc.* **1994**, *116*, 1174−1178.