Rapid Cleavage of Chelated Pyrophosphate using Metal Ion Complexes

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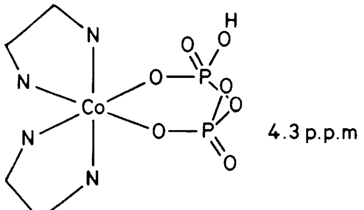
Aspects of the mechanism of the rapid cleavage of $P_2O_7^{4-}$ ion in $Co(en_2)P_2O_7^-$ (en = ethane-1,2-diamine) by $Co(tn)_2(OH)(OH_2)^{2+}$ (tn = propane-1,3-diamine) are outlined to show how the metal ions act in concert, to provide a co-ordinated nucleophile (OH^-) to assist the leaving group in severing a central P–O bond and in general to neutralise charge on the PO_3 residues.

The rapid cleavage and formation of P–O–P bonds in biological systems is effected by enzymes containing metal ions as essential components.[1] However, the observed acceleration of hydrolysis of adenosine triphosphate (ATP) or tripolyphosphate ion ($P_3O_{10}^{5-}$) by metal ions (Mg^{2+}, VO^{2+}, Cu^{2+}, Zn^{2+}, $LaOH_3$, *etc.*) is not especially great (*ca.* 10—100 fold),[2,3] although oxidising VO^{2+} and Mn^{2+} complexes to $VO_{aq.}^{3+}$ or $Mn_{aq.}^{3+}$ does accelerate $P_3O_{10}^{5-}$ hydrolysis[3] in



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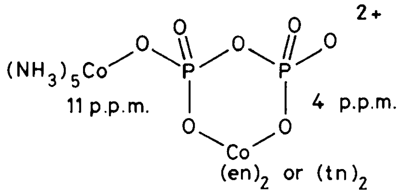

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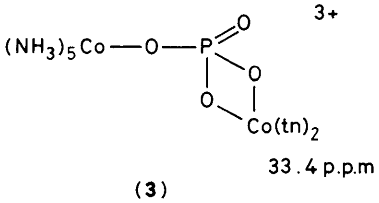

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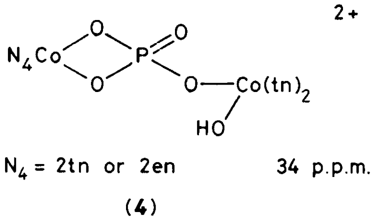

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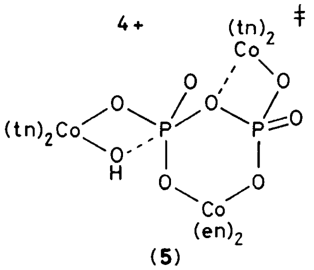

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acidic conditions up to 10^5 fold. Even so, these labile metal ion–phosphate complexes are complicated mixtures and, like the enzymic systems, they are difficult to unravel in a mechanistic sense. For this last reason attention has focused recently on more kinetically inert chelated phosphate molecules as pseudo-substrates for enzymes[4] and as models for understanding how the metal ions influence polyphosphate and phosphate ester reactivity.[5—7] Two roles for the metal ion are evident. One is that co-ordination of the phosphate enhances the approach of a nucleophile to the P atom both by charge neutralisation and polarisation. Another is the prospect of a metal ion providing a co-ordinated nucleophile to carry out a facile intramolecular reaction to cleave a P–O bond.[6]

The hydrolysis of $P_2O_7^{4-}$ to PO_4^{3-} in the presence of excess of the bis(propane-1,3-diamine)hydroxoaquacobalt(III) ion [$Co(tn)_2(OH)(OH_2)^{2+}$] has been reported by Hübner and Milburn[5] to take place relatively rapidly ($t_{\frac{1}{2}}$ *ca.* 2 h) near neutral pH compared with hydrolysis in the absence of the cobalt complex ($<10^{-9}$ s^{-1}). However, ^{31}P n.m.r. spectroscopy has shown that this process is also rather complicated. The present paper attempts to unravel aspects of the mechanism of that process by simplifying the reaction using $P_2O_7^{4-}$ already chelated in a very stable complex, (hydrogen pyrophosphato)bis(ethane-1,2-diamine)cobalt(III) [$Co(en)_2P_2O_7H$] whose structure has been determined as the six-chelate (**1**).[8] Events in the reaction of this molecule with the $Co(tn)_2(OH)(OH_2)^{2+}$ ion are shown in Figure 1 as followed by ^{31}P n.m.r. spectroscopy. The scheme adjacent to the spectra gives structural assignments for the signals observed. The reactant gives a singlet at 4.3 p.p.m. Signals at *ca.* 10 p.p.m. and the broadening at *ca.* 4 p.p.m. are assigned to the reactant with one $Co(tn)_2(OH)^{2+}$ moiety co-ordinated to one end of the bound $P_2O_7^{4-}$ (A). This co-ordination takes place rapidly *via* the fast water exchange[9] on the $Co(tn)_2(OH)(OH_2)^{2+}$ ion (<1 s) and the same types of spectra are

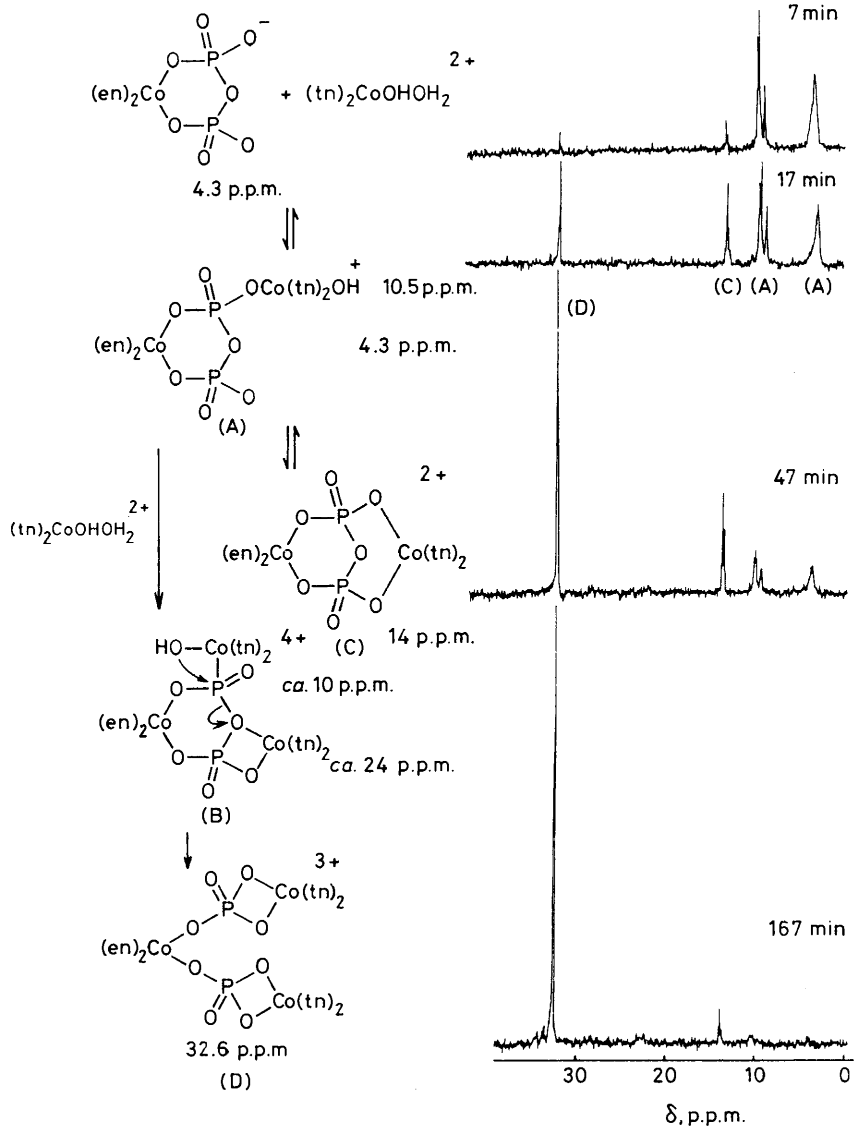


Figure 1. ^{31}P N.m.r. spectra and reaction scheme for the reaction sequence of $Co(en)_2P_2O_7^-$ (0.05 M) and $Co(tn)_2(OH)(OH_2)^{2+}$ (0.15 M) at 25 °C in 1 M triethanolamine buffer. Bruker CXP-200 MHz pulsed spectrometer; 80.98 MHz operating frequency; 30 pulses, 10 s delay; time to middle of accumulation.

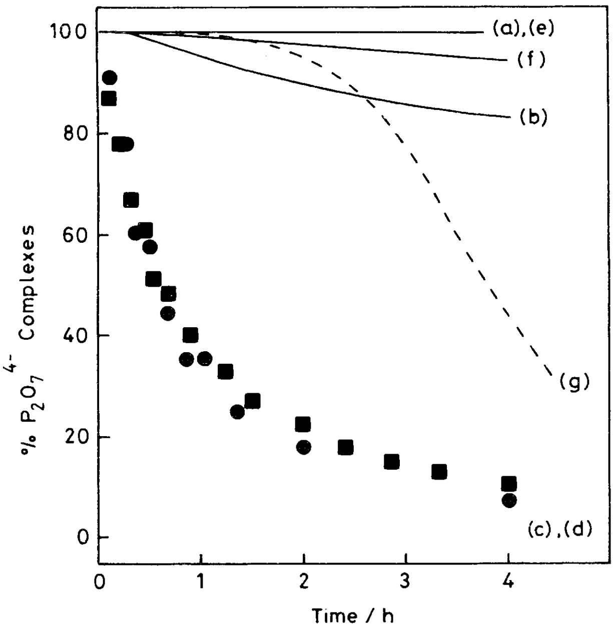


Figure 2. $Co(tn)_2(OH)(OH_2)^{2+}$ induced hydrolysis rates of $N_4CoP_2O_7^-$ complexes at pH 7 and 30 °C in 1 M triethanolamine buffer. The plots are constructed from total integrated ^{31}P n.m.r. signals as shown in Figure 1 minus the integrated 33 p.p.m. signals as the reaction develops. (a) 0.2 M $(en)_2CoP_2O_7^-$ + 0.1 M $Co(tn)_2(OH)(OH_2)^{2+}$. (b) 0.1 M $(en)_2CoP_2O_7^-$ + 0.1 M $Co(tn)_2(OH)(OH_2)^{2+}$. (c) 0.1 M $(en)_2CoP_2O_7^-$ + 0.2 M $Co(tn)_2(OH)(OH_2)^{2+}$. (d) 0.1 M $(tn)_2CoP_2O_7^-$ + 0.2 M $Co(tn)_2(OH)(OH_2)^{2+}$. (e) 0.1 M $(NH_3)_5CoP_2O_7^-$ + 0.1 M $Co(tn)_2(OH)(OH_2)^{2+}$. (f) Solution (e) + 0.1 M $Co(tn)_2(OH)(OH_2)^{2+}$. (g) 0.1 M $Na_4P_2O_7$ + 0.3 M $Co(tn)_2(OH)(OH_2)^{2+}$. Curves (c) (■) and (d) (■) show essentially identical reactivity for $(tn)_2CoP_2O_7^-$ and $(en)_2CoP_2O_7^-$. Points shown as circles (●) were obtained by ^{31}P n.m.r. analysis of CN^- quenched reaction solution aliquots where the Co^{III} complex was decomposed and PO_4^{3-} and/or $P_2O_7^{4-}$ were measured directly (P. Hendry, unpublished work).

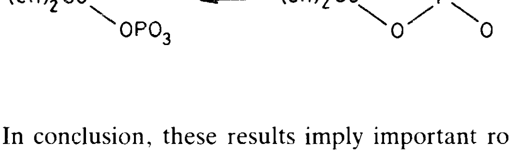
observed when a stable complex (**2**) is formed[10] between $(NH_3)_5Co{-}OP(O)_2OPO_3^-$ and $Co(en)_2(OH_2)_2^{3+}$ or $Co(tn)_2(OH_2)_2^{3+}$.

The complexity of the signals at 10 p.p.m. arises from the isomers involved since the hydroxy group of the added $Co(tn)_2(OH)^{2+}$ moiety can have a *cis* or *trans* configuration in relation to the bound O–P moiety; also the two cobalt centres can be Δ or Λ in diastereoisomeric relationships. The signals at *ca.* 14 p.p.m. are attributed to a non-reactive complex (C) which equilibrates slowly with (A) on the ^{31}P n.m.r. time scale.† The additional chemical shift (4 p.p.m.) is attributed to chelation of $P_2O_7^{4-}$ to both cobalt(III) centres.

The final singlet at *ca.* 33 p.p.m. can be assigned with certainty to a complex of the type (D) where both PO_4 groups are bound to the $(en)_2Co^{2+}$ moiety and each is chelated to a $Co(tn)_2^{2+}$ moiety. Signals of this type can be generated by treating $(NH_3)_5Co{-}O{-}PO_3$ and chelated $(en)_2CoPO_4$ or $(tn)_2CoPO_4$ with $(tn)_2Co(OH)(OH_2)^{2+}$ to give (**3**) and (**4**) directly.

The rate of the cleavage process is very dependent on the $Co(tn)_2(OH)(OH_2)^{2+}$ concentration and the pH. It maximises essentially and the reaction goes to completion with a 2:1 ratio of $Co(tn)_2(OH)(OH_2)^{2+}$ to $Co(tn)_2(P_2O_7)^-$ at pH *ca.* 7. It virtually does not occur with a 1:2 ratio [Figure 2, curves (c) and (a)]. This is consistent with Hübner and Milburn's observation that three molecules of $Co(tn)_2(OH)(OH_2)^{2+}$ were required to cleave $P_2O_7^{2-}$ most efficiently.[5] However, the rates displayed here ($t_{\frac{1}{2}}$ 35 min at 30 °C, pH 7.0) are faster than those observed previously[5,7] (≥4—5 fold). Curve (g) shows the rate of cleavage achieved using $Co(tn)_2(OH)(OH_2)^{2+}$ (3 mol) and $Na_4P_2O_7$ (1 mol) in the buffered conditions at pH 7. Clearly, the rate is considerably slower under these conditions as it is also in the presence of less hydroxoaqua complex. The effective intermediate (B) in the reaction path (Figure 1) is characterized by two features: the use of the co-ordinated OH^- ion as a nucleophile, and chelation of the remaining $Co(tn)_2$ moiety to assist P–O rupture in the resulting five co-ordinate phosphorane. The pK_a of a water molecule in a monodentate phosphate complex would be expected to be in the range 6—7;[6] so the system is nicely poised to maximise the effect of the co-ordinated nucleophile at pH *ca.* 7. The intermediate (B) would be expected to have signals at 10 and 24 p.p.m. but the latter was not observed in the ^{31}P n.m.r. spectrum indicating that the intermediate is shortlived.

The necessity for the chelation of both cobalt ions in the activated complex (**5**) which follows (B) is implied by at least two observations. Firstly, it appears to decompose directly to the product with both phosphate ions chelated to separate Co^{III} ions and bound to a common Co^{III} residue, Figure 1. Secondly, merely binding a cobalt(III) amine complex to the chelated $P_2O_7^{4-}$ ion does not assist cleavage greatly. The last effect can be gauged from curve (e) of Figure 2 where the $(NH_3)_5CoP_2O_7^-$ and $Co(tn)_2(OH)(OH_2)^{2+}$ ions were allowed to react to give complexes like (**2**). These inert molecules, on treatment with additional $Co(tn)_2(OH)(OH_2)^{2+}$, do lead to cleavage of $P_2O_7^{4-}$ [curve (f), Figure 2], but much more slowly than the mixture $CoN_4(P_2O_7)^-$ +2 $Co(tn)_2(OH)(OH_2)^{2+}$. The intermediate (B) has the merit that attack of the intramolecular nucleophile would be efficient and that the second Co^{III} ion chelated would assist the oxygen atom to leave the P centre being attacked. All three metal ions would in general assist the hydrolysis by helping to neutralize the negative charge on the phosphate residues and by polarising the P atoms to allow efficient 'in-line' attack[11] by a nucleophile. The only difficult feature in the assembly is the fact that a very stable 6-chelate ring is broken in the process and that is a rare event in chelate chemistry except for the metal–ligand bond. This unfavourable aspect is offset by at least two factors. One is the greater thermodynamic stability of the two PO_4^{3-} residues relative to the $P_2O_7^{4-}$ ion for the hydrolysis reaction[12] and the other is that two new chelates are generated in the process. These four-chelate rings although strained are clearly more stable than the ring opened complex under the conditions of the experiment.[13]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In conclusion, these results imply important roles for the metal ions in the enzymic reactions where, for polyphosphate

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† This signal grows and decays during the reaction and the complex can be formed without hydrolysis occurring. The only other signal which has been recognised in this position is that for $[Co(en)_2(OH)(OPO_3)]^-$.

cleavages, often more than one metal ion is required. The metal ions are not just spectators in the process nor merely holding the pieces together while they react.

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