Rapid Cleavage of Tridentate Cobalt(III)-co-ordinated Triphosphate

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A tridentate tripolyphosphate cobalt(III) complex is hydrolysed in the presence of one equivalent of $[Co(tn)_2(OH)(OH_2)]^{2+}$ (tn = 1,3-propanediamine) at pH 7.3 *ca.* 10^6 fold faster than unco-ordinated tripolyphosphate.

Despite the extraordinary rapid hydrolysis of polyphosphates in biological systems, hydrolysis of tripolyphosphate is an extremely slow process in aqueous laboratory conditions. At pH 9, the rate is 1.9×10^{-8} s^{-1} at 40 °C, *i.e.* 7×10^{-9} s^{-1} at 25 °C given the activation parameters implied in reference 1. Enhancements of this rate by factors of 10—100 are found in catalysis by aqueous divalent metal ions,[2,3] and rate enhancements of the order of 10^4 to 10^6 times have been observed in the presence of VO^{3+} and Mn^{3+} ions.[4] Hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and orthophosphate is also known to be catalysed by metal ions and metal complex ions. Ca^{2+} Catalysed hydrolysis of ATP, for example, is enhanced 60-fold over free ATP[5] and in the presence of two moles of $[(tn)_2Co(OH)(OH_2)]^{2+}$ (1) (tn = 1,3-propanediamine) the hydrolysis of ATP is enhanced *ca.* 10^5-fold.[6] Hydrolysis of the tripolyphosphate ion in the substitutionally inert α,β-bidentate complex, $[(NH_3)_4$-$CoP_3O_{10}H_2]$, has been observed also using the reagent $[(cyclen)Co(OH)(OH_2)]^{2+}$ (cyclen = 1,4,7,10-tetra-azacyclododecane).[1] At pH 10 and 40 °C and in the presence of three moles of $[(cyclen)Co(OH)(OH_2)]^{2+}$ per mole of phosphate complex the hydrolysis enhancement relative to free $P_3O_{10}H_n$ is 7.5×10^4.[1]
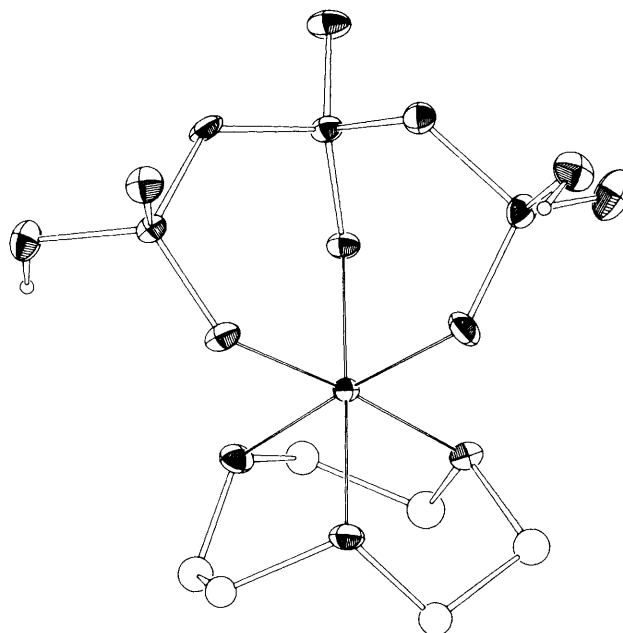
This communication describes the synthesis, structure, and rapid hydrolysis of a tridentate tripolyphosphate complex $[(tacn)CoP_3O_{10}H_2]$ (tacn = 1,4,7-triazacyclononane), and some mechanistic implications for the function of the metal ions in these systems. The tripolyphosphate complex was isolated in good yield in the neutral diprotonated bis(tridentate) form $[(tacn)CoP_3O_{10}H_2]\cdot2H_2O$, following reaction of $[(tacn)Co(OH_2)_3]^{3+}$ and excess of $Na_5P_3O_{10}$ in aqueous solution. The purple product crystallized from water and the

Scheme 1

Figure 1. Molecular geometry of the $[(tacn)CoP_3O_{10}H_2]$ complex.
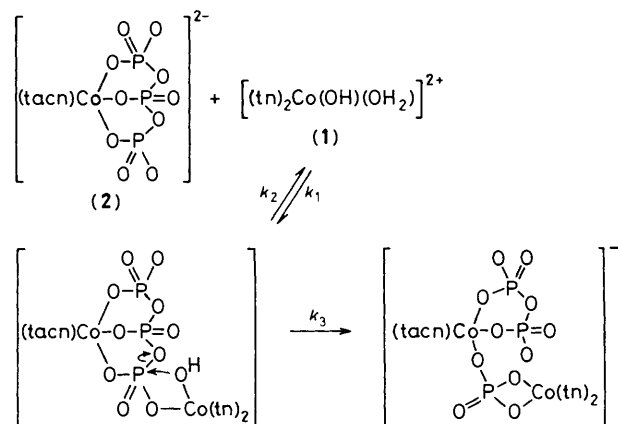
α,β,γ-facial-tridentate co-ordination of the dihydrogen tri-polyphosphate ligand was established by X-ray crystal structure analysis.† An ORTEP drawing of the complex is shown in Figure 1 but only one conformation of the disordered tacn ligand is displayed for clarity. The structure is similar to that reported[7] for $[(NH_3)_3CoP_3O_{10}H_2]$ except that one protonation site is different and as a consequence the α and γ Co–O–P bond angles vary from each other; $138.0°$, $126.3°$, and from the angle in the triammine structure; $131.8°$. The solution ^{31}P n.m.r. spectrum is consistent with mirror symmetry for the molecule. It shows a triplet at -6.3 p.p.m. and a doublet at $+3.8$ p.p.m. (pH 7), $vs.$ external 85% H_3PO_4, $J(P–O–P) = 16.5$ Hz, intensity $1:2$ indicating that the tridentate structure is preserved in solution. This compares well with the ^{31}P n.m.r. spectrum of the $[(NH_3)_3CoP_3O_{10}H_2]$, which displays a doublet at $+4.3$ p.p.m. and a triplet at -5.6 p.p.m.[8] As expected there is a substantial shift of the signals for free $P_3O_{10}^{5-}$ (-19 and -6 p.p.m.) on co-ordination.
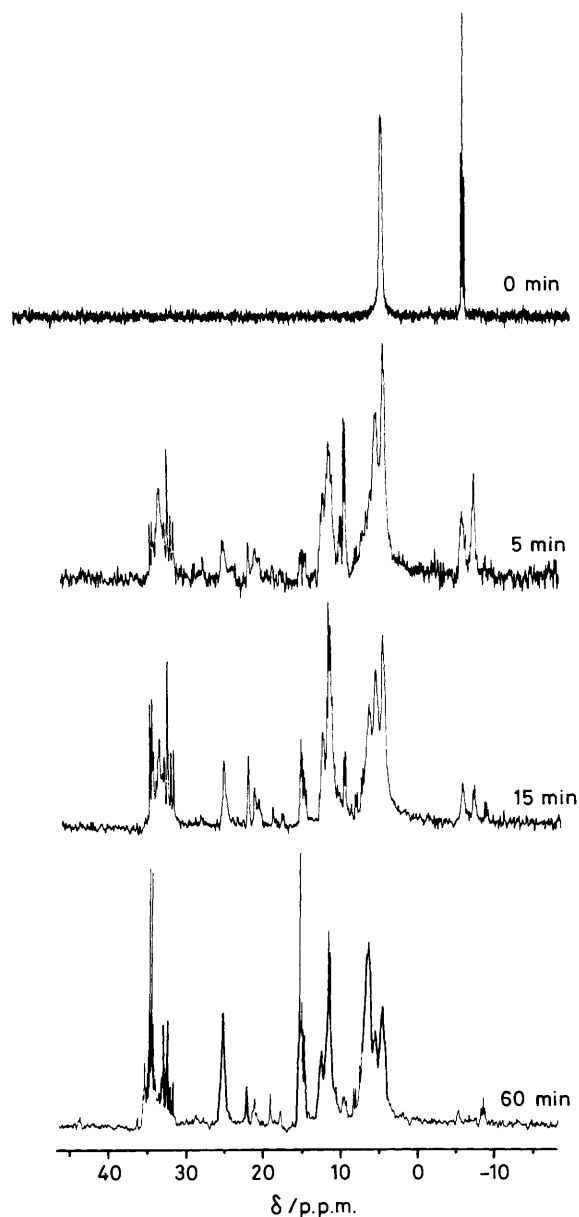
The hydrolysis of $[(tacn)CoP_3O_{10}]^{2-}$ (**2**) by (**1**) was studied in buffered solutions by two methods; ^{31}P n.m.r. spectroscopy of the reacting solution, see Figure 2, and quantitation of the free tri-, di-, and mono-phosphate by ^{31}P n.m.r. spectroscopy after reduction of the Co^{III} amine complex by a trace of Co^{II} and excess of CN$^-$. Both methods gave similar results. Interpretation of the ^{31}P n.m.r. spectra as shown in Figure 2 is aided by applying additivity 'rules' which have been empirically derived by this group,[9,10] and others.[1,8,11—13] These rules consist of δ values (approximate) to be added to the known chemical shift of a particular phosphate moiety following substitution by the metal ions, H$^+$, or PO$_3^-$ residues. Some of
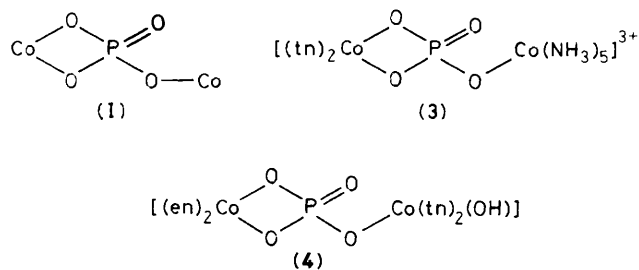
† $C_6H_{21}CoN_3O_{12}P_3$, $M = 479.1$, monoclinic, space group $P2_1/n$, $a = 10.3646(7)$, $b = 12.984(1)$, $c = 12.814(1)$ Å, $\beta = 82.13(1)°$, $U = 1708.2$ Å^3, $D_m = 1.88(2)$, $D_c = 1.863$ g cm^{-3}, $Z = 4$, $\mu(Cu\text{-}K_\alpha) = 114.0$ cm^{-1}, $\lambda(Cu\text{-}K_\alpha) = 1.5418$ Å, $F(000) = 984$ electrons, $T = 293(1)$ K. The 1837 unique observed reflections $[I > 2.5\sigma(I)]$ were measured in the range 3 $< 2\theta < 50°$ on a Picker FACSI four-circle diffractometer. The structure was solved by heavy atom methods and refined by block-matrix least-squares techniques to a final residual of $R = 0.046$ and $R_w = 0.052$. Carbon atoms of tacn, disordered $52:48(2)\%$ over two sites, were refined isotropically. All other non-hydrogen atoms were refined anisotropically. Only hydrogens on phosphate and water were located and refined, a constrained O–H bond length of $0.88(3)$ was used in the refinement. The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Rd., Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this communication.

these δ values are: Co^{3+} $+(6—8)$ monodentate addition, $+10$ chelate addition; PO$_3^-$ $+(11—12)$; H$^+$ $-(1—3)$. Illustrations of these effects appear in the following chemical shifts: PO$_4^{3-}$ $+6$; H$_3$PO$_4$ 0; P$_2$O$_7^{4-}$ -6; P$_3$O$_{10}^{5-}$ -6, -19; $[(NH_3)_5CoOPO_3]^+$ 14;[11] $[(NH_3)_5CoOPO_3H]^+$ $+11$;[11] $[(NH_3)_4\overline{CoOP(O)_2O}]$ $+24$; $[(NH_3)_4\overline{CoOP(O)_2OP(O)_2O}]^-$ $+$ 5 p.p.m.[8] The interpretation of the reaction spectra has been made on the basis of these rules. Signals observed in the region $+31$ to $+35$ p.p.m. are attributed to groups of the type (I) which are numerous because of rapid addition of the $[(tn)_2Co]$ reagent at various sites on the P$_2$O$_7$ moiety in the course of the reaction. Complexes like (**3**) and (**4**) have also been synthesized independently and give ^{31}P n.m.r. signals in the vicinity of 33 p.p.m. The signal at 25 p.p.m. is ascribed to the fragment (II) cleaved completely from the $[(tacn)CoP_2O_7]^-$ moiety. The chemical shifts in the region of $+4$ to $+15$ p.p.m. arise from pyrophosphate and the α and γ phosphorus atoms of tripolyphosphate co-ordinated to at least one and possibly two Co^{III} ions. The β phosphorus of tripolyphosphate bound to one Co^{III} resonates in the region -5 to -8 p.p.m. Relative PO$_4^{3-}$, P$_2$O$_7^{4-}$, and P$_3$O$_{10}^{5-}$ concentrations in the reacting solutions were estimated by integration of spectra of the kind shown in Figure 2. The interpretation of the n.m.r. signals (*vide supra*) was supported by the fact that the intensity attributed to products containing P$_2$O$_7^{4-}$ was always around twice that attributed to PO$_4^{3-}$ products as expected for a simple cleavage of tripolyphosphate. Figure 3 shows the results of analogous experiments with varying (**2**) to (**1**) ratios. The reactions were quenched at intervals with CN$^-$ and unco-ordinated PO$_4^{3-}$, P$_2$O$_7^{4-}$, and P$_3$O$_{10}^{5-}$ ion ratios determined by ^{31}P n.m.r. spectroscopy.

It can be seen from Figure 3 that only one equivalent of (**1**) is required to effect substantial cleavage of the triphosphate and in the presence of more than two moles of (**1**) per mole of triphosphate complex all the triphosphate is hydrolysed. The reason for this is that (**1**) is consumed, *i.e.* bound to basic phosphate oxygens before the triphosphate is fully hydrolysed to PO$_4^{3-}$ and P$_2$O$_7^{4-}$. The hydrolysis of co-ordinated P$_2$O$_7^{4-}$ occurs on a much slower time scale.[14] Under all the conditions

en = ethylenediamine, tn = 1,3-propanediamine

Figure 3. Decrease in $P_3O_{10}^{5-}$ concentration with time, with varying $[(tacn)CoP_3O_{10}]^{2-}$ (**2**) to $[(tn)_2Co(OH)(OH_2)]^{2+}$ (**1**) ratios. Relative $P_3O_{10}^{5-}$ concentrations were determined by ^{31}P n.m.r. spectroscopy of cyanide-quenched samples of the reaction mixture. Reaction conditions: 0.025 M (**2**); 0.025, 0.05, 0.075 M (**1**); 25 °C; pH 7.3 [piperazine-N,N'-bis(2-ethanesulphonic acid) (pipes) buffer, 0.4 M].
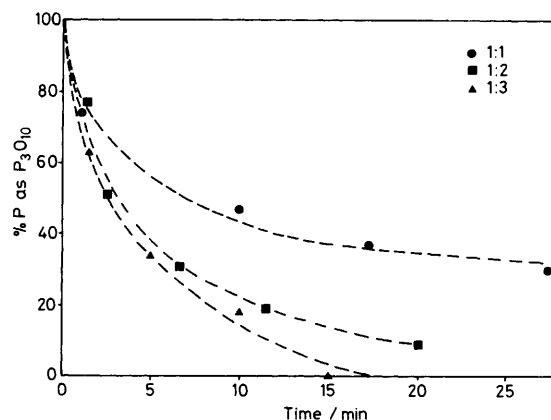

Figure 2. Successive 80.98 MHz ^{31}P n.m.r. spectra of $[(tacn)-CoP_3O_{10}]^{2-}$ (**2**) reacting with 2 moles of $[(tn)_2Co(OH)(OH_2)]^{2+}$ (**1**) (pH 7.3, 20 °C).

studied the hydrolysis of bound triphosphate appears to occur with a crude first order decay and a half life of *ca.* 3 minutes at 25 °C and pH 7.3. Overall, this is a rate enhancement of *ca.* 10^6-fold compared with the unco-ordinated $P_3O_{10}^{5-}$ hydrolysis. Hydrolysis of the triphosphate complex in the absence of (**1**) was not detected even after one week at 25 °C and pH 7.3. More detailed studies of the hydrolysis are in progress using a variety of methods.
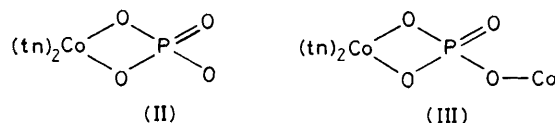
The reaction proceeds with equimolar quantities of the two reactants and the mechanism under consideration as the simplest and most probable is that shown in Scheme 1. It involves fast addition (k_1) of (**1**) to the α-phosphate residue followed by a first order nucleophilic attack (k_3) of the co-ordinated OH^- ion at the α-P centre. This efficient

intramolecular nucleophilic path has been explored in related phosphate ester[9] and pyrophosphate hydrolysis.[14] The fact that substantial hydrolysis occurs with just one mole of (**1**) is interesting since in related studies on the cleavage of chelated pyrophosphate at least two moles of the reagent were required for rapid hydrolysis.[13,14] It seems clear that $P_3O_{10}^{5-}$ anhydride bonds are generally more susceptible to hydrolysis than the $P_2O_7^{4-}$ anhydride bond and it can be argued that this is because the pyrophosphate moiety is a better leaving group than the PO_4^{3-} ion. Additional cobalt reagent is therefore not required to help the $P_2O_7^{4-}$ ion depart especially since it is already bound to a Co^{II} centre and much of its negative charge is thereby neutralised. The effective sites for the reagent seem to be the terminal PO_3 residues since fragments (**II**) and (**III**) clearly dominate the products. Much of the complexity in the ^{31}P n.m.r. spectra however comes from additional binding of the cobalt reagent to basic phosphate oxygen centres during the reaction.

This rapid hydrolysis of the $P_3O_{10}^{5-}$ ion using such metal ion reagents gives an indication of the rôles which metal ions may adopt in enzymic systems involving polyphosphate. Efficient intramolecular co-ordinated nucleophiles can be

greatly assisted by charge neutralisation at the P centre being attacked. The results also imply ways of getting normally slow *in vitro* phosphate chemistry to occur much more rapidly.

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