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Chelation *versus* Hydrolysis in the Cobalt(III)-Assisted Hydrolysis of Polyphosphates

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The mechanisms of metal ion assisted hydrolysis of polyphosphates are of fundamental importance in the study of many metalloenzymes which utilize ATP as an energy source. In order to begin to understand the function of metal ions at the active sites of these enzymes, we [1–3] and other workers [4–9] have made use of simple inorganic model systems, based mainly on cobalt(III), to investigate the effect of metal ions on the hydrolysis reactions of phosphates. These studies support the conclusion that simultaneous coordination of the metal ion by the substrate (polyphosphate) and by an attacking nucleophile (hydroxide ion) is necessary for acceleration of rates of hydrolysis in these inorganic systems. Apart from this conclusion, no hypothesis has been put forward to explain the large number of experimentally observed phenomena occurring in metal ion-polyphosphate systems in aqueous media. In this communication we would like to present a simple concept that explains why very large rate enhancements are observed in some systems but not in others: Nearly all the studies using diaquo cobalt(III) complexes can be explained by a comparison of the nucleophilicity of the coordinated hydroxide ion *versus* the nucleophilicity of the phosphate ion.

Coordination of the polyphosphate ion by the metal ion in solution is necessarily the first step in any metal-catalyzed phosphate hydrolysis reaction, and this coordination has been established by phosphorus-31 NMR spectroscopy in several cases [4, 10]. Given the existence in solution of a cobalt(III)–polyphosphato complex, two subsequent reactions are possible, depending on the exact nature of the species in solution. One of these reactions leads to hydrolysis, the other does not. The scheme shown in Fig. 1 depicts the two possible reaction pathways. In reaction *a*, a phosphate group acts as a nucleophile, displacing the hydroxide ion from the coordination sphere of the cobalt(III) ion. This substitution results in formation of a polyphosphate chelate ring, and little or no hydrolysis will occur. In reaction *b*, the nucleophile is the coordinated hydroxide ion which attacks a phosphorus atom in the phosphate chain to yield a five-coordinate phosphorus atom as an intermediate, a mechanism well established in phosphorus chemistry [11]. The tetrahedral phosphate structure is then reformed by cleavage of a P–O–P bond, so that path *b* leads predominantly to hydrolysis.

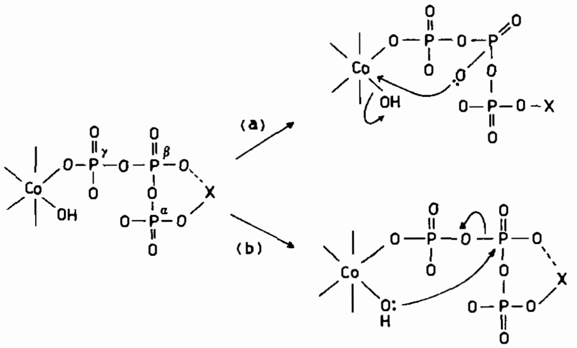

Fig. 1. General scheme for the reaction of polyphosphate with a cobalt(III) amine complex. Path *a* leads to chelation and path *b* leads to hydrolysis. Protons and charges are omitted both for clarity and because they are pH dependent.

Given the two pathways outlined above, it would seem that the criterion that determines whether chelation or hydrolysis will occur is whether the nucleophilicity of the coordinated hydroxide ion is greater than that of the phosphate ion. In any given system, the nucleophilic character of the hydroxide ion should be relatively constant, so the variable parameter in a system will be the nucleophilic character of the phosphate groups in the substrate. Since experiments [1, 10] and examination of Dreiding models both suggest that the β-phosphorus atom (see Fig. 1) is sterically the most favored for either reaction, the nature of chemical modifications to this particular part of the phosphate chain are very important. Thus, the nature of 'X' in Fig. 1 will determine whether chelation or hydrolysis will occur. If 'X' is electron withdrawing then the nucleophilic character of the phosphate will be reduced and hydrolysis will likely occur.

Apparently two conditions must be met for metal ion assisted hydrolysis to occur: 1) simultaneous coordination to the cobalt(III) ion of the substrate polyphosphate and the attacking nucleophile '*cis*' to one another [1] and 2) suitable chemical modification of the phosphate chain to reduce its nucleophilicity. The following paragraphs reveal some specific applications of this concept, and we have noted that many observations of other workers can be explained using similar arguments.

Work in this laboratory on the phosphate hydrolysis reaction of the triphosphato complex [12] $\beta\gamma$-$[Co(NH_3)_4H_2P_3O_{10}]$ in the presence of *cis*-$[Co(cyclen)(H_2O)_2]^{3+}$, a macrocyclic cobalt(III) poly-

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amine with 100% *cis* geometry, has shown [1] that marked accelerations in the rate of hydrolysis of the phosphate species occur at pH greater than 7.5. This rate enhancement occurs upon deprotonation of a coordinated water molecule ($pK \sim 7.9$) on the cyclen complex with subsequent attack of the resulting coordinated hydroxide ion on the polyphosphate chain. Similar studies using either β-$[Co(NH_3)_5HP_2O_7]$ or γ-$[Co(NH_3)_5H_2P_3O_{10}]$ (synthesised in this laboratory [13]) in place of $\beta\gamma$-$[Co(NH_3)_4H_2P_3O_{10}]$ show no tendency for accelerated hydrolysis over the pH range 6–9. The conclusion to be drawn from these two latter observations is that chelation of the polyphosphate chains onto the macrocyclic cobalt(III) amine complex occurs with release of coordinated hydroxide ion into solution and subsequent loss of hydrolytic activity. The hypothesis that chelation occurs in these two systems where hydrolysis is not observed is supported by the observation that if equal volumes of 25 m*M* solutions of *cis*-$[Co(cyclen)(OH)_2]^+$ and β-$[Co(NH_3)_5P_2O_7]^-$, both equilibrated at pH 9.0 and 40 °C, are mixed, the pH of the resulting solution jumps to 11.2. Titration of this solution back to pH 9.0 shows that two mol of hydroxide ion are released into solution for each mol of *cis*-$[Co(cyclen)(OH)_2]^+$ initially present. The same behavior is found when γ-$[Co(NH_3)_5P_3O_{10}]^{2-}$ is used: Clearly displacement of the hydroxide ion from the coordination sphere of the cobalt–cyclen complex has occurred in the reaction of the pyrophosphato complex with $[Co(cyclen)(OH)_2]^+$. This chelation reaction is in place of the hydrolysis observed when β,γ-$[Co(NH_3)_4P_3O_{10}]^{2-}$ or β-$[Co(NH_3)_5P_3O_{10}]^{2-}$ is the polyphosphate substrate. Phosphorus-31 NMR spectra [14] also support the formation of bidentate chelate complexes in the reactions of both β-$[Co(NH_3)_5P_2O_7]^-$ and γ-$[Co(NH_3)_5P_3O_{10}]$ with the macrocyclic dihydroxo species. Conversely, there is little evidence for chelation in the β,γ-$[Co(NH_3)_4P_3O_{10}]^{2-}$/*cis*-$[Co(cyclen)(OH)_2]^+$ system where marked increases in the rates of hydrolysis of triphosphate have been observed [1].

The confines of this letter do not provide sufficient space to extend the argument to other cases, but the importance of the ability of phosphate itself to act as a nucleophile has been demonstrated. The function of cobalt(III) ions coordinated to phosphates in model systems is twofold: 1) They provide a potent coordinated nucleophile (hydroxide ion) and 2) they can block the nucleophilic characteristics of the phosphates themselves. This recognition of the dual role should provide new ideas for the design of model phosphate hydrolysis systems where the phosphate groups could be blocked by agents other than cobalt(III). Perhaps some sites in the enzymatic systems function in this way by preventing chelation of the phosphate to the metal ion at the active site.

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