Novel Ligand Effect on Cobalt(III) Complex Promoted Hydrolysis of a Phosphate Diester

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Bis-p-nitrophenyl phosphate when bound to the cobalt(III) complex [(trpn)Co(OH)(OH$_2$)] [trpn = tris-(3-aminopropyl)amine] is hydrolysed about three hundred times more rapidly than when bound to [(tren)Co(OH)(OH$_2$)] [tren = tris-(2-aminoethyl)amine], and *ca.* ten billion times more rapidly than the free phosphate diester.

Currently there is much interest in developing catalysts that can hydrolyse the phosphate diester backbone in DNA and RNA. Efficient oxidative cleavage of DNA has been achieved.[1] However, hydrolytic cleavage of DNA still remains the task of enzymes. We recently showed that, other than enzymes, the cobalt(III) complex cis-[(trien)Co(OH)(OH$_2$)] (trien = triethylenetetramine) is by far the most active in promoting the hydrolysis of phosphate diesters with good[2] or poor[3] leaving groups. In solution, the cis-cobalt complex is in rapid equilibrium with the trans form which is inactive. Furthermore, [(trien)Co(OH)(OH$_2$)] exists in several different cis forms[4] in solution making it difficult to identify the active form of the cobalt complex. In this paper we report the reactivities of [(tren)Co(OH)(OH$_2$)] [tren = tris-2-aminoethyl)amine] (1)[5] and [(trpn)Co(OH)(OH$_2$)] [trpn = tris-(3-aminopropyl)amine] (2)[6] in promoting the hydrolysis of bis(p-nitrophenyl phosphate) (BNPP). Complexes (1) and (2) are ideal for evaluating the structure–reactivity relationship since both cobalt complexes are locked into the cis form and cannot undergo cis–trans or other similar isomerisation processes.

The mechanism[2] of [(N$_4$)Co(OH)(OH$_2$)] promoted hydrolysis of BNPP, where N$_4$ represents any tetra-amine

ligand, is shown in Scheme 1. Complex (1) and (2) promoted hydrolysis of BNPP was monitored by following the increase in the concentration of the nitrophenolate anion with time. In a typical experiment, a solution of [(tren)Co(OH)(OH$_2$)]/[(tren)Co(OH$_2$)$_2$] (0.01 M) in water was prepared by adding NaOH (1.5 equiv.) to [(tren)CoCl$_2$]Cl. After several minutes, the pH of the solution was adjusted to 7.0. As in previous studies[3,4] maximal rates were obtained under neutral pH conditions. The hydrolysis of BNPP was initiated by addition of BNPP stock solution in water (5 μl, 0.01 M) to the freshly prepared cobalt complex solution (3 ml, 0.01 M) at 50 °C. The hydrolysis reaction was monitored by following the absor-

(1)   (2)

Scheme 1

(1a), N₄ = tren
(2a), N₄ = trpn
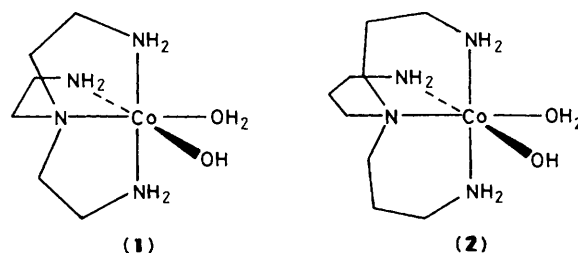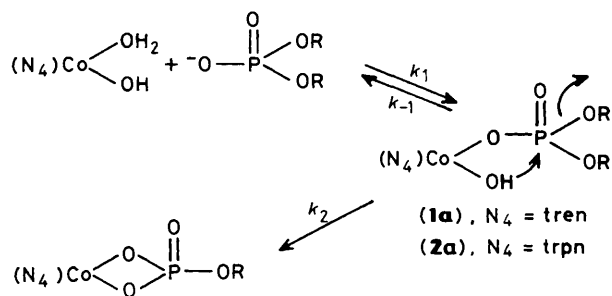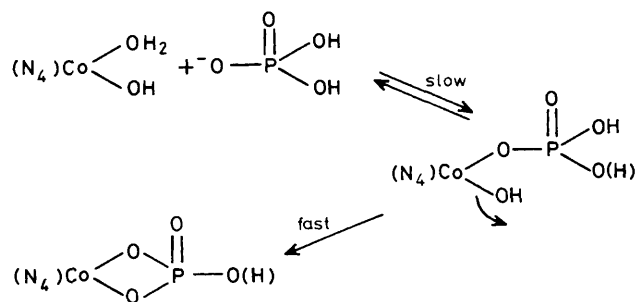
Scheme 2

Table 1. Observed first-order rate constant (s⁻¹) for reactions of BNPP or inorganic phosphate with excess of cobalt complexes (1) or (2) (0.01 M) at 50 °C, pH 7.0.

	(1)	(2)
BNPP	8.1×10^{-5}	2.5×10^{-2}
Phosphate	3.8×10^{-3}	1.2^a

^{a} At 20 °C.

Table 2. First order rate constants (s⁻¹) for hydrolysis of cobalt complex bound BNPP and free BNPP at 50 °C, pH 7.0.

(1a)	(2a)	BNPP
8.1×10^{-3}	2.5	3.0×10^{-10}

bance change at 400 nm. All reactions were carried out under pseudo-first-order conditions with a large excess of the cobalt complex over the phosphate diester. The rate constants were obtained by fitting the first three half lives of the kinetic data according to a first-order kinetic equation. In all cases the rate constants were reproducible to within 3%. The pH of the reaction solution did not change significantly (pH 7.0 ± 0.2) during the course of the hydrolysis reaction owing to the buffering effect of the cobalt complex solution.[2] NaCl had no appreciable effect on the rate of the hydrolysis reaction.

The rate of anation of (1) or (2) with BNPP (Scheme 1, step 1) could not be measured directly. However, anation of (1) and (2) with inorganic phosphate (Scheme 2) was measured following published procedures.[7] The reaction of cis-[(N₄)Co(OH)(OH₂)] with inorganic phosphate involves rate-determining anation followed by rapid ring closure via the Co–O bond cleavage (Scheme 2). Formation of the bidentate phosphato complex was monitored by following the increase in the absorbance at 510 nm. Under identical conditions, the rate of anation of (1) with inorganic phosphate is much greater than the rate of (1) promoted hydrolysis of BNPP (Table 1). Similarly, the rate of anation of (2) is much greater than the rate of (2) promoted hydrolysis of BNPP. The rate-determining step for (1) and (2) promoted hydrolysis of BNPP should be the second step (Scheme 1) since the rate of anation of (1) and (2) with BNPP should be comparable to the rate of anation of (1) and (2) with inorganic phosphate. It is well known that the rate of anation of cobalt(III) complexes is independent of the anionic nucleophile.[8]

The observed rate constant for (1) or (2) promoted hydrolysis of BNPP (Scheme 1) is given by equation (1) when k_2 is the rate-determining step, where $K_1 = k_1/k_{-1}$ (Scheme 1). Under our experimental conditions {[(N₄)Co(OH)(OH₂)] between 0.001 M and 0.01 M}, the rate of p-nitrophenolate anion production increases linearly with increase in the

concentration of (1) or (2). Therefore, $K_1[(N_4)Co(OH)(OH_2)]$ is much less than 1 and $k_{obs.}$ approaches $K_1k_2[(N_4)Co(OH)(OH_2)]$. We have previously estimated the value of K_1 (1 dm³ mol⁻¹).[3] Substituting the value of K_1 into equation (1), k_2 values for (1a) and (2a) (Scheme 1) can be obtained

$$k_{obs.} = \frac{K_1k_2[(N_4)Co(OH)(OH_2)]}{\{K_1[(N_4)Co(OH)(OH_2)] + 1\}} \quad (1)$$

(Table 2). In neutral water, the rate constant for hydrolysis of BNPP at 50 °C is 3×10^{-10} s⁻¹ in agreement with the literature value.[9] The cobalt bound BNPP in (2a) is hydrolysed an unprecedented 10^{10} times faster than the free BNPP. It is surprising that (2a) is some 300 times more reactive than (1a) considering that the two structures are so closely related. This striking dependence of the reactivity on the structure is indicative of an intramolecular nucleophilic mechanism (Scheme 1, step 2).[10] Intramolecular general base mechanism would not result in such a structure–reactivity relationship.[10]

In conclusion, it has been shown for the first time that slight variation of the tetra-amine ligand structure in [(N₄)Co(OH)(OH₂)] can greatly influence the rate of the cobalt complex promoted hydrolysis of phosphate diesters. Furthermore, BNPP bound to [(trpn)Co(OH)(OH₂)] is hydrolysed an unprecedented 10^{10} times more rapidly than the free phosphate diester. We are currently working on detailed mechanistic analysis of the system as well as on improving the efficacy of the cobalt complex.

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