# Cobalt(III) Complex Promoted Hydrolysis of Phosphate Diesters: Change in Rate-Determining Step with Change in Phosphate Diester Reactivity

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*Received April 9, 1987*

**Abstract:** The effects of four Co(III) complexes, $[Co(trien)(OH)(OH_2)]^{2+}$, $[Co(en)_2(OH)(OH_2)]^{2+}$, $[Co(dien)(OH)(OH_2)_2]^{2+}$, and $[Co(en)_2(OH)(NH_3)]^{2+}$, on the rate of hydrolysis of bis(*p*-nitrophenyl) phosphate, *p*-nitrophenyl phosphate, and bis(2,4-dinitrophenyl) phosphate have been examined at 50 °C, under neutral-pH conditions. In neutral water, the cobalt-bound phosphodiester $[Co(en)_2(OH)[OP(O)(OC_6H_4NO_2)_2]]^+$ is cleaved $10^7$ times more rapidly ($2.7 \times 10^{-3}$ s$^{-1}$) than the unbound phosphodiester, whereas the corresponding cobalt-bound phosphomonoester $[Co(en)_2(OH)[OP(O)_2OC_6H_4NO_2]]$ is cleaved only $10^4$ times more rapidly than the unbound phosphomonoester. The reactivity patterns for cobalt complex promoted hydrolysis of bis(*p*-nitrophenyl) phosphate and bis(2,4-dinitrophenyl) phosphate are, respectively, $[Co(trien)(OH)(OH_2)]^{2+} > [Co(en)_2(OH)(OH_2)]^{2+} > [Co(dien)(OH)(OH_2)_2]^{2+}$ and $[Co(dien)(OH)(OH_2)_2]^{2+} > [Co(trien)(OH)(OH_2)]^{2+} > [Co(en)_2(OH)(OH_2)]^{2+} > [Co(en)_2(OH)(NH_3)]^{2+}$. The difference in the reactivity pattern for the hydrolysis of the two phosphodiesters is explained in terms of a change in the rate-determining step with change in the phosphodiester reactivity. The structure of the amine ligand on the cobalt complex has a significant effect on both the rate of binding of the phosphate ester to the cobalt complex and the rate of cleavage of the phosphoester bond.

There is increasing interest in developing artificial nucleases. Currently, the most efficient nonenzymic method of cleaving DNA is by an oxidative procedure.[1-3] In contrast, enzymes cleave DNA by catalyzing the hydrolysis of the phosphodiester bond. Most DNases are activated by metal ions and enhance the rate of hydrolysis of DNA by over 12 orders of magnitude. More recently it has been discovered that self-splicing RNA molecules can function as true enzymes and catalyze the hydrolysis of RNA sequence specifically in the presence of metal ions.[4] There have been numerous enzyme model studies involving metal ion catalyzed hydrolysis of phosphate esters.[5-7] Co(III) complexes are highly efficient in promoting the hydrolysis of phosphomonoesters and have been studied extensively as alkaline phosphatase and ATPase models.[8-11] Sargeson et al.[9] elucidated the mechanism of Co(III) complex promoted hydrolysis of phosphomonoesters through elegant isotope-labeling experiments. Milburn et al.[10,11] showed that dramatic rate acceleration can be obtained for the hydrolysis of ATP when two appropriate cobalt complexes interact with ATP. However, phosphodiesters are very stable compared with phosphomonoesters at neutral pH, and studies on Co(III) complex promoted hydrolysis of phosphodiesters have been few. We[12] recently showed that $[Co(trien)(OH_2)(OH)]^{2+}$ gives a much greater rate acceleration ($10^8$-fold) for the hydrolysis of cyclic AMP, a phosphodiester, than for the hydrolysis of methyl phosphate ($10^2$-fold), a phosphomonoester. In this paper, we report the effects of systematic variations in the structure of the cobalt complex on the mechanism, the rate-determining step, and the efficiency of the cobalt complex promoted hydrolysis of simple phosphate diesters.

**Table I.** Observed First-Order Rate Constants ($k_{obsd}$, s$^{-1}$) for Cobalt Complex (0.01 M) Promoted Hydrolysis of Phosphate Esters at 50 °C, pH 7.0

| cobalt complex | BNPP | BDNPP | NPP |
|---|---|---|---|
| $[Co(en)_2(OH)(NH_3)]^{2+}$ | $<10^{-6}$ | $2.4 \times 10^{-4}$ | $<10^{-6}$ |
| $[Co(en)_2(OH)(OH_2)]^{2+}$ | $2.7 \times 10^{-5}$ | $4.2 \times 10^{-4}$ | $3.0 \times 10^{-4}$ |
| $[Co(trien)(OH)(OH_2)]^{2+}$ | $4.8 \times 10^{-4}$ | $5.2 \times 10^{-3}$ | $5.6 \times 10^{-3}$ |
| $[Co(dien)(OH)(OH_2)_2]^{2+}$ | $<10^{-6}$ | $8.6 \times 10^{-2}$ | $<10^{-6}$ |
| none | $3.0 \times 10^{-10}$ [a] | $2.1 \times 10^{-6}$ [b] | $6.0 \times 10^{-8}$ [c] |

[a] Reference 24; extrapolated from rate measured at 100 °C with $\Delta S^{\ddagger}$ value of −25.5 eu. [b] Reference 25; rate measured at 50 °C. [c] Reference 26; extrapolated from rate measured at 50 °C with $\Delta S^{\ddagger}$ value of 3.5 eu.

## Experimental Section

**Instruments.** Nuclear magnetic resonance spectra were taken on a Varian XL-300 spectrometer. Chemical shifts were measured relative to 1-(trimethylsilyl)-1-propanesulfonic acid, sodium salt (DSS).

Kinetic studies were carried out by a UV method using a Hewlett-Packard 8451A diode array spectrophotometer equipped with a Lauda RM6 thermostat.

**Materials.** Disodium *p*-nitrophenyl phosphate (NPP), and sodium bis(*p*-nitrophenyl) phosphate (BNPP) were purchased from Sigma. Ethylenediamine (en), diethylenetriamine (dien), and triethylenetetramine (trien) were purchased from Aldrich.

*cis*-$[Co(trien)Cl_2]Cl$,[13] *cis*-$[Co(en)_2Cl_2]Cl$,[14] $[Co(dien)Cl_3]$,[15] and *cis*-$[Co(en)_2(NH_3)Br]Br_2$[16] were prepared according to well-known procedures. Bis(2,4-dinitrophenyl) phosphate (BDNPP) was synthesized by the method of Milburn et al.[17]

**Kinetics. UV Methods.** The hydrolysis of NPP, BNPP, and BDNPP was monitored by following the UV change at 400 nm. All the reactions were carried out under pseudo-first-order conditions with a large excess of the cobalt complex over the phosphate ester. The rate constants were obtained by fitting the first 3 half-lives of the kinetic data according to

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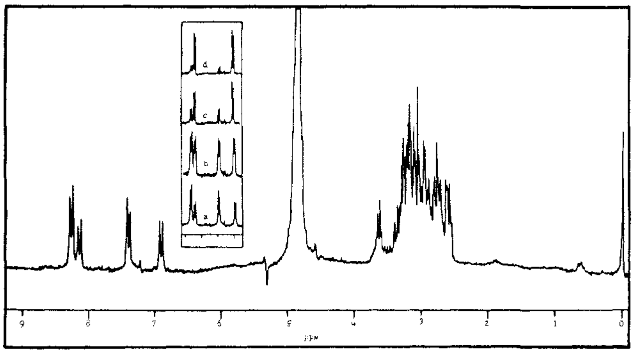


**Figure 1.** $^1H$ NMR (300-MHz) spectrum taken 1 h after mixing BNPP (0.01 M) with $[Co(trien)(OH)(OH)_2]^{2+}$ (0.1 M) in $D_2O$, pD 7, 25 °C. Inset shows time dependence of aromatic C–H signal: a 1, b 3, c 7, d 10 h.

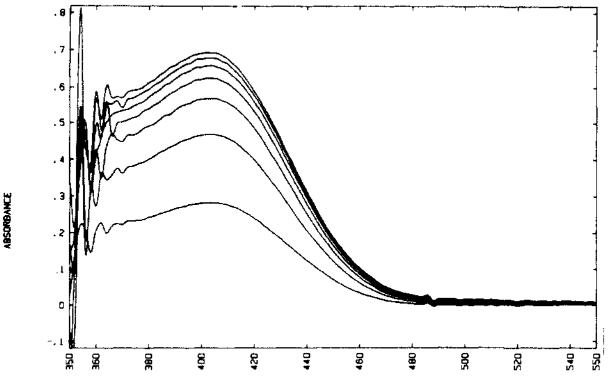


**Figure 2.** Change in UV spectrum due to $[Co(trien)(OH)(OH)_2]^{2+}$ (0.01 M) promoted hydrolysis of BDNPP ($10^{-5}$ M) at 50 °C, pH 7, recorded at 3-min intervals.

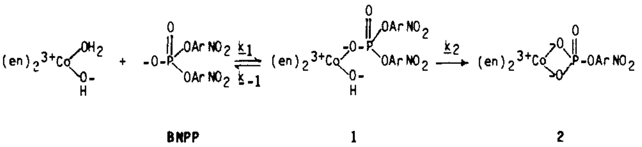
a first-order kinetics equation (correlation coefficient >0.995). Each rate constant shown in Table I is an average of rate constants obtained from at least three kinetic runs. In all cases, rate constants obtained from individual kinetic runs did not deviate from the average value by more than 3%. In a typical UV experiment, 0.01 M $[Co(trien)(OH)(OH)_2]^{2+}/[Co(trien)(OH_2)_2]^{3+}$ solution in water was prepared by adding 1.5 equiv of NaOH to $[Co(trien)Cl_2]Cl$. After 10 min, the solution was adjusted to pH 7.0. The hydrolysis of BNPP was initiated by addition of 5 μL of a 0.01 M BNPP stock solution in water to 3 mL of the freshly prepared 0.01 M cobalt complex solution at 50 °C. The cobalt complexes gradually lose activity in solution [$[Co(dien)(OH)(OH_2)_2]^{2+}$ being least stable] because of dimerization and polymerization processes.[13,18] The pH of the reaction solution did not change significantly (pH 7.0 ± 0.2) during the course of the hydrolysis reaction due to the buffering effect of the cobalt complex solution (the first and second $pK_a$s of the cobalt diaquo complexes are 6 and 8).[8b] Addition of NaCl had no appreciable effect on the rate of the hydrolysis reaction. However sodium phosphate inhibits the hydrolysis reaction.

**NMR Method.** In a typical NMR experiment, the hydrolysis of 0.01 M BNPP in 0.1 M $[Co(trien)(OH)(OH_2)]^{2+}/[Co(trien)(OH_2)]^{3+}$ solution in $D_2O$ (pD 7.0, 25 °C) was followed by monitoring the $^1H$ NMR signals of the free *p*-nitrophenol (6.973, d; 8.172, d) and BNPP (7.414, d; 8.271, d).

## Results and Discussion

Cobalt complex promoted hydrolysis of the phosphodiesters BNPP and BDNPP each gives 2 equiv of substituted phenols. Kinetic studies by UV and NMR methods showed that there is no buildup of the phosphomonoester intermediate during the phosphodiester hydrolysis reaction. The rate of hydrolysis of BNPP increases linearly with increase in the concentration of $[Co(trien)(OH)(OH_2)]^{2+}$ (0.001–0.1 M), suggesting that there is only one cobalt complex involved in the hydrolysis of BNPP. Figure 1 shows the NMR change and Figure 2 shows the UV change that accompanies $[Co(trien)(OH)(OH_2)]^{2+}$-promoted hydrolysis of BNPP and BDNPP, respectively. The observed rate constants for the hydrolysis of NPP, BNPP, and BDNPP with and without the cobalt complexes are summarized in Table I.

**Scheme I**



In nature, Zn(II) ion is the cofactor for many hydrolytic metalloenzymes. Numerous zinc–enzyme model studies have been reported.[5,19] Co(III) ion is not involved in hydrolytic metalloenzymes. However, in simple systems, Co(III) complexes are often more active[20] than other metal ions in hydrolyzing amides and esters, because Co(III) complexes, being substitutionally inert, bind more tightly to substrates. One drawback of using Co(III) complexes as catalysts is that, in general, the hydrolytic products once formed do not dissociate from the Co(III) ion. This may not be a serious problem for using Co(III) complexes as catalysts for hydrolyzing phosphate esters, since Lincoln[21] showed that inorganic phosphate bound to Co(III) complexes can be freed hydrolytically.

**Cobalt Complex Promoted Hydrolysis of BNPP.** Our experimental data on *cis*-$[Co(en)_2(OH)(OH_2)]^{2+}$-promoted hydrolysis of BNPP can be explained in terms of the mechanism shown in Scheme I. We can rule out direct intermolecular Co(III)–hydroxide attack on BNPP, since the substitutionally inert cobalt complex $[Co(en)_2(NH_3)(OH)]^{2+}$ has minimal activity (Table I). Hydrolysis of **2** must be more rapid than the rate of its formation, since under our experimental conditions, 2 equiv of *p*-nitrophenol is produced with no accumulation of the phosphomonoester intermediate **2**. Sargeson et al.[9] showed by isotope-labeling experiments that the hydrolysis of the phosphomonoester bond in $[Co(en)_2(OH)(OP(O)_2OC_6H_4NO_2)]$ occurs by a four-membered ring formation analogous to the case in Scheme I. Anation of $[Co(en)_2(OH)(OH_2)]^{2+}$ with phosphates has been studied by Lincoln.[21] The $k_2$ step in Scheme I is the rate-determining step, since anation of the cobalt complex is faster than our observed hydrolysis reaction. *cis*-$[Co(en)_2(OH)(OH_2)]^{2+}$ is in rapid equilibrium with *trans*-$[Co(en)_2(OH)(OH_2)]^{2+}$ during the course of the phosphoester hydrolysis reaction. At neutral pH the equilibrium favors the cis form ($K = 1.5$).[22]

The rates of anation of $[Co(trien)(OH)(OH_2)]^{2+}$ and $[Co(dien)(OH)(OH_2)_2]^{2+}$ are greater than the rates of the cobalt complex promoted hydrolysis of BNPP (Table I). Therefore, the mechanism shown in Scheme I is applicable for all three cobalt complex promoted hydrolyses of BNPP. It is interesting to note that the structure of the amine ligand has a significant effect on the step for hydrolysis of the phosphodiester bond ($k_2$, Scheme I) [$[Co(trien)(OH)(OH_2)]^{2+} > [Co(en)_2(OH)(OH_2)]^{2+} > [Co(dien)(OH)(OH_2)_2]^{2+}$; Table I]. This implies that the exact position of the cobalt-bound hydroxide relative to the position of the cobalt-bound phosphate ester is important for $k_2$ to be large. If the mechanism of cobalt complex promoted hydrolysis of BNPP involved anation followed by rate-determining intermolecular hydroxide attack rather than intramolecular cobalt hydroxide attack, the above three cobalt complexes would be expected to have similar reactivities.

**Cobalt Complex Promoted Hydrolysis of BDNPP and NPP.** The reactivity pattern of the cobalt complex promoted hydrolysis of BDNPP closely resembles the reactivity pattern of the cobalt complex anation reaction [$[Co(dien)(OH)(OH_2)_2]^{2+} > [Co(trien)(OH)(OH_2)]^{2+} > [Co(en)_2(OH)(OH_2)]^{2+} > [Co(en)_2$-

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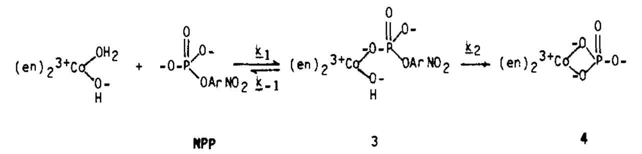
**Scheme II**



$(OH)(NH_3)]^{2+}$]. Furthermore, the observed rate constant for anation [$9 \times 10^{-4}$ s$^{-1}$, 50 °C (lit.[21] $4 \times 10^{-4}$ s$^{-1}$, 47.9 °C)] of $[Co(en)_2(OH)(OH_2)]^{2+}$ (0.01 M) with inorganic phosphate ($5 \times 10^{-4}$ M) is within the same order of magnitude as the observed rate constant for the cobalt complex promoted hydrolysis of BDNPP (Table I). Similarly, we have measured the rate of anation of $[Co(trien)(OH)(OH_2)]^{2+}$ ($3 \times 10^{-2}$ s$^{-1}$, 50 °C). On the basis of this result we propose that the mechanism of $[Co(dien)(OH)(OH_2)_2]^{2+}$-, $[Co(trien)(OH)(OH_2)]^{2+}$- and $[Co(en)_2(OH)(OH_2)]^{2+}$-promoted hydrolysis of BDNPP involves at least partially rate-determining anation followed by cleavage of the phosphoester bond.

NPP and BNPP have comparable reactivities in the presence of $[Co(trien)(OH)(OH_2)]^{2+}$ or $[Co(en)_2(OH)(OH_2)]^{2+}$ (Table I). Therefore, $[Co(trien)(OH)(OH_2)]^{2+}$- and $[Co(en)_2(OH)(OH_2)]^{2+}$-promoted hydrolysis of NPP should involve at least partially rate-determining anation followed by cleavage of the phosphoester bond (Scheme II). Consistent with this finding, Sargeson et al.[9] showed that **3** decomposes more rapidly to **4** than to the starting material. This shows that the transition-state energy of the first step is higher than that of the second step.

Although anation of $[Co(dien)(OH)(OH_2)_2]^{2+}$ with phosphate takes place rapidly, the cobalt complex is relatively inactive in promoting the hydrolysis of NPP or BNPP. However, $[Co(dien)(OH)(OH_2)_2]^{2+}$ is very efficient in cleaving BDNPP. In this last case, binding of BDNPP to the cobalt complex may be followed by intermolecular attack of the solvent molecule, since 2,4-dinitrophenolate is a very good leaving group.

Ligand exchange of $[Co(en)_2(NH_3)(OH)]^{2+}$ is slower than the cobalt complex promoted hydrolysis of BDNPP. In this case, the cobalt complex promoted hydrolysis of BDNPP involves an intermolecular Co(III)–hydroxide mechanism. The cobalt complex is inefficient in cleaving NPP, since the phosphomonoester has one more negative charge than BDNPP.

**Comparison of Cobalt Complex Promoted Hydrolysis of BNPP and NPP.** The observed rate constant for $[Co(en)_2(OH)(OH_2)]^{2+}$-promoted hydrolysis of BNPP is given by $k_{obsd} = [Co(en)_2(OH)(OH_2)]k_1k_2/k_{-1}$, since $k_2$ is the rate-determining step (Scheme I). We[12] previously estimated the equilibrium constant for displacement of the aquo ligand in $[Co(trien)(OH)(OH_2)]^{2+}$ with a phosphodiester ($k_1/k_{-1} = 1$ M$^{-1}$). Assuming that the equilibrium constant ($k_2$) for the anation of $[Co(en)_2(OH)(OH_2)]^{2+}$ is also 1 M$^{-1}$, the rate constant for cleavage of the phosphodiester bond in **1** is $2.7 \times 10^{-3}$ s$^{-1}$. The cleavage reaction is $10^7$ times faster than the hydrolysis of the uncoordinated ester under the same conditions. Sargeson[9] showed that the rate constant for cleavage of the phosphoester bond in **3** is $1.6 \times 10^{-4}$ s$^{-1}$ at 25 °C, pH 7.0. In neutral water, the hydrolysis of **3** (P–O bond cleavage) is $10^4$-fold faster than the hydrolysis of the uncoordinated phosphomonoester. It appears that the reactivity of **1** ($2.7 \times 10^{-3}$ s$^{-1}$, 50 °C) is comparable to that of **3** ($1.6 \times 10^{-4}$ s$^{-1}$, 25 °C). The phosphoryl group in **1** is a better electrophile than the phosphoryl group in **3**, but the former is sterically more hindered. A larger rate acceleration obtained for $[Co(en)_2(OH)(OH_2)]^{2+}$-promoted hydrolysis of BNPP over that of NPP (Table I) is mainly due to the availability of the metaphosphate mechanism[23] for the uncatalyzed hydrolysis of NPP. The rate acceleration is even less for cobalt complex promoted hydrolysis of phosphomonoesters with poor leaving groups[12] (e.g., methyl phosphate), since the metaphosphate mechanism is even more important for these phosphomonoesters.

## Conclusions

The mechanism as well as the rate-determining step of the cobalt complex promoted hydrolysis of the phosphoester bond is determined by the reactivity of the phosphate ester and the structure of the amine ligand on the cobalt complex. The rate-determining step for the hydrolysis of reactive phosphate esters at least partially involves binding of the phosphate ester to the cobalt complex. For less reactive phosphate esters, the rate-determining process is the phosphoester bond cleavage step itself. The amine ligand not only affects the rate of binding of phosphate esters to the cobalt complex but also has a singificant effect on the rate of cleavage of the phosphoester bond.

There is a much greater rate acceleration for cobalt complex promoted hydrolysis of phosphodiesters compared with that for phosphomonoesters. Cobalt-bound BNPP can be $10^8$ times more reactive than the unbound phosphodiester. Although this is by far the best rate acceleration attained for hydrolysis of phosphodiesters, when compared with other nonenzymic systems, further improvement is necessary to hydrolyze the phosphodiester backbone in DNA efficiently.

**Acknowledgment.** This work was supported by the Natural Sciences and Engineering Council of Canada.

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