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Hydrolysis of 2,4-Dinitrophenylphosphate Promoted by Hydroxoaquatetraaminecobalt(III) Ions

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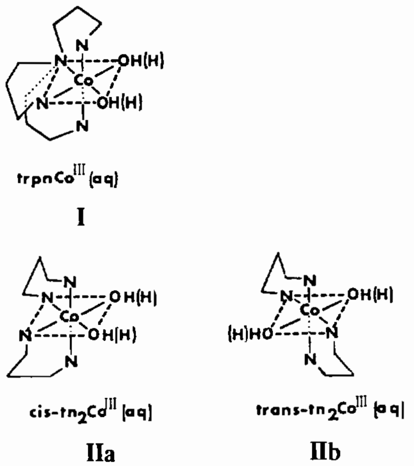
Abstract

The hydrolysis of 2,4-dinitrophenylphosphate (DNPP) to orthophosphate and 2,4-dinitrophenolate (DNP) is accelerated in the presence of excess $tn_2Co(H_2O)_2^{3+}$ or $trpnCo(H_2O)_2^{3+}$ at rates which maximize at pHs close to those at which the hydroxoaquatetraaminecobalt(III) complex concentrations peak (tn_2, pH ~6.4; trpn, pH ~6.0; tn = trimethylenediamine; trpn = 3,3′,3″-triaminotripropylamine). For dilute DNPP solutions (~10^{-4} M) the hydrolysis rates (25°C, 0.50 M $NaClO_4$) increase with increasing Co/DNPP ratio in ways that are qualitatively as well as quantitatively different for the two systems (trpn: steady increase moving toward rate saturation, higher rates; tn_2: 'S'-shaped curve with very low rates at low ratios, lower rates compared to trpn for comparable ratios). For the trpn system the results are interpreted on the basis of pre-equilibrium formation of the 1:1 monodentate-DNPP cobalt complex by substitution of the labile water on cobalt, and rate-determining attack by the *cis*-coordinated hydroxide on the phosphorus center to affect hydrolysis. For the tn_2 system the main path to hydrolysis is through a 2:1 cobalt to DNPP complex in which attack by a *cis*-coordinated hydroxide is again involved. The more complex rate behavior and the slower hydrolysis rates observed for tn_2 system result from the formation of *cis* and *trans* isomers in which *trans* arrangements of coordinated DNPP and hydroxide leave the latter unavailable to participate in intramolecular hydrolysis. Computer fitting of the observed rate data provides values of equilibrium and rate constants for the two systems. Detailed mechanistic schemes are proposed. For the trpn system at pH 6.0 and a 25:1 cobalt to DNPP ratio (5×10^{-5} M DNPP) the observed acceleration over hydrolysis in the absence of the cobalt complex is ~3×10^3; the calculated specific rate constant for hydrolysis in the reactive 1:1 complex (k ~0.2 s^{-1}) represents an acceleration over the unpromoted rate of ~3×10^4.



$trpnCo^{III}$ (aq)

I

cis-tn_2Co^{III} (aq)

IIa

trans-tn_2Co^{III} (aq)

IIb

Fig. 1. Diaquatetraaminecobalt(III) complexes used in this study. Overall charge depends on number of deprotonated waters and is thus omitted. The symbol 'aq' refers to $(H_2O)_2$, $(OH^-)(H_2O)$ or $(OH^-)_2$.

Introduction

The role of metal ions in promoting the hydrolysis of phosphate esters is of broad interest, especially because of the importance of divalent metal ions in biological phosphoryl transfer processes [1–3]. For model systems involving phosphate ester hydrolysis, or related phosphoryl transfer, determining the detailed functions of divalent ions is a difficult task due to the high labilities of the aqua metal ions and the multiplicity of species which can be involved. Some of these difficulties can be alleviated, and increased reactivities can be observed, with the use of certain semi-labile aqua/amine trivalent cobalt complexes [4–10]. Two such complexes which have been found to be effective in this regard are diaquabis(trimethylenediamine)cobalt(III) ion [4, 5, 8, 9] and diaqua-(3,3′,3″-triaminotripropylamine)cobalt(III) ion [6, 7] (**I** and **II**, Fig. 1). In the hydroxoaqua form of each of these complexes the coordinated water is remark-

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ably labile, and the interaction with a phosphate ester can lead to rapid formation of a monodentate phosphate ester complex. Such complexes then undergo reactions which can include cleavage of the ester function (hydrolysis). The present paper reports on the hydrolysis of the monoester 2,4-dinitrophenylphosphate (abbreviated as 2,4-DNPP) promoted by complexes **I** and **II**. The hydrolysis of 2,4-DNPP in the absence of multivalent metal ions has been previously examined [11, 12].

Experimental

Analytical grade reagents were used except where specified otherwise. 2,4-Dinitrophenol (2,4-DNP), which was further purified by recrystallizing twice from acetone (melting point (m.p.) 115–116 °C) [13], and the buffers tris(hydroxymethyl)aminomethane (Tris) and 2,2-bis(hydroxymethyl)-2,2′,2″-nitrilotriethanol (Bis-tris) were obtained from Aldrich Chemical Co. 1,4-Bis(3-sulphopropyl)piperazine (PIPPS) was synthesized as described by Jermyn [14].

Published methods were used to prepare sodium bis(2,4-dinitrophenyl)phosphate [15], 2,6-lutidinium 2,4-dinitrophenyl hydrogen phosphate [15], $Na_3[Co(CO_3)_3]\cdot 3H_2O$ [16], $[Co(tn)_2CO_3]ClO_4$ [17], and $trpn\cdot 4HCl\cdot 0.5H_2O$ [18].

Pyridinium Bis(2,4-dinitrophenyl)phosphate

Pyridinium bis(2,4-dinitrophenyl)phosphate was prepared in a modification of a reported method [19]. 5.52 g of 2,4-DNP were dissolved in 55 ml acetonitrile. The yellow solution was cooled and maintained in an ice-bath, and 5 ml pyridine were added with stirring followed by 10 ml of phosphorus oxychloride. After 15 min of continuous stirring, the mixture was poured into an ice-water mixture (320 ml) and kept refrigerated overnight. The resulting pale yellow solid was washed several times with ice water to remove pyridine. The pyridinium salt of bis(2,4-dinitrophenyl)phosphate was obtained as a monohydrate after recrystallization from absolute ethanol (4.46 g) (see 'Supplementary Material').

trans-$[Co(tn)_2(OH)H_2O](ClO_4)_2$

trans-Hydroxoaquabis(trimethylenediamine)cobalt(III) perchlorate was synthesized according to a literature method [17]. In our hands this procedure produces the anhydrous complex (see 'Supplementary Material').

$[Co(trpn)CO_3]ClO_4\cdot 0.5H_2O$

Carbonato(3,3′,3″-triaminotripropylamine)cobalt(III) perchlorate was synthesized* as a hemihydrate (see 'Supplementary Material') by modifying a preparation for $trenCoCO_3^+$ [20]. Details on the precedure have been reported elsewhere [21].

$[Co(trpn)(H_2O)_2](ClO_4)_3\cdot 2H_2O$

Diaqua(3,3′,3″-triaminotripropylamine)cobalt(III) perchlorate was prepared as a dihydrate. 2.5 ml of 5 M $HClO_4$ were added slowly and with stirring to 0.6 g of $[Co(trpn)CO_3]ClO_4\cdot 0.5H_2O$. The resulting red solution turned violet during the stirring. It was kept under vacuum overnight to ensure removal of all CO_2. Small needle-like deep purple crystals of the diaquo complex were formed. The product was washed with dry ether and dried over P_2O_5. The yield was 0.67 g (73%) (see 'Supplementary Material').

Acid Dissociation Constants

The two acid dissociation constants for both $[Co(tn)_2(H_2O)_2]^{3+}$ and $[Co(trpn)(H_2O)_2]^{3+}$ were determined by potentiometric pH titration with sodium hydroxide [22]. A solution of the former complex (5.00×10^{-3} M) was prepared by dissolving *trans*-$[Co(tn)_2(OH)H_2O](ClO_4)_2$ in perchloric acid, while that of the latter complex (1.40×10^{-3} M) was prepared by dissolving $[Co(trpn)(H_2O)_2](ClO_4)_3\cdot 2H_2O$ directly. The ionic strength was maintained by including 0.50 M $NaClO_4$, and the temperature was 25.0 (±0.1) °C. The values obtained were: $[Co(tn)_2(H_2O)_2]^{3+}$, $pK_{a1} = 4.97$, $pK_{a2} = 7.83$; $[Co(trpn)(H_2O)_2]^{3+}$, $pK_{a1} = 4.52$, $pK_{a2} = 7.48$. The values for $[Co(tn)_2(H_2O)_2]^{3+}$ represent only an averaged pair of acidity constants for the *cis* and *trans* isomers because of the rapid (seconds) rate of isomerization for the hydroxoaqua form [23, 24]. These averaged values are reasonably close to those reported by Fowless and Stranks [24] for slightly different conditions (22 °C and ionic strength 1.0 M).

Kinetic Studies

Rate data were collected with a Cary 210 UV–Vis recording spectrophotometer which was connected to a thermostated circulating water bath. Two quartz mixing cells (path lengths established in separate experiments) were used for the kinetic runs. For each series of kinetic runs, fresh solutions of the ester and the cobalt complex were prepared using deionized water. The ester stock solutions were prepared in fresh $NaClO_4$ solution (used to maintain ionic strength). The buffer solutions were prepared with use of the tables of Dawson and McKenzie [25]. The ester and the cobalt complex solutions were pipetted, one into each compartment of a mixing cell, and the cell was allowed to equilibrate for 15 min at the reaction temperature in the thermostated cell holder, after which the absorbance due to the unmixed solutions was recorded. The reaction was then started by rapid mixing of the two solutions. In most cases reaction progress was monitored

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*Prepared by Dr Markus Hediger.

at 344 nm where the absorbance increases due to the production of 2,4-dinitrophenolate anion[*]. Reaction progress was followed for several half-lives. Most kinetic runs were carried out under pseudo-first-order conditions in which the cobalt was in excess, and first-order kinetics were observed for at least two to three half-lives. An ionic strength of 0.50 M was maintained with $NaClO_4$. Buffers were used to control pH: Tris for pH 7.0 to 9.0, Bis-tris for pH 5.5 to 8.0, and PIPPS for pH 3.0 to 5.0. Effective extinction coefficients were required for 2,4-dinitrophenol(ate) over the pH range 4 to 9; for this purpose seven solutions 5.10×10^{-5} M in 2,4-DNP (previously twice recrystallized from acetone), 0.05 M in appropriate buffer and 0.50 M in $NaClO_4$ were prepared, and the absorbances at 344 nm were measured.

Results and Discussion

The hydrolysis of 2,4-DNPP was monitored by following the increases in absorbance at 344 nm as 2,4-DNP is released[*]. Since the pK_a for 2,4-DNP is 3.93 at 25 °C and ionic strength 0.10 M [26], and a slightly lower value will prevail at ionic strength 0.50 M, the 2,4-dinitrophenolate anion is the predominant form of free 2,4-DNP for most experiments in the present study. The majority of the kinetic runs were carried out under pseudo-first-order conditions with the cobalt complexes in excess. Observed first-order rate constants were obtained by linear least-squares fitting of $\log(A_\infty - A_t)$ *versus* time data using a calculated value of A_∞; measured and calculated values of A_∞ differed by no more than 3%[**]. For kinetic runs in which the ratio of cobalt complex to 2,4-DNPP was 7.5/1.0 or less, rate constants were obtained from the initial rates; for these cases the data used was restricted to hydrolysis of not more than 3% of the ester.

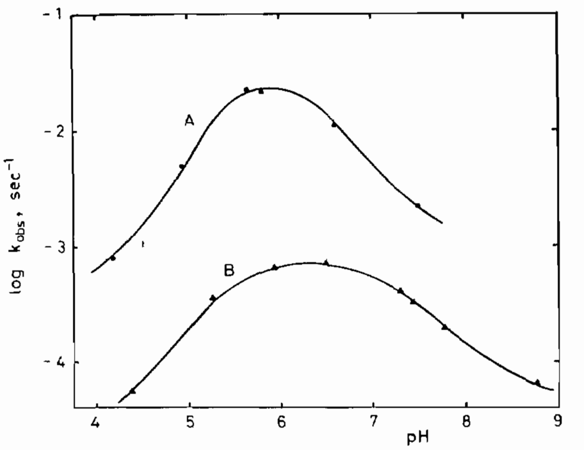

Fig. 2. pH–rate profiles for $N_4Co^{III}(aq)$ promoted hydrolysis of 2,4-DNPP. $N_4Co^{III}(aq) = 1.25 \times 10^{-3}$ M, 2,4-DNPP $= 5.00 \times 10^{-5}$ M, buffer = 0.025 M, temperature = 25.0 °C, ionic strength = 0.50 M ($NaClO_4$). A: N_4 = trpn; B: $N_4 = (tn)_2$.

The pH–rate profiles for the two $N_4Co^{III}(aq)$/2,4-DNPP systems are shown in Fig. 2[†]. In these bell-shaped profiles the rate maxima are at pH ~5.9 for the trpn system, and at pH ~6.3 for the tn_2 system. For the two systems at the pHs of their rate maxima we calculate that 94% of $trpnCo^{III}(aq)$ and 93% of $tn_2Co^{III}(aq)$ will be present as the hydroxoaqua form. Also, these pHs of the rate maxima correspond closely to those at which concentrations of the hydroxoaqua complexes will maximize; namely pH 6.0 and pH 6.4, respectively. These results are consistent with the view that for each system the hydroxoaqua complex constitutes the most reactive form of the diaqua species.

The dependence of the 2,4-DNPP hydrolysis rate on the concentration of $N_4Co^{III}(aq)$ was investigated for each system; the results are summarized in Figs. 3 and 4.

For the $trpnCo^{III}(aq)$/2,4-DNPP system we see an increase in cobalt to ester ratio is accompanied by a steady increase in rate, with a trend towards rate saturation. This behavior, together with direct observations on unusually high rates at which $trpnCo(OH)(H_2O)^{2+}$ undergoes complex formation by water substitution [27], are in accord with the view that the hydrolysis mechanism involves rapid formation of the monodentate 2,4-DNPP complex followed by rate-determining reaction of this complex in the ester hydrolysis step. A skeleton representation of this mechanism, which omits coordinated (and released) water/hydroxide, is given in reactions (1) and (2), where $M = trpnCo^{III}$, $P\Phi$ = 2,4-DNPP, $MP\Phi$ = the monodentate $P\Phi$ complex, Φ = 2,4-DNP, P = orthophosphate, and K_1 and k_1 are equilibrium and rate constants.

$$\mathrm{M} + \mathrm{P}\phi \overset{K_1}{\rightleftharpoons} \mathrm{MP\Phi} \tag{1}$$

$$\mathrm{MP\Phi} \xrightarrow{k_1} \mathrm{MP} + \Phi \tag{2}$$

This scheme leads to

$$k_{obs} = \frac{k_1 K_1 [\mathrm{M}]_0}{1 + K_1 [\mathrm{M}]_0} \tag{3}$$

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[*] The wavelength 344 nm was chosen because it is an isosbestic point in the UV–Vis spectra which exhibits small changes when $tn_2Co^{III}(aq)$ and DNP are allowed to interact.

[**] The measured values are less reliable because of secondary reactions which begin to contribute at longer time.

[†] We use $N_4Co^{III}(aq)$ to represent any of the complexes $N_4Co(H_2O)_2^{3+}$, $N_4Co(OH)(H_2O)^{2+}$ and $N_4Co(OH)_2^{+}$, where N_4 = trpn or tn_2.

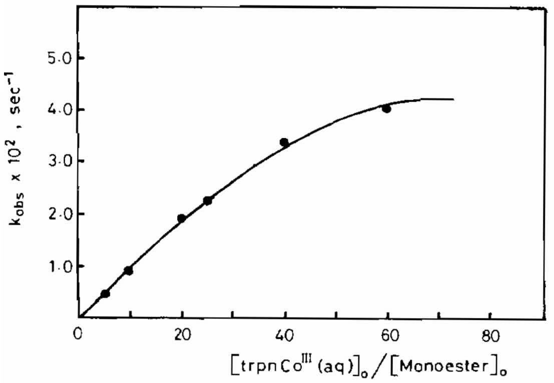


Fig. 3. Concentration–rate profile for trpnCo^{III}(aq) promoted hydrolysis of 2,4-DNPP. Conditions: 2,4-DNPP = 5.00×10^{-5} M, temperature = 24.9 (±0.1) °C, pH = 5.97 (±0.04), ionic strength = 0.50 M ($NaClO_4$).

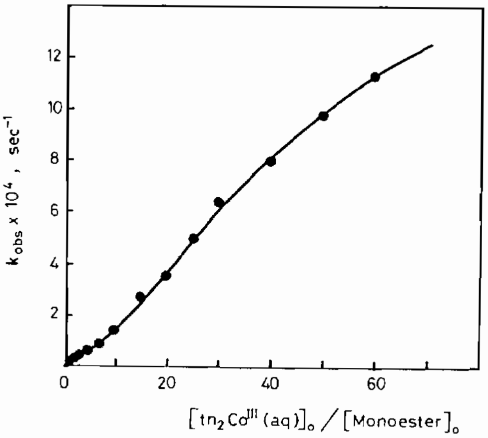
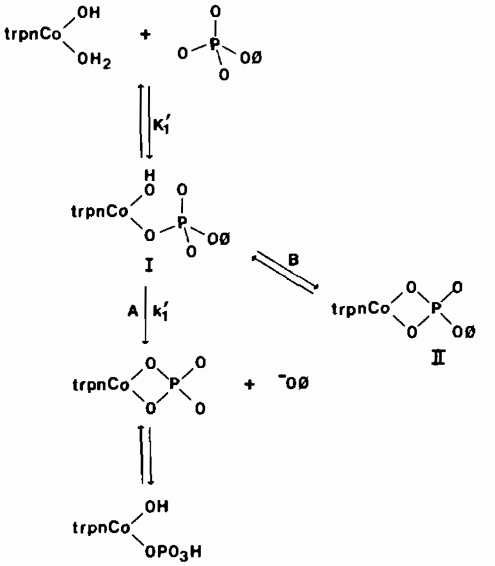


Fig. 4. Concentration–rate profile for tn_2Co^{III}(aq) promoted hydrolysis of 2,4-DNPP. Conditions: 2,4-DNPP = 1.00×10^{-4} M, pH = 7.60 (±0.05), temperature = 25.0 (±0.1) °C, ionic strength = 0.50 M ($NaClO_4$).

where k_{obs} is the observed first-order rate constant, defined by $d[\Phi]/dt = k_{obs}[P\Phi]_T$, with $[P\Phi]_T = [P\Phi] + [MP\Phi]$, and $[M]_0$ = total cobalt(III) concentration. Computer least-squares fitting of k_{obs} *versus* $[M]_0$ yielded values of k_1 and K_1 to be 0.106 s^{-1} and 216, respectively, for the reaction conditions (pH 6.0, 25 °C, ionic strength 0.50 M). The curve in Fig. 3 has been calculated using these values.

The results for the tn_2Co^{III}(aq)/2,4-DNPP system (Fig. 4, S-shaped curve) cannot be explained on the basis of a 1:1 complex providing the major hydrolysis path, and point to the participation of a reactive complex containing more than one cobalt(III) center. The simplest reaction scheme that can accommodate the observations is shown below ($M = tn_2Co^{III}$, *cis* and *trans* forms; coordinated and released water/hydroxide omitted).



Scheme 1. Proposed mechanistic pathway for the trpnCo^{III}-(aq) promoted hydrolysis of 2,4-DNPP (charges mostly omitted).

$$M + P\Phi \overset{K_2}{\rightleftharpoons} MP\Phi \tag{4}$$

$$M + MP\Phi \overset{K_3}{\rightleftharpoons} M_2P\Phi \tag{5}$$

$$MP\Phi \xrightarrow{k_2} MP + \Phi \tag{6}$$

$$M_2P\Phi \xrightarrow{k_3} M_2P + \Phi \tag{7}$$

This scheme, with $MP\Phi$ and $M_2P\Phi$ as the reactive intermediates, leads to expression (8)

$$k_{obs} = \frac{k_2K_2[M]_0 + k_3K_2K_3[M]_0^2}{1 + K_2[M]_0 + K_2K_3[M]_0^2} \tag{8}$$

where $[M]_0$ = total cobalt(III) concentration. Computer least-squares fitting of the data provided the following values: $K_2 = 314$; $K_3 = 212$; $k_2 = 1.39 \times 10^{-4}$ s^{-1}; $k_3 = 2.81 \times 10^{-3}$ s^{-1}. The curve in Fig. 4 has been calculated with using these values.

In Scheme 1 we provide a detailed mechanistic interpretation for the reaction of trpnCo^{III}(aq) with 2,4-DNPP in the middle pH region. Reaction between $trpnCo(OH)(H_2O)^{2+}$ (the major species and the most reactive towards water substitution) and the 2,4-DNPP dianion* leads to rapid preequilibrium formation of the monodentate intermediate complex **I**. Intermediate **I** may react intramolecularly via path A, which leads to hydrolysis, or via path B, which is an unproductive side reaction.

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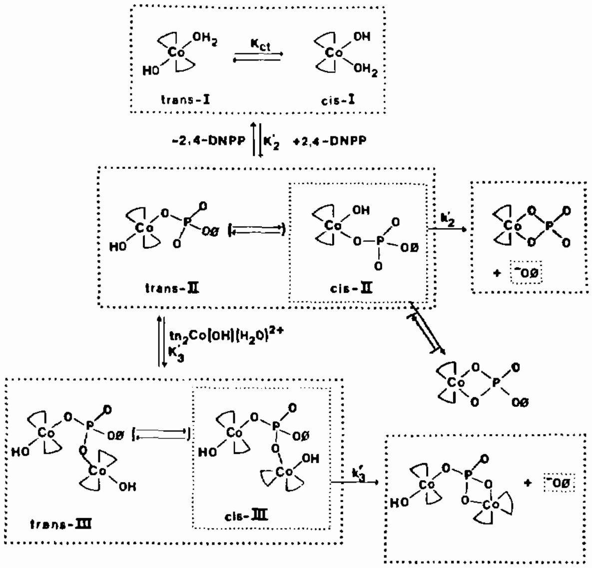
*For 2,4-DNPP, pK_a ~4.5 at 1 °C; see ref. 12(a).



Scheme 2. Proposed mechanistic pathway for the tn_2Co^{III}(aq) promoted hydrolysis of 2,4-DNPP (charges mostly omitted).

This side path B does not prevent the ester from being hydrolyzed; in fact close to 100% of the available 2,4-DNPP is released around pH ~6. The orthophosphate chelate arising through path A may also undergo reversible ring-opening in a similar manner to the corresponding en_2Co^{III}(aq)/orthophosphate system [28]. The key step in the hydrolysis (path A, rate constant k_1') is presumed to involve attack by the coordinated hydroxide on the phosphorus center in a manner analogous to that demonstrated in related systems [1, 4–7, 9, 10, 29, 30]. The rate constant for this step (k_1') is specific for the hydrolysis and the numerical value must be larger than the rate constant measured for reaction (2) (k_1) since the latter must also accommodate the unproductive side path.

Scheme 2 provides a mechanistic interpretation for the reaction of tn_2Co^{III}(aq) with 2,4-DNPP. The hydroxoaqua complex **I** (*trans* and *cis* forms, which can be considered to be in equilibrium [23, 24]) reacts with 2,4-DNPP to produce the monodentate 1:1 complex (*trans*/*cis*-**II**). *cis*-**II** can react with attack by the coordinated hydroxide on the phosphorus center to produce 2,4-DNP, or with ring closure with loss of the coordinated hydroxide/water. But the more important path to hydrolysis involves reaction of *trans*-**II** with additional $tn_2Co(OH)(H_2O)^{2+}$ to produce the binuclear species **III**. *cis*-**III** provides the hydrolysis route with attack of the phosphorus by the proximate coordinated hydroxide. The computed rate constants are consistent with the mechanistic scheme displayed.

We thus see that the hydrolysis of 2,4-DNPP is enhanced by both $trpnCo^{III}$(aq) and tn_2Co^{III}(aq), although by different degrees. At the pH of the rate maxima, a 2,4-DNPP total concentration of 5×10^{-5} M, and a metal to ester ratio of 25:1, the enhancements for hydrolysis over the unpromoted rate [12a] are $k_{obs} \sim 3 \times 10^3$ and ~90 for the trpn and tn_2 systems, respectively. However, when the computed rate constants for the hydrolysis steps are considered, $trpnCo^{III}$(aq) is seen to enhance the hydrolysis by a factor of $\sim 1 \times 10^4$ while tn_2Co^{III}(aq) shows an enhancement by $\sim 3 \times 10^2$ (for the step involving the 2:1 intermediate. Since k_1, k_2 and k_3 are not specific to the hydrolysis step, and include competing reactions, the true enhancements inherent to the specific hydrolysis steps, must be even greater. This will be especially true for the tn_2 complex, where the isomeric forms in which the coordinated hydroxide is *trans* to the monodentate phosphate ester will be inactive towards hydrolysis. In fact, *trans*-**III** may well be the predominant form of **III**, in part because of the repulsion between the formally negative coordinated hydroxide and phosphate ester groups. According to this analysis, k_3' (Scheme 2), and k_1' (Scheme 1) could easily be of comparable magnitude.

These considerations have general relevance to the roles of aqua ions (such as Cu^{2+}, Zn^{2+}, Cd^{2+}, La^{3+}) in promoting the hydrolysis of phosphate esters and polyphosphates in simple model systems, and to related roles which certain metal centers may play in biological phosphoryl transfer [1, 5]. In simple model system complexes, in which a metal center is coordinated to both phosphate ester (or polyphosphate) and hydroxide, it may be common to have only a small proportion of isomer(s) in which the hydroxide is appropriately positioned for attack on the phosphorus center. Indeed, it has been shown that quite special structures are often required to provide significant concentrations of complexes in which coordinated hydroxide is appropriately positioned [1]. However, in enzymatic systems the special additional ligating groups and coordination environment may be able to provide stereochemistry in which coordinated hydroxide is optimally positioned in relation to a phosphate substrate, thus providing a clear path for metal ion/coordinated hydroxide promoted phosphoryl transfer.

Supplementary Material

Results for elemental analysis are available from the authors on request.

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