# Metal-ion catalysed phosphate diester transesterification: quantifying double Lewis-acid activation

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**Double Lewis-acid activation provides a substantial (*ca.* 4 × $10^5$ fold) rate acceleration for the cleavage of phosphate diesters.**

Metal ions play enormously important roles in both enzymic and non-enzymic hydrolysis of phosphate diesters. Many enzymes that hydrolyse phosphate ester bonds (*e.g.* DNases,[1] RNases,[2] phosphatases[3] and phospholipases[4]) and those that synthesize them (*e.g.* DNA polymerases[5]) are activated by two or more metal ions, and over the years there has been considerable interest in understanding the mechanistic role of metal ions in these metalloenzymes. It has been shown that metal ions can accelerate the rate of phosphate ester hydrolysis by coordinating a phosphoryl oxygen (Lewis-acid activation[6,8]), by coordinating a water molecule and generating a metal-hydroxide nucleophile (metal-hydroxide activation[7,8]) and by coordinating the leaving-group oxygen (leaving-group activation[9]). Since there can be more than one metal centre in a catalytic system and each metal centre can have multiple contacts with the phosphate, it is fundamentally important to be able to dissect quantitatively the rate accelerations observed in metal-ion catalysed cleavage of phosphate esters to understand the importance of each interaction. Here we report on quantifying double Lewis-acid activation for cleaving phosphate diesters.

In order to quantify this effect for cleaving phosphate diesters we investigated the base catalysed transesterification (Scheme 1) of 2-hydroxypropyl phenyl phosphate (HPP) bridged to two $Co^{III}$ centres **1** to give the bridged cyclic phosphate diester **2** and phenol. Thus, the effect of the metal ions is unambiguously associated with double Lewis-acid activation. As a control experiment, phenyl methyl phosphate bridged to the dinuclear complex **3** was also examined.

Perchlorate salts of **1** and **3** were synthesized following the procedure for making the perchlorate salt of the dinuclear complex with a bridging acetate,[10] and showed the characteristic ≈ 14 ppm shift[11] relative to the unbound diesters in their $^{31}P$ NMR spectra.† The production of **2** was confirmed by synthesis from a genuine sample of propylene phosphate.‡ The transesterification reaction (Scheme 1) was monitored by $^{31}P$ NMR and by HPLC.

$^{31}P$ NMR reveals that **1** (7.2 mmol $dm^{-3}$, δ7.0) reacts in a buffered solution [50 mmol $dm^{-3}$ 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid (EPPS)] at pH 8.0, 25 °C to initially give **2** (δ30.4) and free HPP (δ−6.9). Compound **2** gradually hydrolyses to give **4a** (δ14.3) and **4b** (δ13.7) in a ratio of *ca.* 1 : 1 with some dissociation to free propylene phosphate (δ15.0). Under our experimental conditions, neither dissociation of the monoesters from **4a** and **4b** nor reaction of the unbound diesters was observed.

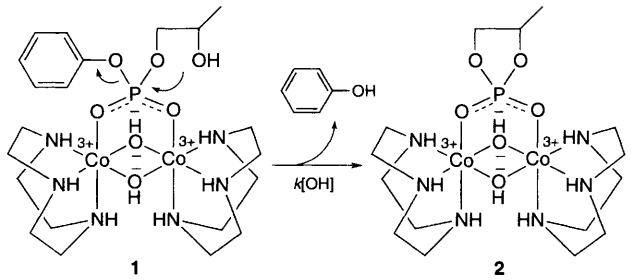
HPLC analysis by repeated injection of a solution of **1** [0.2 mmol $dm^{-3}$ in 50 mmol $dm^{-3}$ aqueous buffer, $I$ = 0.1 mol $dm^{-3}$ ($NaClO_4$), at 25 °C] shows that the peak due to **1** (≈ 12 min) decreases with a concomitant increase in the peaks due to phenol (8.4 min) and HPP (10.2 min). At pH > 8, aliquots of the reaction mixture were quenched with pH 5.5 buffer (0.2 mol $dm^{-3}$ ammonium phosphate) and the amount of phenol measured for each sample. By measuring the ratio of phenol to the free diester (HPP) produced (after 10 half-lives for the quenched reactions), rate constants for both transesterification and dissociation were obtained at each pH.§ The pH-rate profiles (Fig. 1) for the transesterification and dissociation reactions were fit according to eqns. (1) and (2) respectively.

$$k_{obs} = k_1[OH^-] \quad (1)$$

$$k_{obs} = k_2[OH^-] + k'_2 \quad (2)$$

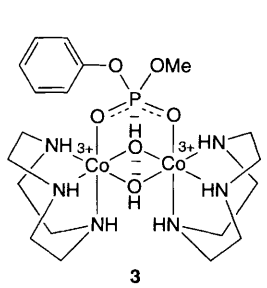
While both processes are hydroxide catalysed ($k_1$ = 430 ± 20 $dm^3$ $mol^{-1}$ $s^{-1}$; $k_2$ = 130 ± 10 $dm^3$ $mol^{-1}$ $s^{-1}$), a water rate ($k'_2$ = 3.4 ± 0.4 × $10^{-5}$ $s^{-1}$) for the dissociation reaction is also observed. Under the same conditions, there is less than 3% phenol produced from **3** indicating that the phenol produced from **1** is due to the transesterification reaction shown in Scheme 1. Thus, alternative mechanisms for phenol production from **1** involving metal-bound nucleophiles followed by formation of **2** are unlikely. The rate of dissociation (across the same pH range) of phenyl methyl phosphate from **3** ($k_2$ = 133 ± 7 $dm^3$ $mol^{-1}$ $s^{-1}$ and $k'_2$ = 3.0 ± 0.3 × $10^{-5}$ $s^{-1}$) is the same within experimental error as the rate of dissociation of HPP from **1** (and hence phenol is produced > 100 times faster from **1** than **3**).

Pioneering work by Sargeson's research team established the value of studying the reactivity of phosphate esters coordinated to substitutionally inert metal complexes. They showed that single Lewis activation can provide a rate acceleration of about 400 fold for hydrolysis of a phosphate triester.[6] Furthermore,

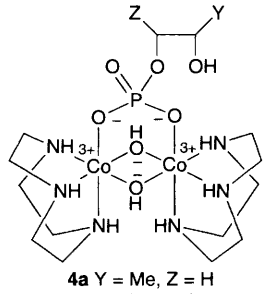



**1** **2**

**Scheme 1**




**3**




**4a** Y = Me, Z = H
**4b** Y = H, Z = Me

they have also shown that the addition of a second $Co^{III}$ to a phosphate monoester already coordinated to a $Co^{III}$ leads to a $\approx 50$ fold increase in the rate of hydrolysis.[12] Similarly, they conclude that coordination of a phosphate diester to a $Co^{III}$ centre provides a comparable rate enhancement.[12] Despite the modest rate accelerations provided by single Lewis-acid activation, we reasoned that double Lewis-acid activation should have a substantially greater effect on phosphate diester hydrolysis. In the single Lewis-acid activation mode, the developing negative charge in the phosphate diester cleavage reaction is not stabilized by the metal ion [Scheme 2, **a**]; however, in the double Lewis-acid activation mode, it is stabilized by the two metal ions [Scheme 2, **b**]. It should also be noted that even with double Lewis-acid activation, the developing negative charge in phosphate monoester hydrolysis is not stabilized by the two metal ions [Scheme 2, **c**] and this reaction is more akin to single Lewis-acid activation of a phosphate diester.

Dinuclear metal complexes that can doubly coordinate phosphate diesters have recently been shown to be particularly reactive for cleaving them;[13] is double Lewis-acid activation a plausible explanation for this reactivity? In order to quantify double Lewis-acid activation for cleaving phosphate diesters, we have determined the second-order rate constant for hydroxide catalysed transesterification of **1** as $430 \pm 20$ dm$^3$ mol$^{-1}$ s$^{-1}$ at 25 °C. The second-order rate constant for hydroxide catalysed cleavage of HPP is $9.8 \times 10^{-4}$ dm$^3$ mol$^{-1}$ s$^{-1}$ at 25 °C.[14] Remarkably, double Lewis-acid activation in the model system provides about $4 \times 10^5$ fold-rate acceleration for cleaving the diester.

Unlike single Lewis-acid activation which only provides modest rate accelerations for cleaving phosphate esters, double Lewis-acid activation provides a substantial rate enhancement. Double Lewis-acid activation should be particularly useful for cleaving RNA, which has already a very efficient intramolecular nucleophile to facilitate cleavage. The second-order rate constant for hydroxide catalysed cleavage of ApA is about $3.2 \times 10^{-3}$ dm$^3$ mol$^{-1}$ s$^{-1}$ at 25 °C.[15] Therefore the half-life for RNA cleavage due to hydroxide catalysis at pH 7 and 25 °C should be about 70 years. The rate-acceleration observed here would reduce this to about 90 min. Thus, this mode of activation has much potential in the design of catalysts capable of hydrolysing RNA efficiently. Further detailed study is in progress on this model system and mode of activation.

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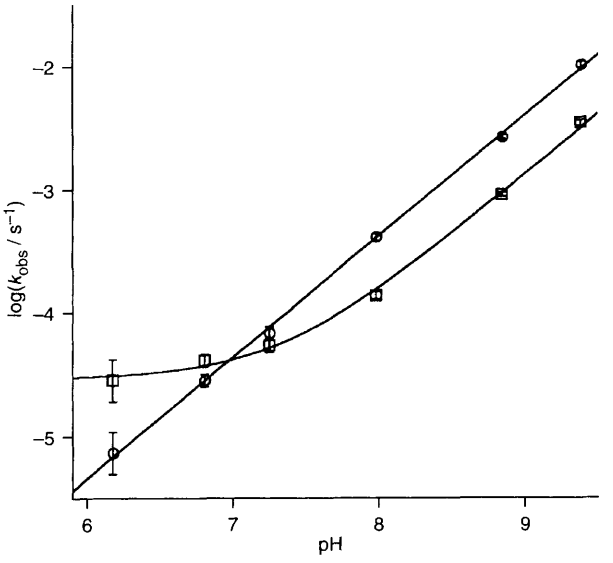
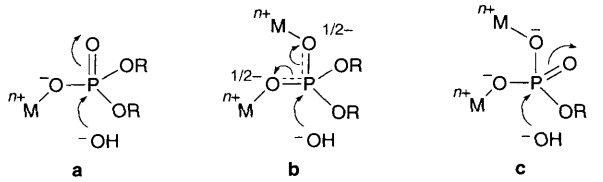



**Fig. 1** The pH rate profile for transesterification (■) and dissociation (●) of **1** at 25 °C, 50 mmol dm$^{-3}$ buffer, $I$ = 0.1 mol dm$^{-3}$ ($NaClO_4$). Solid lines are fitted using equations and constants given in the text.




**Scheme 2**

## Footnotes

† Analysis for **1**: Found C, 26.33; H, 5.00; Co, 12.13; N, 8.61. $C_{21}H_{44}Cl_3Co_2N_6O_{19}P{\cdot}H_2O$ requires C, 26.33; H, 4.84; Co, 12.31; N, 8.77%. For **3**: Found C, 24.78; H, 4.78; Co, 12.92; N, 9.04 $C_{19}H_{40}Cl_3Co_2N_6O_{18}P{\cdot}H_2O$ requires, C, 24.97; H, 4.63; Co, 12.90 N, 9.20%. **1** and **3** were also characterised by $^1$H, $^{13}$C and $^{31}$P NMR.

‡ $\delta_P$ (121.5 MHz; $D_2O$) bound 30.4, free 15.0, hydrolysis products at 14.3 and 13.7 relative to 10% trimethyl phosphate in $D_2O$.

§ At the low buffer concentrations used, buffer catalysis does not provide a significant contribution to the observed rate of reaction. This was confirmed by repeating runs at 50 and 100 mmol dm$^{-3}$ total buffer concentration for 50% free-base [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] (HEPES; pH 7.52) and EPPS (pH 7.91 respectively; in both cases, the observed rate constants obtained did not differ within the experimental error.

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