A Remarkable Rate-accelerating Metal Ion Effect on the Intramolecular Transesterification Reaction of a Phosphodiester in Acetonitrile†

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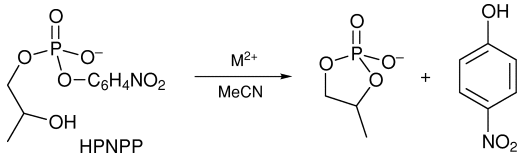
The intramolecular transesterification reaction of 2-hydroxypropyl 4-nitrophenyl phosphate is greatly accelerated (10^5–10^6 fold) by the addition of divalent metal ions such as Zn^{2+} and Cu^{2+} in MeCN without a base; the metal ion effect is quite sensitive to the content of H_2O in MeCN.

Hydrolytic cleavage of phosphodiesters attracts considerable attention from the viewpoint of preparing artificial nucleases.[1] X-Ray crystallographic data have revealed that metal ions and hydrogen bonding play crucial roles in the hydrophobic active sites of some nucleases.[2] Thus, to investigate these two functions concurrently in model systems by employing functional molecules bearing both metal-binding and hydrogen-bonding sites, the choice of solvent is very important. These two electrophilic interactions should be more favourable in organic solvents.[3] In fact, transesterification reactions of activated phosphodiesters have been reported to be greatly facilitated *via* hydrogen bonding of the guanidinium receptors in organic solvents such as MeCN[4] and DMF.[5] On the other hand, to our surprise, the metal ion influence on phosphodiester cleavage in organic solvents has been scarcely reported, although transesterifications of 2-hydroxypropyl 4-nitrophenyl phosphate[6] (HPNPP[7]) as well as RNA[8] have been extensively investigated with metal complexes in aqueous solutions. This prompted us to study the action of metal ions on the transesterification reaction of HPNPP in organic solvents. In this paper, we report large rate-accelerating metal ion effects (Zn^{2+} and Cu^{2+}) on the transesterification of HPNPP in MeCN without a base.



Scheme 1 $M^{2+} = Zn^{2+}$, Cu^{2+}

Pseudo-first-order rate constants (k_{obs}) for the transesterification of HPNPP were determined spectrophotometrically by following the UV–VIS absorption increase of 4-nitrophenol at 324 nm in organic solvents. The rates followed first-order kinetics up to at least three half-lives. Concentration effects of the metal ions on the rates were examined. As can be seen in Fig. 1, the rates increase dramatically on adding an equimolar amount of the metal ion to HPNPP solutions, and then reach saturation. This suggests strong complexation of the metal ion with HPNPP. The rate constants (k_{obs}) determined under various reaction conditions are collected in Table 1. In MeCN, the rate accelerations at $[M^{2+}] = 2.5 \times 10^{-4}$ M are 3.8×10^6-fold for Zn^{2+} and 2.7×10^5-fold for Cu^{2+}, respectively. It is reported that the transesterification of HPNPP is accelerated by a factor of 20–70-fold in the presence of Zn^{2+} (with a few molar excess over HPNPP) in aqueous solutions.[6c,h] In fact, the rates were found to decrease dramatically with an increase of the H_2O content in MeCN (Fig. 2). The rate constant with 0.01% H_2O, for example, decreases by a factor of 1/250 in the presence of 2.01% H_2O. Adding other solvents such as MeOH and DMF also decreases the rate due to weakening of the electrophilicity of the metal ions. In addition, the hydrolysis of bis(4-nitrophenyl) phosphate with no internal hydroxy group is very slow ($k_{obs} < 10^{-6}$ s^{-1}) in the presence of Zn^{2+} in MeCN. These results can be explained by the electrophilic catalysis of metal ions which

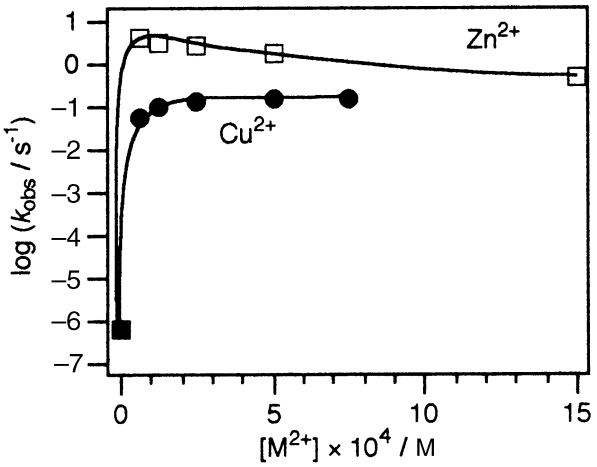


Fig. 1 Plots of k_{obs} *vs.* $[M^{2+}]$. [HPNPP] $= 5.0 \times 10^{-5}$ M in MeCN (0.01% H_2O v/v) at 25 °C

Table 1 Pseudo-first-order rate constants for the transesterification of HPNPP in organic solvents

Solvent[a]	Metal ion[b]	Additive[c]	k_{obs}/s^{-1} [d]
MeCN	None		6.8×10^{-7} [e]
MeCN	Zn^{2+} (2.5)		2.55[f]
MeCN	Zn^{2+} (2.5)	18C6 (1.0)	2.41[f]
MeCN	Zn^{2+} (2.5)	bpy (2.5)	2.09×10^{-1} [e]
MeCN–MeOH (50%)	Zn^{2+} (2.5)		9.87×10^{-4} [e]
MeOH	Zn^{2+} (2.5)		1.18×10^{-6} [e]
DMF	Zn^{2+} (2.5)		3.74×10^{-6} [e]
MeCN	Cu^{2+} (2.5)		1.26×10^{-1} [e]
MeCN	Cu^{2+} (1.25)		9.74×10^{-2} [e]
MeCN	Cu^{2+} (1.25)	18C6 (1.0)	4.77×10^{-2} [e]
MeCN	Cu^{2+} (2.5)	bpy (2.5)	2.61×10^{-3} [e]
MeCN–MeOH (50%)	Cu^{2+} (2.5)		6.41×10^{-4} [e]
MeOH	Cu^{2+} (2.5)		6.32×10^{-5} [e]

[a]In the presence of 0.01% H_2O (v/v) in the reaction mixture. [b]Concentration ($\times 10^{-4}$ M) of metal ions in parentheses. [c]Concentration ($\times 10^{-4}$ M) of additives in parentheses. In all cases, additional 18C6 (5.0×10^{-5} M) was present in the reaction mixture. [d][HPNPP] $= 5.0 \times 10^{-5}$ M at 25 °C. [e]Determined by UV–VIS spectroscopy. [f]Determined by the stopped flow method.

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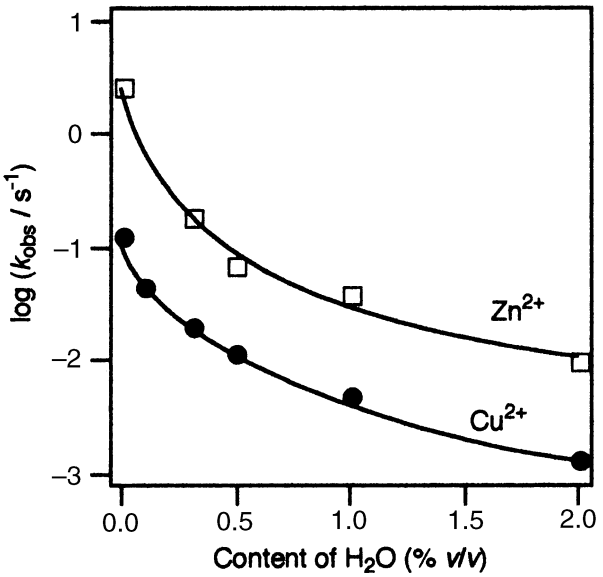

Fig. 2 The dependence of k_{obs} on the content of H_2O in MeCN. [HPNPP] = 5.0×10^{-5} M, [M^{2+}] = 2.5×10^{-4} M at 25 °C

activate the internal hydroxy group, wherein the Lewis acidity of the metal ion is more obvious in organic solvents of low donor numbers.[3]

18-Crown-6, used to dissolve HPNPP in organic solvents, decreases the rate slightly probably due to weak complexation with Zn^{2+} and Cu^{2+}.[9] However, the addition of a stronger metal-binding ligand, 2,2′-bipyridine (bpy), was found to decrease the rate by one order of magnitude.

Based on an experiment with an excess of HPNPP over Zn^{2+}, the metal ion was found not to be a turnover catalyst, suggesting that the metal ion coordination to the product is stronger than that to HPNPP. Furthermore, it is noteworthy that the transesterification of HPNPP in the presence of metal ions proceeds without a base. This strongly suggests that the hydroxy group of HPNPP is involved in metal ion coordination, reducing the pK_a of the hydroxy group to provide a nucleophilic alkoxide ion.[1]

Rate accelerations of phosphate ester hydrolysis due to metal ions are known to be interpretable in terms of electrophilic catalysis: (i) activation of the substrate and the nucleophile, (ii) stabilization of the transition state and/or the hypervalent phosphorus(v) intermediate, and/or (iii) facilitation of the cleavage of the leaving group. All of these may be facilitated in organic solvents. To our knowledge, this is the first example of the observation of such a large rate acceleration upon addition of the metal ions without any ligand for intramolecular transesterification of the phosphate diester. The present results should be informative for the construction of artificial nuclease systems.

Experimental

Barium 2-hydroxypropyl 4-nitrophenyl phosphate (HPNPP)[7] and pyridinium bis(4-nitrophenyl) phosphate[10] were prepared according to the literature procedures.

Rate measurement.—To a distilled organic solvent containing an appropriate amount of $Cu(NO_3)_2 \cdot 6H_2O$ or $Zn(NO_3)_2 \cdot 6H_2O$ was added 15 μl of a stock solution of HPNPP [1.0×10^{-2} M in MeCN (2% H_2O) containing 18-crown-6 (1.0×10^{-2} M)]. For fast reactions ($k_{obs} > ca.$ 10^{-1} s^{-1}), the rates were followed by the stopped flow method. Released 4-nitrophenol was followed with a JASCO Ubest -560 spectrophotometer and Otsuka Electronics RA-401. Formation of 4-nitrophenol was confirmed by TLC analysis.

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