

Cooperative Effect of a Metal Ion and Hydrogen Bonds on Phosphodiester Cleavage in Acetonitrile

Shin-ichi Kondo,* Yuichi Sakuno, Takashi Yokoyama, and Yumihiko Yano*
 Department of Chemistry, Gunma University, Kiryu, Gunma 376-8515

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Cleavage of an activated phosphodiester by $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ was accelerated (9×10^3 -fold) by cooperative effect of a metal ion and hydrogen bonds in the presence of a bismelamine derivative bearing a 2,2'-bipyridine moiety in MeCN.

Hydrolytic cleavage of phosphodiester attracts considerable attention from a view point of artificial nucleases,¹ probably because of therapeutic applications.² For construction of artificial enzymes,³ it would be required to arrange all the necessary functional groups for catalysis in the reaction site. It is known that both metal ions and hydrogen bonds play a crucial role in active sites of some nucleases.⁴ However, the roles of metal ions and hydrogen bonds have been investigated separately in model systems.⁵ To our knowledge, there have been few reports which deal with both the effects together, although such an idea seems to be not novel.^{5d} For example, Krämer et al. reported the first synthetic models of rate-enhancing cooperativity of a metal ion and hydrogen bonds provided by cationic ammonium in phosphodiester hydrolysis in ethanol–water (19:1).^{1,6} For investigation of the cooperativity, it is of primary importance to design a molecule which provides a metal-binding and hydrogen-bonding sites. We wish to report rate-accelerating cooperativity of a metal ion and hydrogen bonds in cleavage of phosphodiester, bis(2,4-dinitrophenyl) phosphate⁷ (BDNPP) in MeCN by employing a bismelamine derivative bearing a 2,2'-bipyridine moiety (Chart 1).

Melamine derivatives **1**–**5** were prepared from cyanuric chloride by stepwise substitutions with the corresponding amines and the structures were confirmed by NMR, MS, and elemental analyses. Hydrolysis of BDNPP by $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (10 molar excess) was spectrophotometrically examined in MeCN, giving 2,4-dinitrophenol passing through isosbestic points. Pseudo-first-order rate constants (k_{obs}) were determined by following absorption increases at 340 nm (at least 3 half lives). Successive hydrolysis of 2,4-dinitrophenyl phosphate was confirmed to be negligible under the same conditions (HPLC analysis).

Concentration effects of Zn(II) on the rates in the presence of melamine derivatives are shown in Figure 1. Sigmoidal kinetics for melamine derivatives bearing a 2,2'-bipyridine moiety (**2**–**4**) were observed because **2**–**4** formed 2 : 1 and 1 : 1

complexes with Zn(II). Association constants for **2** with Zn(II) in MeCN at 298 K were estimated to be $\log K_1 = 5.6$ ($2 + \text{Zn(II)} \rightleftharpoons 2 \cdot \text{Zn(II)}$) and $\log K_2 = 5.1$ ($2 + 2 \cdot \text{Zn(II)} \rightleftharpoons 2 \cdot 2 \cdot \text{Zn(II)}$) by UV-vis titration. This allowed us to calculate the amount of the 1 : 1 complex [$2 \cdot \text{Zn(II)}$] at a given concentration of Zn(II) (Figure 1). A fairly good correlation was observed between k_{obs} and the amount of $2 \cdot \text{Zn(II)}$. It should be noted that the rate accelerations of **2** with four H-donors are much larger than those of **3** and **4**, suggesting that positioning of the metal ion and four hydrogen bonds are responsible for the rate acceleration.

Hydrogen-bonded complex formation of the melamine derivatives (**2** and **3**) and tetrabutylammonium diphenyl phosphate (DPP^-)⁸ with Zn(II) was examined by ^1H NMR and ESI-MS in MeCN. As shown in Figure 2, the melamine NH protons (5.2 and 5.7 ppm) were shifted to downfield and overlapped with the aromatic protons on addition of 1 equiv Zn(II), and the signals were broadened. However, the signals were sharpened again on addition of 2 equiv Zn(II) probably because ca. 70% of **2** exists as $2 \cdot \text{Zn(II)}$. It was difficult to determine the shift of the NH signals due to broadening in the presence of DPP^- . A mixture of **2**, Zn(II), and DPP^- in MeCN was found to form a ternary complex [$2 \cdot \text{Zn(II)} \cdot \text{DPP}^-$] by ESI-MS (data not shown).

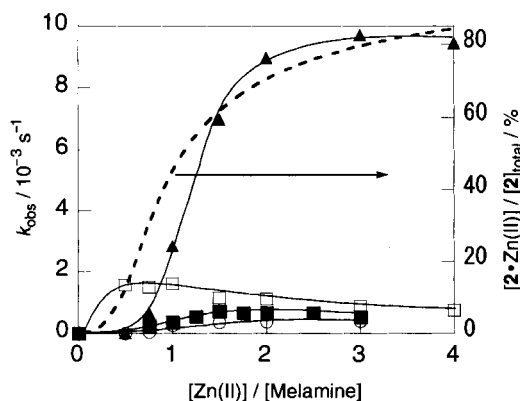
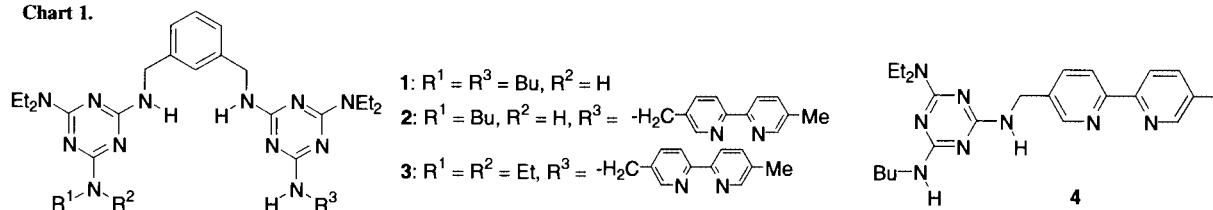


Figure 1. Effect of [Zn(II)] on hydrolysis of BDNPP with melamine derivatives **1** (\square), **2** (\blacktriangle), **3** (\blacksquare), and **4** (\circ). [Melamine] = 2.5×10^{-4} mol dm^{-3} , [BDNPP] = 5.0×10^{-5} mol dm^{-3} , in MeCN, at 298 K. The amount of 1 : 1 complex for **2** and Zn(II) in total amount of **2** calculated from $\log K_1 = 5.6$ and $\log K_2 = 5.1$ is imposed (dashed line).

Chart 1.



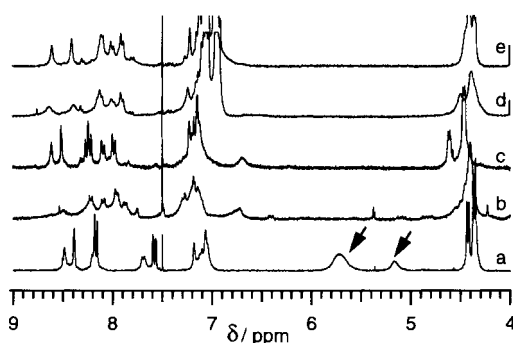


Figure 2. Partial ^1H NMR spectra of the aromatic and NH parts of **2** and **3** in the presence of Zn(II) and diphenyl phosphate (DPP^-) in CD_3CN at 298 K. $[\mathbf{2}] = [\mathbf{3}] = [\text{DPP}^-] = 2.5 \times 10^{-3} \text{ mol dm}^{-3}$. (a) **2**; (b) **2** + Zn(II) (1 equiv); (c) **2** + Zn(II) (2 equiv); (d) **2** + Zn(II) (2 equiv) + DPP^- ; (e) **3** + Zn(II) (2 equiv) + DPP^- .

Table 1. Effect of melamine derivatives on hydrolysis of phosphodiester in the presence of Zn(II)

Melamines	$k_{\text{obs}} / \text{s}^{-1}$ ^a	Rel. rates
None	1.0×10^{-6}	1
None ^b	1.29×10^{-4}	130
None ^c	8.64×10^{-5}	86
1	1.12×10^{-3}	1100
2	8.98×10^{-3}	9000
2 ^b	6.86×10^{-3}	6900
3	6.80×10^{-4}	680
4	3.86×10^{-4}	390

^a $[\text{Melamine}] = 2.5 \times 10^{-4} \text{ mol dm}^{-3}$, $[\text{Zn(NO}_3)_2 \cdot 6\text{H}_2\text{O}] = 5.0 \times 10^{-4} \text{ mol dm}^{-3}$, $[\text{BDNPP}] = 5.0 \times 10^{-5} \text{ mol dm}^{-3}$, in MeCN, at 298 K. All measured k_{obs} values had less than 10% deviation. ^b[2,6-Lutidine] = $2.5 \times 10^{-4} \text{ mol dm}^{-3}$. ^c[**5**] = $2.5 \times 10^{-4} \text{ mol dm}^{-3}$.

At $[\text{Zn(II)}] / [\text{melamine}] = 2$, k_{obs} 's and relative rates are listed in Table 1. Since no hydrolysis occurs without Zn(II) , the reactive species might be Zn(II)-OH^- . Thus, effect of a base on the rate was also examined. As can be seen in Table 1, addition of a base such as 2,6-lutidine and 2,4,6-tris(diethylamino)-1,3,5-s-triazine (**5**) accelerates the rates due to formation of Zn(II)-OH^- . The fact that **5** acts as a base indicates melamine derivatives themselves (**1**–**4**) also act as a base in the present system. However, the protonated melamine derivatives did not act as a rate-accelerating hydrogen-bonds donor, because the addition of pyridine hydrogen chloride ($2.5 \times 10^{-4} \text{ mol dm}^{-3}$) as an acid reduced the rate of hydrolysis with **2** ($k_{\text{obs}} = 1.75 \times 10^{-3} \text{ s}^{-1}$ in the same conditions described in Table 1). The rate was considerably accelerated (1.1×10^3 -fold) with **1**, suggesting that four hydrogen bonds concern with stabilization of the transition state of the hydrolysis. More interestingly, the rate constant for **1** is larger than that for **3**. This indicates that the four hydrogen bonds and free Zn(II)-OH^- ion is more effective than three hydrogen bonds and Zn(II)-OH^- bound at 2,2'-bipyridine moiety of **3**.

Although the structure of the ternary complex is not clarified at present, the large rate acceleration for **2** and Zn(II) allows us to propose that a trigonal bipyramidal and anionic phospholane-like transition state is stabilized by four hydrogen

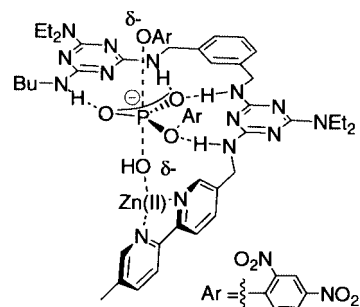


Figure 3. A proposed structure of the transition state of hydrolysis for BDNPP with **2**- Zn(II) .

bonds as illustrated in Figure 3, where Zn(II)-OH^- could locate in a suitable position. It is worthy to note that such a complex can be constructed with the CPK models without any difficulty.

In summary, we have demonstrated that a large rate acceleration for hydrolysis of the activated phosphodiester is achieved by a cooperative effect of a metal ion and hydrogen bonds by employing an H-bond-donor molecule bearing a metal-binding site in MeCN. Such a molecule could be regarded to exhibit an apoprotein function.

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- The rate of hydrolysis of DPP^- was negligible under the conditions of Table 1.