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# Acceleration of Acid-Catalyzed Transesterification of 2-Hydroxypropyl-*p*-nitrophenyl Phosphate by Organic Solvents

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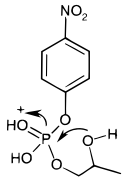
## ABSTRACT



**Transesterification of 2-hydroxypropyl-*p*-nitrophenyl phosphate in the presence of 0.092 M $HClO_4$ is 50–5000 times faster in acetonitrile, 1,4-dioxane, methanol, ethanol, *N,N*-dimethylformamide, or dimethyl sulfoxide than in water. This demonstrates the importance of tuning the microenvironments in designing synthetic nucleases.**

The drive to develop synthetic nucleases has stimulated intensive studies in catalysis of hydrolysis of phosphate diesters in recent years. Phosphate diesters are very difficult to hydrolyze. The half-life for spontaneous hydrolysis of phosphate diester linkages of DNA at 25 °C and pH 7 has been estimated as $10^{11}$ years.[1] Hydrolysis of phosphate diester linkages of RNA is much easier than those of DNA as it involves the intermediacy of cyclic phosphate diester formed by the nucleophilic attack of 2′-hydroxyl group at the phosphorus atom. Yet, half-life for the spontaneous hydrolysis of phosphate diester linkages of RNA at 25 °C and pH 7 is about 100 years.[1]

Artificial nucleases designed to date include nonmetallic compounds[2−6] as well as transition metal complexes[7−25] and lanthanide complexes.[26−28] In view of the high stability of DNA and RNA, various activated analogues of DNA

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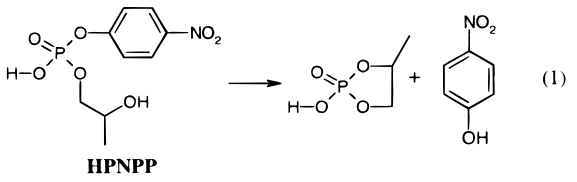
and RNA have been used as substrates for artificial nucleases. For example, bis(*p*-nitrophenyl)phosphate (BNPP) has been regarded as a doubly activated analogue of DNA, and 2-hydroxypropyl-*p*-nitrophenyl phosphate (HPNPP),[13,19,20,22−25,28,29] which undergoes transesterification (eq 1), has been frequently exploited as an activated analogue of RNA.




(1)

To design effective artificial nucleases, knowledge on various aspects of phosphate diester hydrolysis is needed. Enzymes optimize the microenvironment of their active sites to maximize the catalytic capability.[30] Similarly, adjustment of microenvironments around catalytic centers of artificial enzymes is important for improvement of the catalytic efficiency. In the case of phosphate diester hydrolysis, little is known of the solvent effects. This is mainly due to the difficulties encountered in the kinetic measurement of the very slow hydrolysis of phosphate diesters. BNPP, the aforementioned activated DNA analogue, has a half-life of about 2000 years for spontaneous hydrolysis at 25 °C and pH 7.[31] This can be compared with the half-life of 500−1000 years for hydrolysis of unactivated peptides at 25 °C and pH 7.[32−34] In this regard, we chose HPNPP as an activated analogue of not only RNA but also DNA and examined solvent effects on its transesterification to obtain information on designing microenvironments of effective artificial nucleases.

In the present study, kinetic data were obtained for the acid (HCl or $HClO_4$)-catalyzed transesterification of HPNPP ($5 \times 10^{-5}$ M) at 25 °C in various solvents such as acetonitrile (MeCN), 1,4-dioxane, methanol (MeOH), ethanol (EtOH), *N*,*N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), and water by spectrophotometric measurement (324 nm) of the release of *p*-nitrophenol. Products of the reactions were identified as the cyclic phosphate ester on the basis of $^{31}P$ NMR measurement.[35]

The values of pseudo-first-order rate constants ($k_o$) for transesterification of HPNPP measured with 0.092 M $HClO_4$ or HCl in MeCN, 1,4-dioxane, MeOH, EtOH, DMF, or DMSO containing 1% (v/v) water as well as water are summarized in Figure 1. Transesterification of HPNPP with

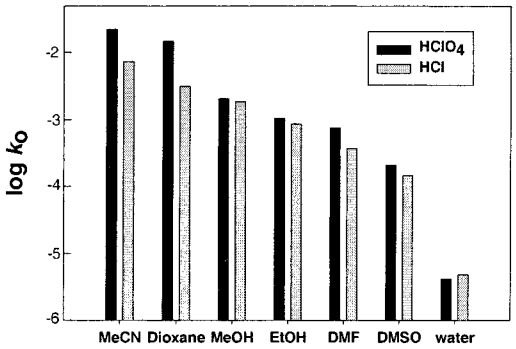



**Figure 1.** log $k_o$ measured for transesterification of HPNPP with 0.092 M $HClO_4$ or HCl in various solvents. Organic solvents contain 1%(v/v) water.

0.092 M $HClO_4$ or HCl is 50−5000 times faster in the organic solvents than in water. The rates measured in water, MeOH, or EtOH are not affected significantly by the nature of the acid used. On the other hand, the reaction with $HClO_4$ is faster than that with HCl by 3−4 times in MeCN or 1,4-dioxane. This difference might be related to stronger acidity of $HClO_4$. In basic solvents such as water, MeOH, and EtOH, both HCl and $HClO_4$ are converted to the conjugate acid of the solvent. If the acid is not fully ionized in less basic organic solvents,[36] the different acidity of the acid would be reflected in the rate. The $k_o$ values measured in MeCN−water cosolvents with varying composition are illustrated in Figure 2.

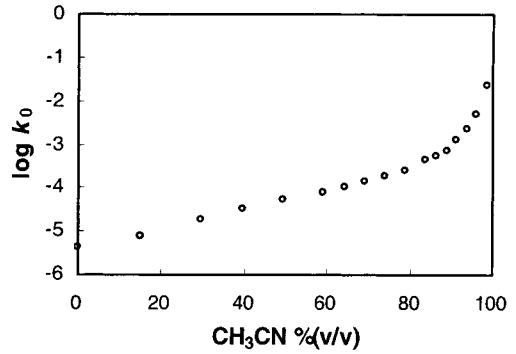



**Figure 2.** log $k_o$ for transesterification of HPNPP in the presence of 0.092 M $HClO_4$ in MeCN−water cosolvents.

In Figure 3 is illustrated the dependence of $k_o$ on the concentration of $HClO_4$ measured in various solvents. Slopes of the straight lines of Figure 3 are 1.0, within experimental error for organic solvents. The kinetic data measured in organic solvents are consistent with Scheme 1 which assumes

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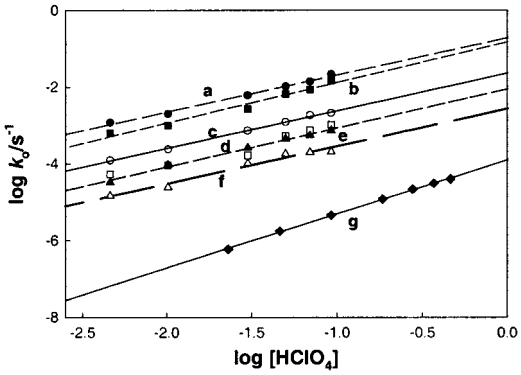
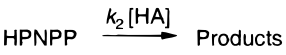



**Figure 3.** Plot of log $k_o$ against log [$HClO_4$] for transesterification of HPNPP in 99% (v/v) organic solvents (**a** (●), MeCN; **b** (■), 1,4-dioxane; **c** (○), MeOH; **d** (□), EtOH; **e** (▲) DMF; **f** (△), DMSO) or in water (**g** (◆), ionic strength maintained at 0.5 M with $NaClO_4$). Slopes of the straight lines are 0.97 ± 0.04 for **a**, 1.06 ± 0.09 for **b**, 0.98 ± 0.03 for **c**, 1.01 ± 0.12 for **d**, 1.02 ± 0.04 for **e**, 0.98 ± 0.08 for **f**, and 1.41 ± 0.02 for **g**.

**Scheme 1**




participation of one acid (HA; HCl, $HClO_4$, or conjugate acid of the solvent) and the neutral form of HPNPP in the rate-determining step.

The p$K_{a1}$ values of monoalkyl phosphates and the p$K_a$ values of dialkyl phosphates are 1.3−1.9 in water and are not much different from the p$K_{a1}$ (2.1) of phosphoric acid, indicating that effects of alkyl moieties are not great.[37] The p$K_a$ of phosphoryl hydroxy group of HPNPP in water, therefore, may be taken as near 1.3−1.9. Then, HPNPP can ionize at the acid concentration employed for kinetic measurements in water. Ionization of HPNPP should be suppressed in organic solvents compared with water, and HPNPP would be present mostly as neutral species in the organic cosolvents in the presence of strong acids.

For the reaction carried out in water, the slope (1.41 ± 0.02) of the straight line drawn in Figure 3 is considerably lager than 1. This can be attributed to ionization of the phosphoryl hydroxyl group of HPNPP at lower acid concentrations which reduces the molar fraction of the neutral form of HPNPP. In this regard, the data points obtained in water were analyzed again in terms of Scheme 2, considering

**Scheme 2**


ionization of HPNPP. Results of the analysis are summarized in Figure 4. Much better fit is obtained by analysis with

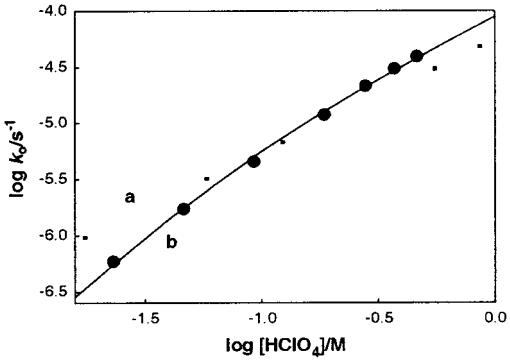
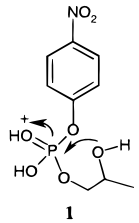



**Figure 4.** Plot of log $k_o$ against log [$HClO_4$] measured in water with ionic strength maintained at 0.5 M with $NaClO_4$. Regression of the data points led to $k_2 = (5.58 \pm 0.96) \times 10^{-5}$ $M^{-1}$ $s^{-1}$ for Scheme 1 (curve a), and $k_2 = (9.44 \pm 0.46) \times 10^{-5}$ $M^{-1}$ $s^{-1}$ and p$K_a$ = 1.16 ± 0.05 for Scheme 2 (curve b).

Scheme 2 than with Scheme 1. The p$K_a$ value (1.16 ± 0.05) estimated from analysis with Scheme 2 is consistent with that estimated above. When the reactivity of the un-ionized form of HPNPP in acid-catalyzed transesterification is considered, the reaction is 30−3000 times faster in organic solvents than in water (Figure 1).

On the basis of the kinetic data, the mechanism of **1** may be proposed for the acid-catalyzed transesterification of HPNPP in water and organic solvents. Here, the protonated species is formed by equilibration between HPNPP and HA of Scheme 1 or 2. In solvents such as alcohols and water, another solvent molecule may participate as a general base to assist the intramolecular attack by the hydroxy group.




**1**

Many organic reactions respond sensitively to changes in media. Various sets of parameter values have been proposed for quantitative analysis of solvent effects on rates of organic

(35) Chemical shift (85% phosphoric acid used as the external standard) for the product obtained in $D_2O$ with 0.092 M $HClO_4$ was 18.8 ppm in agreement with that[25] for the authentic cyclic ester, whereas that for HPNPP in $D_2O$ was −4.2 ppm. The product obtained in an organic solvent was compared with that obtained in water. For example, when the product solutions obtained in 99% (v/v) $CD_3CN$ or $D_2O$ with 0.092 M $HClO_4$ were diluted with equal volume of $D_2O$ or $CD_3CN$, respectively, identical chemical shifts (0.55 ppm) were obtained for the phosphorus atom of the product. In the same solvent, chemical shift for HPNPP was −5.2 ppm.

(36) In MeCN, HCl is partially ionized, whereas $HClO_4$ is fully ionized: Kolthoff, I. M.; Bruckenstein, S.; Chantooni, M. K. Jr. *J. Am. Chem. Soc.* **1961**, *83*, 3927.

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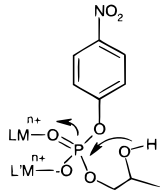
reactions. Attempts to correlate kinetic data summarized in Figure 1 with the solvent parameters[38] such as dielectric constant, $Z$, $E_T$, DN, AN, and Swain A/B were unsuccessful. This suggests that the solvents effects examined in the present study are not directly related to the physical phenomena associated with those parameter sets. When charge becomes more dispersed in the transition state as in some nucleophilic substitution reactions, the rate is enhanced in less polar media.[38] It is noteworthy that the charge is more dispersed in the transition state than in the protonated HPNPP for the mechanism of **1**.

Several effective catalysts have been designed for transesterification of HPNPP by using various types of metal complexes. The best synthetic catalyst reported to date for transesterification of HPNPP is a poly(ethylenimine) (PEI) derivative containing Ni(II) complexes of terpyridine (TP) and lauryl pendants.[25] The highest catalytic rate was observed when the contents of $Ni^{II}TP$ and lauryl pendants were 5% and 12%, respectively, of the amino groups of PEI. Effective catalysis was also achieved by binuclear metal complexes derived from a calixarene.[20,24] The catalytic action of the metal complexes in transesterification of HPNPP has been explained in terms of the activation of the two phosphoryl−oxygen bonds as indicated by **2**.[11,19,23] In some cases, the general base assistance for the intramolecular attack of the hydroxyl group by a metal-bound hydroxide ion is also assumed.[23] The mechanism of **2** is analogous to that of **1** since the two P−O oxygen atoms are bound by Lewis acids in both mechanisms.

The remarkable acceleration of the acid-catalyzed transesterification of HPNPP in organic solvents suggests that




**2**

the metal-catalyzed transesterification may be also subject to considerable solvent effects.[39] The high catalytic power of the PEI derivative containing $Ni^{II}TP$ has been partly attributed to the hydrophobic microenvironment created in the vicinity of the $Ni^{II}TP$ centers.[25] It is also possible that the effective catalysis by the calixarene-based binuclear metal complexes[20,24] originates partly from the hydrophobic microenvironment created in water. Complexes of transition metal ions or lanthanides are the most effective synthetic catalysts reported so far for hydrolysis of phosphate diester linkages of DNA and RNA.[8,13,14,21,26] Results of the present study implicate that adjustment of microenvironments of the catalytic metal centers is important in designing effective artificial nucleases.

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(39) The degree of acceleration of HPNPP transesterification in the presence of bis(guanidinium) receptors was higher in acetonitrile than in water (Oost, T.; Filippazzi, A.; Kalesse, M. *Liebigs Ann.* **1997**, 1005). In this case, strengthening the hydrogen bonding between HPNPP and the host by the organic solvent would make a major contribution to the observed solvent effect.