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Micellar Effects on the Co-catalysis of Magnesium Ion and Imidazole for the Hydrolysis of Phenyl Phosphatosulfate

Toshio Eiki and Waichiro Tagaki*, †

Department of Chemistry, Faculty of Engineering, Gunma University, Kiryu, Gunma 376
†*Department of Applied Chemistry, Faculty of Engineering, Osaka City University,*
Sugimoto 3, Sumiyoshi-ku, Osaka 558

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The effects of Mg^{2+} ion and imidazole were examined on the hydrolysis of phenyl and *p*-decylphenyl phosphatosulfate (PPS and DPPS) in the presence of micelle of sodium dodecyl sulfate (SDS). It was observed that Mg^{2+} ion and imidazole increased the rates of hydrolysis cooperatively and the rate enhancement was much larger in the case of DPPS than in the case of PPS. The difference in the reactivities of two esters was discussed in terms of micellar effects.

Active sulfates[1] containing the phosphatosulfate linkage (P–O–S) are the key intermediates in the biological sulfur metabolism.[2] Yet a little has been understood for the mechanism of enzymic catalyses involving P–O–S linkage. In the model reactions, the P–O–S linkage is now known to be susceptible to the hydronium ion catalysis to give a selective S–O bond cleavage.[3,4] Such an acid catalysis can be enhanced by Mg^{2+} ion when the water content of the medium is low.[5,6] However, under the neutral conditions, the effect of Mg^{2+} ion alone is small even in the medium of low water content. Related fact is the requirement of bivalent metal ions as the cofactors in some hydrolytic enzymes of active sulfates which are active under the neutral conditions.[2e] Thus it is interesting to know a mechanism of activation of P–O–S linkage by Mg^{2+} ion under the neutral conditions. In this paper, we describe our observation that Mg^{2+} ion activates the P–O–S linkage toward the attack of imidazole nucleophile and the activation is enhanced under the micellar conditions.

Results and Discussion

Products. The substrates used were phenyl phosphatosulfate (PPS) and *p*-decylphenyl phosphatosulfate (DPPS). Their aminolysis was reported previously.[6,7] In the absence of Mg^{2+} ion and in the neutral or basic media, amines were observed to attack phosphorus to give phosphoramidates and inorganic sufate with a selectivity of 70—85% P–O cleavage. In the presence of Mg^{2+} ion, the selectivity of P–O cleavage increased further up to almost 100%.

In the case of imidazole, the corresponding phosphoramidates were not detected, but presumed to undergo rapid hydrolysis (Eq. 1). This presumption appears to be supported by the formation of methyl phenyl phosphate from PPS when the reaction was conducted in aqueous methanol (Eq. 1), while methyl hydrogensulfate was not detected which would be formed if the alternative cleavage of S–O bond occurred to give imidazole-*N*-sulfonic acid.

Kinetics. *Effect of SDS*: The kinetics of hydrolysis of PPS and DPPS were generally conducted in the micellar solution of SDS (6×10^{-2} M, 1 M=1 mol dm^{-3}), although the latter DPPS and its hydrolysis product diammonium *p*-decylphenyl phosphate (DPP)

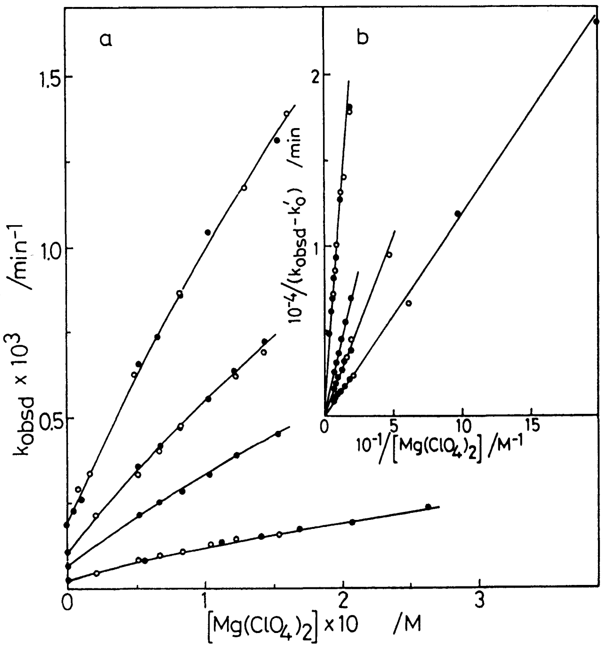
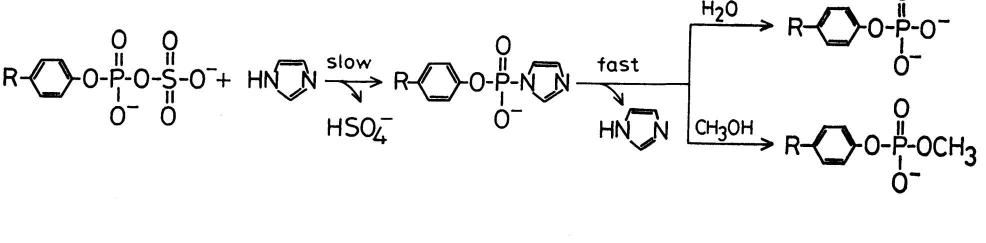


Fig. 1. a) Mg^{2+} ion effects on the imidazole catalyzed hydrolysis of PPS (2×10^{-2} M) in *N*-ethylmorpholine buffer (0.02 M, pH 7.90) at 55.0 °C: ●, in the presence of SDS (6×10^{-2} M); ○, in the presence of NaCl (6×10^{-2} M). Initial total concentrations of imidazole are from the bottom, 0.0, 1.0×10^{-2}, 2.0×10^{-2}, and 4.0×10^{-2} M, respectively. Solid lines are theoretical curves calculated from the values in Table 1 and Eq. 3; b) the reciprocal plots.

 (1)

both form micelles above their CMC.[7] The reason of using SDS was the following. In the absence of SDS, an initially homogeneous reaction mixture of DPPS and Mg^{2+} ion became cloudy as the reaction proceeded due to the formation of an insoluble DPP–Mg^{2+} ion complex which could be solubilized by SDS micelle. The presence of SDS micelle, however, did not cause any detectable effect on the rates (k_{obsd}) of hydrolysis of DPPS.[8] The effect of SDS was also negligible on the hydrolysis of PPS as shown in Fig. 2. Thus in each case of PPS and DPPS, the effect of SDS on the rates was not apparent except for solubilization of the insoluble product. Nevertheless, a higher reactivity of DPPS than PPS appears to be due to a micellar effect as discussed later.

Hydrolysis of PPS: *N*-Ethylmorpholine used as the buffer reagent was confirmed to be unreactive toward the P–O–S linkage.[6,7] The effects of imidazole and Mg^{2+} ion concentration on the rates were then examined as shown in Fig. 1. The figure indicates that there was no difference in the rates between the medium of non-micellar NaCl (0.06 M) and micellar SDS (0.06 M). The figure also indicates that the rates tend to be saturated with increasing Mg^{2+} ion concentration at each fixed concentration of imidazole ($0-4\times10^{-2}$ M). On the other hand, the rates were linear with the increase in imidazole concentration at a fixed concentration of Mg^{2+} ion. These observations appear to be accounted for by assuming a reaction scheme involving preequilibrium formation of 1 : 1 complex (M.S) of substrate (S) and Mg^{2+} ion (M), where $[\mathrm{M.S}] \ll [\mathrm{M}]_T$, according to Eq. 2. From Eq. 2, the pseudo-first-order rate constant, k_{obsd} is given by Eq. 3, where $[\mathrm{Im}]_T$ and $[\mathrm{M}]_T$ are the initial stoichiometric concentration of imidazole and Mg^{2+} ion, respectively; k_o, k_o^{Im}, k_m^{Im}, and K_m (association constant) are defined in Eq. 2. Equation 3 gives Eq. 4 (Fig. 1b).

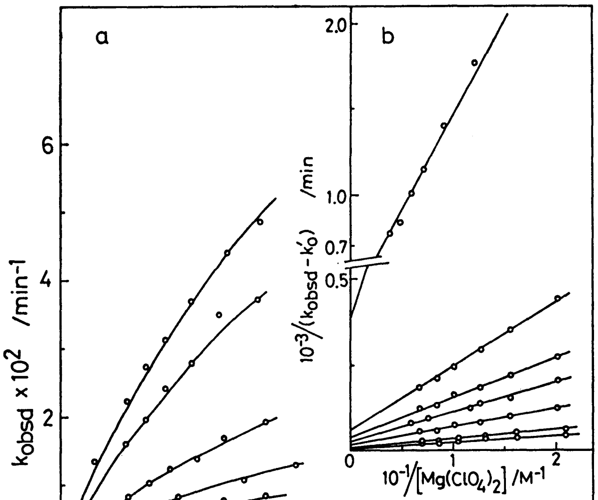


Fig. 2. a) Mg^{2+} ion effects on the imidazole catalyzed hydrolysis of DPPS (2×10^{-2} M) in the presence of SDS (6×10^{-2} M) in *N*-ethylmorpholine buffer (0.2 M, pH 7.90) at 55.0 °C. Initial total concentration of imidazole are from the bottom, 0.0, 2.4×10^{-3}, 4.0×10^{-3}, 6.0×10^{-3}, 1.0×10^{-2}, 2.1×10^{-2}, and 2.8×10^{-2} M, respectively. Solid lines are theoretical curves calculated from the values in Table 1 and Eq. 3; b) the reciprocal plots.

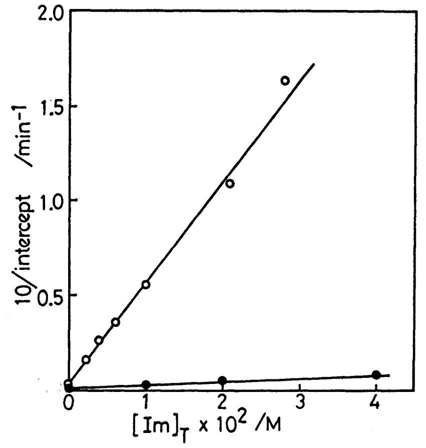


Fig. 3. Plots of 1/intercept *vs.* initial total concentration of imidazole ($[\mathrm{Im}]_T$) for DPPS (○) and PPS (●), see text.

$$\begin{tikzcd} \mathrm{M}+\mathrm{S} \arrow[r, shift left, "K_m"] \arrow[rr, bend right, "k_o'"'] & \mathrm{M.S} \arrow[l, shift left] \arrow[r, "k_m'"] & \text{products} \end{tikzcd} \qquad (2)$$

$$k_m' = k_m + k_m^{Im}[\mathrm{Im}]_T;\ k_o' = k_o + k_o^{Im}[\mathrm{Im}]_T$$

$$k_{obsd} = \frac{k_o' + k_m' K_m[\mathrm{M}]_T}{1 + K_m[\mathrm{M}]_T} \qquad (3)$$

$$\frac{1}{(k_{obsd}-k_o')} = \frac{1}{(k_m'-k_o')} + \frac{1}{(k_m'-k_o')K_m}\cdot\frac{1}{[\mathrm{M}]_T} \qquad (4)$$

$$\frac{1}{(\text{intercept})} = (k_m - k_o) + (k_m^{Im} - k_o^{Im})[\mathrm{Im}]_T \qquad (5)$$

The intercepts of Eq. 4 (Fig. 1b) should be linearly related to the imidazole concentration (Eq. 5) as verified in Fig. 3. Thus the k_{obsd} values could be analyzed based on these equations to give the kinetic parameters for PPS as listed in Table 1.

Hydrolysis of DPPS: The kinetic procedures and the analysis were essentially the same as in the above hydrolysis of PPS. The results are shown in Figs. 2 and 3, and in Table 1.

TABLE 1. RATE PARAMETERS FOR THE HYDROLYSIS OF PPS AND DPPS[a)]

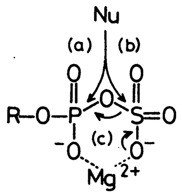
Constant	PPS	DPPS	k_{DPPS}/k_{PPS}
k_o/min^{-1}	2.57×10^{-5}	2.31×10^{-5}	0.898
k_m/min^{-1}	8.98×10^{-4}	37.6×10^{-4}	4.18
k_o^{Im}/M^{-1} min^{-1}	4.14×10^{-3}	13.3×10^{-3}	3.21
k_m^{Im}/M^{-1} min^{-1}	0.171	5.29	30.9
K_m/M^{-1}	1.19	3.39	

a) pH 7.90, 55 °C, in the presence of 6×10^{-2} M SDS; other conditions, see Figs. 2 and 3.

Comparison of PPS and DPPS Reactivities. The k_o values in Table 1 indicates that the reactivities of two esters toward water are nearly the same at pH 7.9 and

55 °C and in the absence of Mg^{2+} ion and imidazole. These water rates are enhanced by 35 and 163 fold (k_m/k_0 ratio) for PPS and DPPS respectively, when the ester are complexed with Mg^{2+} ion. Similarly the nucleophilic reactivities of imidazole (k_0^{Im}) in the absence of Mg^{2+} ion are enhanced by 41 and 400 fold (k_m^{Im}/k_0^{Im} ratio) for PPS and DPPS respectively by the complexation. Thus these rate enhancements are much larger for DPPS than PPS. Such comparison can also be made by relative rates, k_{DPPS}/k_{PPS} shown in Table 1.

Since DPPS is a surfactant,7) it is reasonable to assume that DPPS forms a co-micelle with SDS.9) On the other hand, it is known to be difficult to incorporate such a hydrophilic anion as PPS into an anionic micelle like SDS.9) Therefore, it can be considered that the reaction of DPPS occurs in the micellar phase, while that of PPS in the bulk water phase. It is known that the binding of a counter ion on an ionic micelle is stronger in general than in the corresponding ion pair formation in the bulk water phase.9) As expected, the K_m value of DPPS is larger than that of PPS. A plausible structure for the M.S complex (Eq. 3) is a cyclic one as illustrated in **1**, in which the bonding of P–O$^-$···Mg^{2+} is presumably stronger than that of S–O$^-$···Mg^{2+}.



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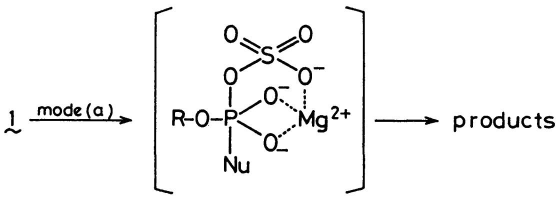

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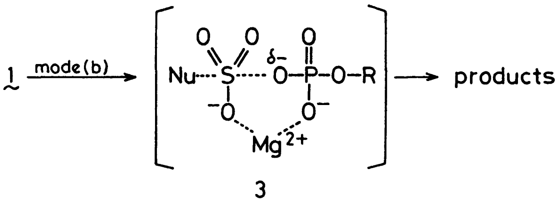

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Three modes (a—c) may be conceivable for the cleavage of P–O–S linkage of such a complex **1**. The modes (a) and (b) are the biomolecular nucleophilic attack of imidazole on phosphorus and sulfur, respectively, to give either the P–O or the S–O bond cleavage. The mode (c) is the unimolecular cleavage of the S–O bond. In each case, Mg^{2+} ion is assumed to act as an electrophilic catalyst to polarize the reacting bonds or to neutralize the developing negative charge of the intermediate or the transition state. As mentioned already, what actually observed is the mode (a). Presumably, the formation of pentacovalent intermediate or transition state (**2**) through the mode (a) (Eq. 6) is energetically more favored than to make a S_N2 transition state (**3**) by mode (b) (Eq. 7). The stabilization by charge neutralization by Mg^{2+} ion seems to be more effective in **2** than in **3**, because the developement of negative charge must be more pronounced in **2** than in **3**. Such electrostatic interaction must be stronger in the micellar phase than in the bulk water phase, because of the hydrophobic nature of the micellar phase.10)

As for the micellar effects on the metal ion catalysis, a positive micellar effect, although small, was reported by Melhado and Gutsche for the catalysis of cationic micellar chelates of 4-alkyl-3,6-diazaoctane-1,8-diamine in the decomposition of acetyl phosphate.11) Very recently, we also reported a larger micellar effect on the catalytic activity of a lipophilic metal ion chelate in the hydrolysis of carboxylic acid esters in a cationic micelle.12) In these reactions, the reacting bond undergoing nucleophilic attack is the ester carbonyl group. The present results indicate that a large micellar effect is also possible in the metal ion assisted nucleophilic attack on phosphorus. For enzyme catalysis, the present results suggest that if an enzyme require a bivalent metal ion as the cofactor in the hydrolysis of active sulfates, the medium of the active site is somewhat like a micellar phase and the site of cleavage of the P–O–S linkage is the P–O bond, as suggested by Roy.2e) The results also suggest the importance of the histidyl residue at the active site of enzymes as the nucleophilic catalyst toward the phosphate substrates·

Experimental

Material. The ammonium salts of DPPS and PPS were prepared by our previous method.4,7) Inorganic salts, EDTA and SDS were of commercial analytical grade and were used without further purification. Mg^{2+} ion was the perchlorate. All liquid reagents were purified by distillation. Imidazole was recrystallized from benzene. Demineralized water was treated with KMnO$_4$ and distilled twice before use.

Kinetics. A Hitachi-Horiba F-7DE pH meter and a Shimadzu UV-200 spectrophotometer were used for the kinetics. Kinetic procedures were the same as previously described.7)

Methanolysis. The substrate PPS (144 mg) or DPPS (214 mg) was dissolved in 80% aqueous methanol (25 cm^3) containing imidazole (340 mg) and Mg(ClO$_4$)$_2$ (1.11 g). This reaction mixture was kept at 55 °C for 24 h and concentrated to dryness. The residue was dissolved in an alkaline solution (pH 11) and centrifuged to remove magnesium hydroxide. The supernatant was concentrated to dryness. The residue dried on P$_2$O$_5$ was washed with ether and again dried. The final residue was dissolved in D$_2$O and analyzed by NMR6) for methyl phenyl phosphate: δCH$_3$=3.74 (3H, doublet). The yield of inorganic sulfate was quantitative as determined by Ba-chloranilate method.7)

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8) The SDS concentration was varied in a range of 0—0.1 M under the following conditions: pH 6.86 (0.2 M imidazole/HCl buffer), 55 °C; DPPS$=2\times10^{-2}$ M, $Mg(ClO_4)_2=2.03\times10^{-2}$ M.

9) For example, see J. H. Fendler and E. J. Fendler, "Catalysis in Micellar and Macromolecular Systems," Academic Press, New York (1975).

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