Bull. Chem. Soc. Jpn., 64, 1235—1242 (1991)

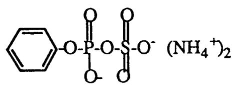
Metal Ion Dependent Site Selective Cleavage of P-O-S Linkage in the Methanolysis of Phenyl Phosphatosulfate

Toshio Eiki* and Waichiro Tagaki†
Department of Chemistry, Faculty of Technology, Gunma University, Kiryu, Gunma 376
†Department of Bioapplied Chemistry, Faculty of Engineering, Osaka City University,
Sugimoto 3-3-138, Sumiyoshi-ku, Osaka 558
(Received December 13, 1990)

Effects of various metal ions on the methanolysis of phenyl phosphatosulfate having P-O-S linkage were investigated. Metal ions examined were found to be divided into two different groups in the manner of cleavage of P-O-S linkage: Mg^{2+}, Cr^{3+}, Cu^{2+}, Mn^{2+}, and Ca^{2+} with larger ionic radii catalyzed the selective P-O bond cleavage, while Be^{2+}, Al^{3+}, Fe^{2+}, and Zn^{2+} with smaller ionic radii promoted the selective S-O bond cleavage. Such selectivity was discussed in terms of the difference in the mechanisms of P-O and S-O bond cleavage.

In the biological sulfur metabolism, the key intermediates are 3′-phosphoadenosine-5′-phosphosulfate (PAPS) and adenosine-5′-phosphosulfate (APS).1–9) Both intermediates have a P-O-S linkage as the reactive bond. The enzymes catalyzing the cleavage of this P-O-S bond require a metal ion as the essential cofactor. For example, Mg^{2+} is known to be an essential cofactor for the enzymes catalyzing the sulfuryl group transfer from PAPS to numerous acceptors such as polysaccharides, phenols, steroids, and other nucleophiles.1–5) For such reactions to occur, the S-O bond must be selectively cleaved. It is also known that an APS reductase contains a non-heme iron with an FAD coenzyme.4,9) Here again, the S-O bond must be selectively cleaved. On the contrary, it was suggested that the enzyme-catalyzed hydrolysis of PAPS occurs with the selective P-O bond cleavage in the presence of Mn^{2+} or Co^{2+}.3,6) Yet much remains to be clarified as to the mechanism of metal-ion catalysis, in particular as to the question how a metal ion affects the rate and the selectivity in the cleavage of the S-O vs. P-O bond of P-O-S linkage.

In our previous model studies, Mg^{2+} was found to enhance the rate of acid-catalyzed hydrolysis of phenyl phosphatosulfate (PPS) and suggested to cause the selective S-O bond cleavage.10–12) On the other hand, the same Mg^{2+} was observed to catalyze the selective P-O bond cleavage with amine nucleophiles at neutral pH.13,14) More explicitly, it was disclosed that metal ions such as Mg^{2+} and Zn^{2+} catalyze the attack of a nucleophile on the phosphorus of 2-pyridyl phosphonosulfate to result in the selective P-O bond cleavage at neutral pH.15) Although this mysterious gap between the effects of Mg^{2+} on the model and enzyme reactions has remained unfilled, we reported previously that there are two different groups of metal ions which promote the selective cleavage of either the S-O or the P-O bond of PPS and the selectivity appears to depend on the ionic radii of the metal ions.17) However, things are not so simple that Zn^{2+} has now been found to promote the selective S-O bond cleavage in the methanolysis, contrary to the expected selective P-O bond cleavage based on the ionic radius. With this additional finding, we describe herein the full account of the previous communication16,17) on the metal ion-dependent site selective cleavage of P-O-S linkage in the methanolysis of PPS.



PPS

Experimental

Materials and Methods. UV and visible spectra were recorded on a Shimadzu UV-200 spectrophotometer and NMR spectra with a Varian A-60 spectrometer. pH was measured with a Hitachi-Horiba F-7DE pH meter. Water used for kinetics was purified by treating deionized water with $KMnO_4$ followed by double distillation. Buffer and other reagents for analysis were obtained from commercial sources as extra pure reagents. Metal salts used were of analytical grade. Organic solvents, *N*-ethylmorpholine and *N*,*N*-diethylaniline were distilled before use according to the standard procedures.

The diammonium salt of PPS was prepared by the reaction of phenyl phosphate with DMF-SO_3 complex in DMF at room temperature according to our method.18) Bis(triethylammonium) salt of PPS used for the sulfonation of *N*,*N*-diethylaniline was prepared as follows. An excess of triethylamine was added to the methanol solution of diammonium salt of PPS and the exchanged NH_3 was removed by evaporation under reduced pressure to give an oily residue which was recrystallized from a methanol/ether mixed solvent. Anal. Calcd. for $C_{18}H_{37}O_7N_2PS$: C, 47.39; H, 8.11; N, 6.13%. Found: C, 46.94; H, 8.11; N,6.08%.

Alcoholyses. Although the methods of kinetics and product analyses were essentially the same as those reported previously,13–15,18) the details are now described below.

a) Kinetics. In ampoules, the reaction was carried out in a mixed solvent of DMF-methanol (1 : 1, v/v) containing the diammonium salt of PPS (1.0×10^{-2} mol dm^{-3}), a metal ion (0—0.1 mol dm^{-3}), and *N*-ethylmorpholine (0.55 mol dm^{-3}) at 55 °C. The rate was determined by quenching two samples at appropriate time intervals: one for the monitor of inorganic sulfate released by colorimetric barium chloranilate or barium chromate method, and the other for the monitor of phenyl phosphate and methyl phenyl phosphate formed by the UV method described below.

b) Product Analysis. Amounts of the liberated inorganic sulfate was determined colorimetrically as mentioned above and the amounts of phosphates formed were determined on the basis of UV absorption intensity. Constants employed for the analyses are listed in Table 1.

(i) UV Method. Two methods were employed. In one method, a reaction mixture (0.5 ml) was mixed with 1 mol dm^{-3} NaOH (0.5 ml) and water (4 ml), and the resulting metal hydroxide precipitate was removed by centrifuge, and the supernatant was subjected to UV analysis (for Be^{2+}, Al^{3+}, Fe^{3+}, Zn^{2+}, Mg^{2+}, Mn^{2+}, and Ca^{2+}). In the other method, a reaction mixture (0.5 ml) was mixed with 0.2 mol dm^{-3} Na_2HPO_4 (4.5 ml), and the precipitated metal ion salt of orthophosphate was removed by centrifuge, and the supernatant was subjected to the UV analysis (for Be^{2+}, Al^{3+}, Fe^{3+}, Zn^{2+}, Cr^{2+}, and Cu^{2+}). The two methods gave the same results.

(ii) NMR Method. (ii-a). For the competitive solvolysis by methanol and 2-propanol (Be^{2+}, Al^{3+}, Fe^{3+}, and Zn^{2+}): A reaction mixture (50 ml) consisting of PPS $(NH_4^+)_2$ (1×10^{-2} mol dm^{-3}), *N*-ethylmorpholine (0.55 mol dm^{-3}), and a metal ion (3×10^{-2} mol dm^{-3}) in a mixed solvent of methanol, 2-propanol, and DMF was kept for ten half-lives at 55 °C. Then the reaction mixture was concentrated to remove the solvent, and the residual oil was dissolved in water (3 ml), and to this was added 0.2 mol dm^{-3} Na_2HPO_4 (10 ml) and 1 mol dm^{-3} NaOH (2 ml). The resulting precipitate of metal ion salt of orthophosphate was separated from the supernatant by centrifuge and washed with water. The combined supernatant and the washings were concentrated to give a solid residue, and the residue was washed with ether to remove remaining *N*-ethylmorpholine. Dried final residue was dissolved in D_2O and subjected to the NMR analysis.

(ii-b). For the cases of Mg^{2+} and Ca^{2+}: A reaction mixture consisting of PPS $(NH_4^+)_2$ (1×10^{-2} mol dm^{-3}), *N*-ethylmorpholine (0.55 mol dm^{-3}), and a metal ion (5×10^{-2} mol dm^{-3}) in a mixed solvent of methanol and DMF (1 : 1, v/v %) was concentrated and the residual oil was dissolved in water (5 ml) and mixed with 0.5 mol dm^{-3} NaOH (5 ml). The metal hydroxide was separated from the supernatant by centrifuge and the precipitate was washed with 0.5 mol dm^{-3} NaOH (5 ml). The combined supernatant and the washings were treated as in the above procedure for the NMR analysis.

(ii-c). For the case of Cu^{2+}: A similar reaction mixture ($Cu^{2+}=2\times10^{-2}$ mol dm^{-3}) was concentrated to give a residual oil, and this was dissolved in 1 mol dm^{-3} HCl (20 ml). To the HCl solution was introduced H_2S gas and the copper sulfide was removed by filtration. The filtrate was treated with active carbon and the colorless filtrate was concentrated after pH adjustment (pH 11). The dried residue was subjected to the NMR analysis as mentioned above.

(iii) HPLC Method (for the reactions of Mg^{2+}, Cu^{2+}, Cr^{3+}, and Mn^{2+}): The column and the elution solvent were YANACO PELAX and 20% H_2O-80% CH_3OH, respectively. A kinetic reaction mixture (0.5 ml) was mixed with 1 mol dm^{-3} NaOH (0.5 ml) and water (4 ml), and the metal hydroxide was removed by centrifuge, and the supernatant was subjected to the NMR analysis.

(iv) Paper Chromatography. Both a raw reaction mixture and the final NMR sample were analyzed. In all the cases, only a single spot corresponding to either methyl phenyl phosphate (MPP), phenyl isopropyl phosphate or phenyl phosphate (PP) was detected.

Sulfonation. Ammonium *p*-(diethylamino)benzenesulfonate used as the authentic sample was prepared by the reaction of *N*,*N*-diethylaniline (3 ml, 19.2 mmoles) with DMF-SO_3 complex (9.6 mmoles) in DMF (6 ml). The reaction mixture was left for 10 h at ambient temperature and quenched with an excess of NH_3 in methanol. The product was precipitated by the addition of ether and recrystallized from methanol–ether. Anal. Calcd. for $C_{10}H_{18}O_3N_2S$: C, 48.79; H, 7.31; N, 11.37%. Found: C, 48.76; H, 7.36; N, 11.37%. Mp. 168—170 °C. UV (see Tables 1).

The $AlCl_3$-promoted sulfonation was conducted by the reaction of bis(triethylammonium) PPS (200 mg, 0.43 mmoles) with *N*,*N*-diethylaniline (4 ml, 25.7 mmoles) in the presence of anhydrous $AlCl_3$ (70.6 mg, 0.53 mmoles) in dry acetonitrile solvent (3 ml) at ambient temperature for 45 h. The product, ammonium *p*-(diethylamino)benzenesulfonate, was obtained in a quantitative yield by treating the reaction mixture according to the above procedure, and identified by comparing UV, ^1H NMR, and IR spectra with those of the authentic sample.

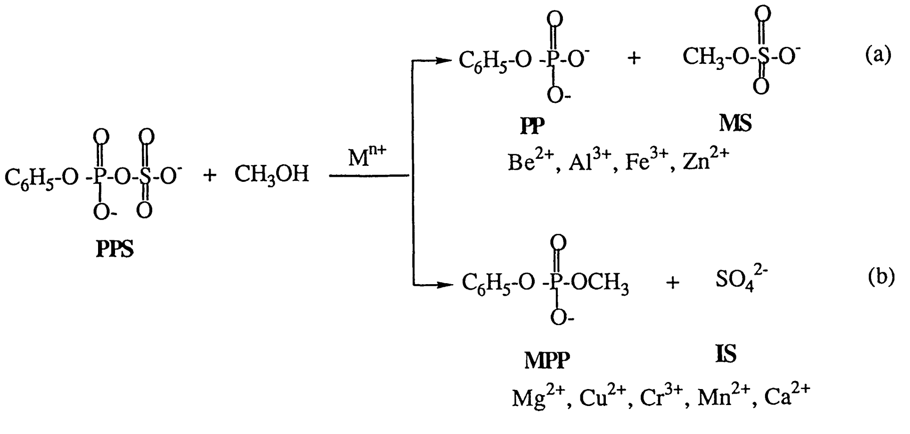
Results

Metal Ion Effects in the Methanolysis. (a) Product Analysis. Methanolysis of diammonium PPS (1×10^{-2} mol dm^{-3}) was carried out in a mixed solvent of methanol and DMF (1 : 1, v/v) under basic conditions containing *N*-ethylmorpholine (0.55 mol dm^{-3}) at

Table 1. Analytical Data for the Product Analyses

Salts	UV data[a)]		^1H NMR[b)]		R_f[c)]
	λ_{max}/nm	(ε)	δ CH_3	δ CH	
Phenyl phosphatosulfate (PPS)	262	(440)	—	—	0.65
Methyl phenyl phosphate (MPP)	262	(580)	3.74 (d)	—	0.85
Phenyl phosphate (PP)	267	(740)	—	—	0.48
Methyl sulfate (MS)	—		3.80	—	—
Isopropyl sulfate (PS)	—		1.28 (d)	4.63 (m)	—
p-(Diethylamino)benzenesulfonate	278	(22700)	—	—	

a) In 0.1 equiv NaOH. b) In D_2O and from sodium 2,2-dimethyl-2-silapentane-5-sulfonate. c) Toyo Filter Paper, No. 50; PrOH-NH_3(28%)-H_2O (6 : 3 : 1, v/v).



Scheme 1.

55 °C. Previously, *N*-ethylmorpholine was confirmed to be unreactive toward PPS either in the presence or absence of a metal ion examined.[10,13] The reaction was very slow in the absence of a metal ion. All metal ions tested in this study enhanced the rate of reaction. Fortunately, the product analyses described in the experimental section could be performed readily and quantitatively. The reason for the ease of analyses was first due to the simplicity of the products because of the site-selective S-O or P-O bond cleavage of P-O-S linkage. The second was due to the ease of quantitative analyses of the methylated products based on the ^{1}H NMR signals of methyl protons (Table 1) as well as their HPLC analysis. The results are summarized in Scheme 1. Scheme 1 indicates that the metal ions are divided into two different groups in the selectivity of bond cleavage. That is, Be^{2+}, Al^{3+}, Fe^{3+}, and Zn^{2+} promoted an exclusive S-O bond cleavage to form phenyl phosphate (PP) and methyl sulfate (MS) in quantitative yields, whereas Mg^{2+}, Cu^{2+}, Cr^{3+} Mn^{2+}, and Ca^{2+} catalyzed an exclusive P-O bond cleavage to form methyl phenyl phosphate (MPP) and inorganic sulfate (IS) in quantitative yields. The metal ions were used as the chlorides containing hydrated water, but the results obtained with the hydrated metal ions were essentially the same as those obtained with anhydrous metal ions. The counterions of the metal ions such as chloride, nitrate, or perchlorate did not affect the selectivity.

(b) Kinetics. The pseudo-first-order rate constants (k_{obsd}) were obtained by following spectrophotometrically the formation of either one of PP, MPP, or IS.[13,18] In the P-O bond cleavage to form MPP and IS, the metal ions performed as catalysts, i.e. a good peudo-first-order rate constants were obtained regardless of using an excess of substrate or a metal ion over the other. However, in the S-O bond cleavage to form PP and MS, precipitation occurred owing to the formation of a strongly bound PP-metal ion complex in the absence of DMF. This was one of the reasons to use DMF for a component of the mixed solvent. Thus, in the S-O bond cleavage, the rates were of pseudo-first-order in the presence of an excess of a metal ion over the substrate PPS in the mixed solvent, but under reversed conditions of an excess of PPS over a metal ion, burst kinetics were observed, i.e. an initial fast reaction followed by the slow production of PP, as exemplified in Fig. 1 (lower two curves). Most kinetics were carried out with metal ion concentrations below 0.03 mol dm^{-3}. Within this concentration range, the k_{obsd} values were first-order with respect to the metal ion concentration to give second-order rate constants, k_2(S-O) and k_2(P-O) as listed in Table 2. However, at higher metal ion concentrations, saturation kinetics were observed as shown in Fig. 2 for the three representative metal ions, Zn^{2+}, Mg^{2+}, and Ca^{2+}. The saturation curves in Fig. 2 seem to be accounted for by

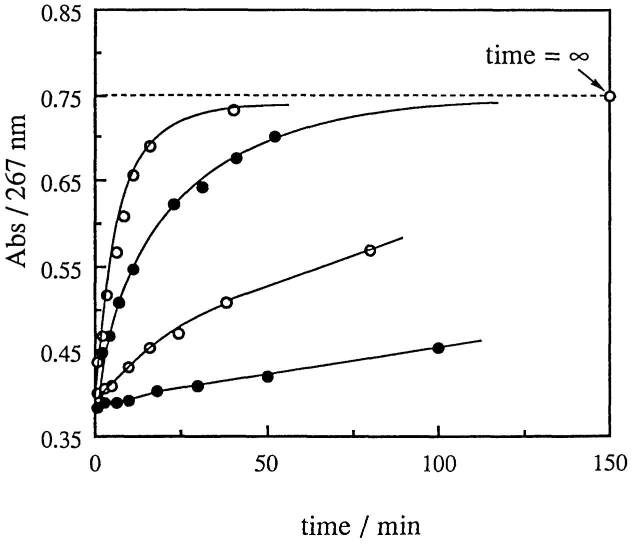


Fig. 1. Absorbance change vs. reaction time in the Zn^{2+} promoted methanolysis of PPS (1×10^{-2} mol dm^{-3}) in MeOH-DMF (1 : 1, v/v) at 55 °C: Zn^{2+} concentration (mol dm^{-3}) from the top, 1×10^{-1}, 3×10^{-2}, 1×10^{-2}, 5×10^{-3}.

Table 2. The Second Order Rate Constants, k_2(S-O) and k_2(P-O), and Ionic Radii of the Metal Ions

Metal ion	Ionic radii[a]/Å	k_2(S-O) or k_2(P-O)/mol^{-1} dm^3 s^{-1}
Be^{2+}	0.3	1.40×10^{-2}
Al^{3+}	0.53	9.56×10^{-2}
Fe^{3+}	0.63	3.33×10^{-2}
Zn^{2+}	0.74	2.45×10^{-2}
Mg^{2+}	0.71	1.31×10^{-3}
Cu^{3+}	0.71	3.51×10^{-2}
Cr^{3+}	0.76	5.80×10^{-2}
Mn^{2+}	0.8	4.13×10^{-3}
Ca^{2+}	1.14	2.81×10^{-4}

a) Ref. 20.

Table 3. Kinetic Parameters for the Metal Ion Catalyzed and/or Promoted Methanolysis[a]

Metal ion[b]	k_m/s^{-1}	K/mol^{-1} dm^3	$k_mK/(1+K[S]_T)$/mol^{-1} dm^3 s^{-1}
Zn^{2+}	8.33×10^{-3}	3.03	2.45×10^{-2}
Mg^{2+}	2.60×10^{-5}	102	1.31×10^{-3}
Ca^{2+}	1.01×10^{-5}	38.6	2.81×10^{-4}

a) Based on the data shown in Fig. 2: [PPS]=1.0×10^{-2} mol dm^{-3}, [N-ethylmorpholine]=0.55 mol dm^{-3} in CH_3OH/DMF (1 : 1, v/v), 55 °C. b) Chloride.

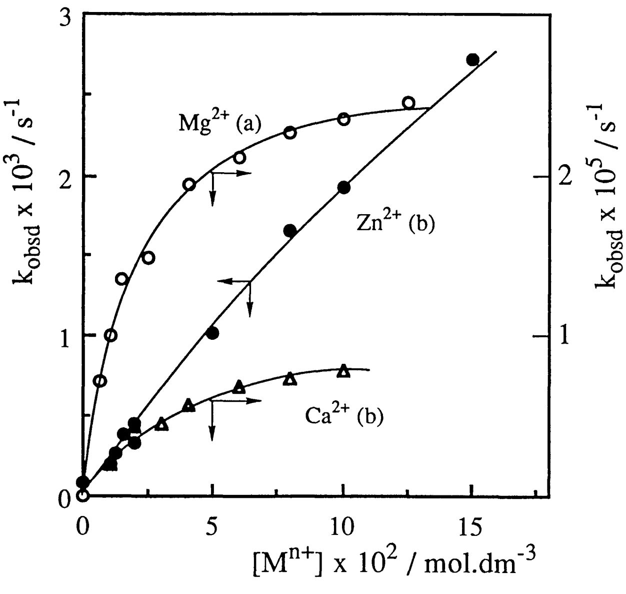


Fig. 2. Plots of k_{obsd} vs. metal ion concentration (see text for the kinetic conditions and the methods): a, SO_4^{2-} method; b, UV method.

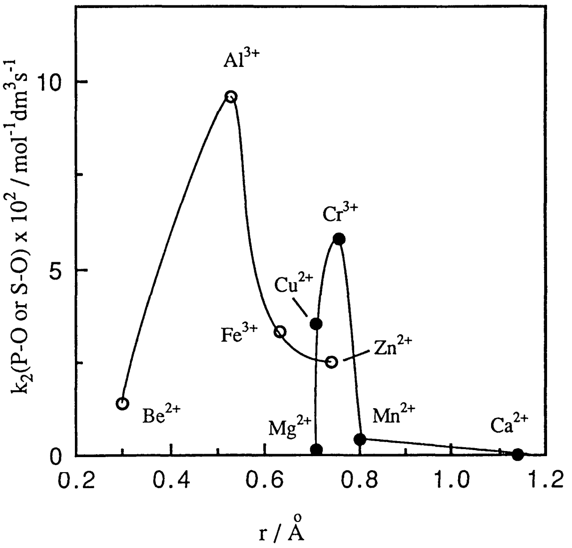


Fig. 3. Plots of the second-order rate constants vs. ionic radii of the metal ions: ○ k_2(S-O); ● k_2(P-O).

$$\begin{tikzcd} M+S \arrow[r, "K", shift left] \arrow[rr, "k_0"', bend right] & C \arrow[l, shift left] \arrow[r, "k_m"] & \text{products} \end{tikzcd} \qquad (1)$$

$$k_{obsd}=k_0+\frac{(k_m-k_0)K[M]_T}{1+K[M]_T+K[S]_T\left(1-\dfrac{k_{obsd}-k_0}{k_m-k_0}\right)} \qquad (2)$$

assuming the preequilibrium formation of a 1 : 1 complex (C) of the substrate (S) and metal ion (M) on the basis of Eq. 1. Eq. 2 is formulated in accordance with Eq. 1,[15,19] where $[M]_T$ and $[S]_T$ are the initial stoichiometric concentration of metal ion and substrate, respectively, k_o and k_m are the rate constants for the methanolyses of the metal-free and metal-coordinated substrate, respectively, and K stands for the formation constant for the 1 : 1 complex (C). In practice, the k_0 value was negligibly small and could be neglected in the rate calculation. The calculated curves in Fig. 2 are obtained by Eq. 2, using the evaluated K and k_m values given in Table 3, where the $k_mK/(1+K[S]_T)$ values in the last column correspond to the above mentioned k_2 values. Table 3 indicates that the k_m value for the Zn^{2+}-promoted S-O bond cleavage is 320- and 824-fold larger than those for the Mg^{2+} and

Ca^{2+} catalyzed P-O bond cleavage, respectively. It is interesting to note that the K value for Mg^{2+} with PPS in the present mixed solvent is much larger than that in water reported previously (K=1.19 mol^{-1} dm^3, pH 7.9, 55 °C),[14] suggesting for a strong solvation (hydration) to inhibit Mg^{2+} from complexation with PPS in water.

(c) The k_2 Values vs. Ionic Radii of the Metal Ions. In Fig. 3 are shown the k_2 values in Table 2 as a function of ionic radii of the metal ions.[20] The figure shows that the ionic radii of the metal ions which promote the O-S bond cleavage are shorter than those of the metal ions which catalyze the P-O bond cleavage, although Zn^{2+} appears to be an exception. It is interesting to note that there are two maxima in the vicinity of 0.53 and 0.75 Å corresponding to the S-O and P-O bond cleavage, respectively. These observations suggest that the ionic radius of a metal ion is a key factor for both selectivity and rate enhancement of the bond cleavage.

(d) Effect of Methanol Concentration on the Rates. The results are shown in Fig. 4 for the Al^{3+}-promoted S-O bond cleavage and the Mg^{2+} catalyzed P-O bond cleavage. Fig. 4 shows that the k_2 values of Mg^{2+} increase linearly with increasing methanol concentration, but those of Al^{3+} tend to be saturated with increasing methanol concentration. Since methanol is the nucleophile, it is no wonder that the rate is linear to the concentration of nucleophile if the rate-limiting step is the attacking of nucleophile. However, the rate is presumed to be indifferent to the methanol concentration if the rate-limiting step is the unimolecular cleavage of the terminal S-O bond. This argument may be too simple, because the increasing of methanol concentration should affect the rate through the change of solvent property, perhaps nonlinearly. Nevertheless, the linearlity in the case of Mg^{2+} suggests that the attacking of methanol on phosphorus to cleave the P-O bond is the rate-limiting step. In the case of Al^{3+} the situation seems to be more complex, but the data appear to support unimolecular process, at least at higher methanol concentration.

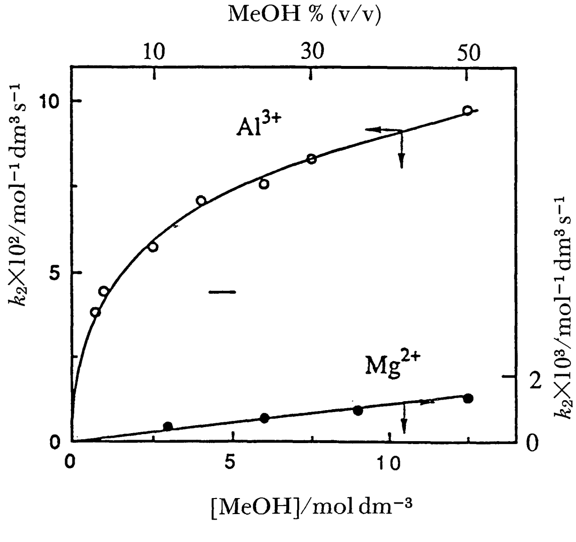


Fig. 4. Dependency of k_2 on methanol concentration in DMF.

(e) Alcoholysis by Methanol vs. 2-Propanol. The metal ions, Be^{2+}, Al^{3+}, Fe^{3+}, and Zn^{2+} were also found to promote the S-O bond cleavage of PPS in the alcoholysis by 2-propanol to give PP and isopropyl sulfate (PS) in quantitative yields. The k_2 value for the Al^{3+}-promoted alcoholysis by 2-propanol in a mixed solvent of 2-propanol and DMF (1 : 1, v/v%) at 55 °C was 1.3 times smaller than that of the corresponding methanolysis. In the case of Mg^{2+}, neither phenyl isopropyl phosphate (P-O bond cleavage) nor isopropyl sulfate (S-O bond cleavage) was obtained, but the detected products were only PP and IS, presumably due to the hydrolysis by a trace of water. In the case of Ca^{2+}, the expected phenyl isopropyl phosphate could be detected after a prolonged reaction as in the methanolysis due to the P-O bond cleavage. Thus, there seemed to be a severe steric hindrance to the attack of 2-propanol on the phosphorus to result in the P-O bond cleavage, whereas such steric hindrance seemed to be less important in the S-O bond cleavage.

In order to obtain more information, the competitive alcoholysis by methanol vs. 2-propanol was examined in the mixed solvents of methanol and 2-propanol in DMF, in the presence of either one of

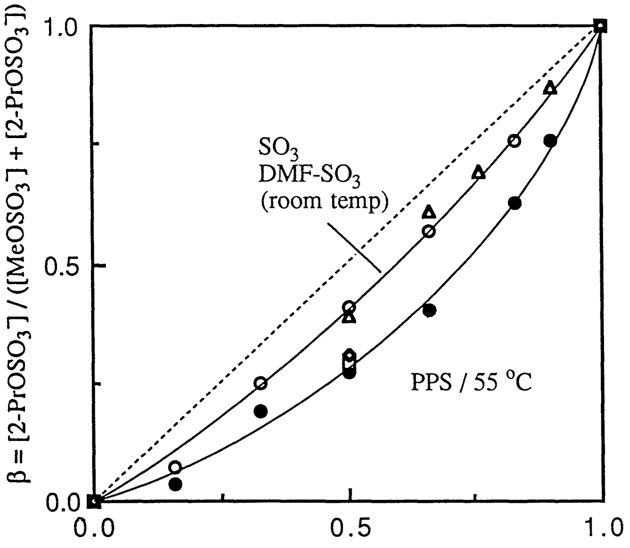
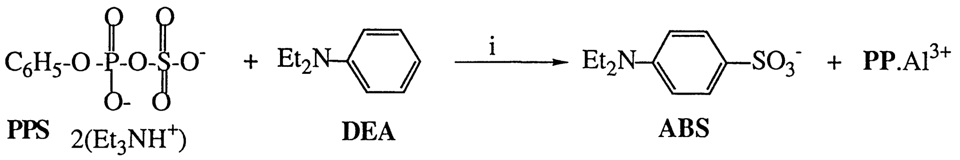


Fig. 5. Competitive solvolyses of PPS, SO_3, and DMF-SO_3 in the mixed solvents of MeOH, i-PrOH, and DMF: concentrations(mol dm^{-3}), [MeOH]+[2PrOH]=6.0, [N-ethylmorpholine]=0.55, ○ [SO_3]=0.15—1.0×10^{-2}, △ [DMF-SO_3]=1.6×10^{-2}, [PPS]=1.0×10^{-2} ([Be^{2+}]=3.0×10^{-2} (□), [Al^{3+}]=2.5×10^{-2} (●), [Fe^{3+}]=2.5×10^{-2} (□), [Zn^{2+}]=2.8×10^{-2} (◇)).



Scheme 2. Reagents: i, $AlCl_3$ in CH_3CN; room temp.

Be^{2+}, Al^{3+}, Fe^{3+}, or Zn^{2+}. The substrates examined were SO_3 and DMF-SO_3 in the absence of a metal ion, and PPS in the presence of a metal ion. The former two may be the same, because their difference was only in that whether SO_3 gas was introduced into a mixed solvent of methanol, 2-propanol and DMF or a mixture of methanol and 2-propanol was mixed with a DMF-SO_3 solution prepared prior to the mixing. The results are shown in Fig. 5. In Fig. 5, the diagonal straight line would be expected if methanol and 2-propanol are equally reactive. The observed line in the absence of a metal ion indicates that methanol is slightly more active than 2-propanol. Namely it shows that the product ratio was PS : MS=1 : 1.5 (β=0.4) when a mixture of equal amount of methanol and 2-propanol (γ=0.5) was used. The solid line was observed for the reaction of PPS in the presence of a metal ion. The four metal ions gave almost the same results in product ratio. When γ=0.5, the observed β value was about 0.3 for each metal ion, corresponding to a ratio of PS : MS=1 : 3. These results indicate that although there is a sizable difference in reactivity between methanol and 2-propanol, it is much less in the S–O bond cleavage than in the above mentioned P–O bond cleavage.

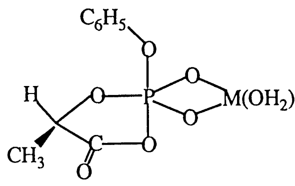
Sulfonation. The different behavior of the two groups of the metal ions was further manifested in the reaction of PPS with *N*,*N*-diethylaniline (DEA) in the presence of Al^{3+} or Mg^{2+} as a representative of each group of the metal ions. In acetonitrile, PPS (bis(triethylammonium) salt) and DEA did not react at room temperature irrespective of the presence or absence of Mg^{2+}. By contrast, Al^{3+} readily promoted the reaction to give *p*-(diethylamino)benzenesulfonate and PP in quantitative yields as shown in Scheme 2.

Discussion

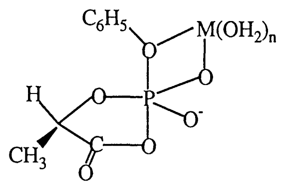
The rate of Mg^{2+}-catalyzed methanolysis of PPS (k_2(P–O)) was first-order with respect to the methanol concentration (Fig. 4), suggesting the rate-limiting nucleophilic attack of methanol on phosphorus of PPS. This is in accordance with our previous observation that in aqueous media Mg^{2+} catalyzes the nucleophilic attack of primary and secondary amines on phosphorus of PPS to give the corresponding phosphoramidates and IS, but the reaction does not occur with a sterically crowded amine such as *N*-ethylmorpholine and 2,6-lutidine irrespective of the presence or absence of Mg^{2+}.[10,13]

The dependency of rates of Al^{3+}-promoted methanolysis (k_2(S–O)) on the methanol concentration (Fig. 4) was quite different from that mentioned above. The rates tended to become independent of methanol concentration at higher concentration. The two different pathways can be conceivable for the S–O bond cleavage: (1) the unimolecular elimination of sulfur trioxide (SO_3) or its solvated species, and (2) the bimolecular nucleophilic attack of methanol on sulfur as in the case of above mentioned phosphorus attack. However, the latter possibility seems to be unlikely on the following grounds in addition to the above concentration effect. As shown in Fig. 5, the difference between the rates for methanol and 2-propanol is rather small in the S–O bond cleavage as compared to a large difference in the case of P–O bond cleavage, suggesting the formation of a very reactive species, i.e. SO_3 in the former case. Further supporting evidence is the Al^{3+}-promoted sulfonation of DEA with PPS in acetonitrile, where SO_3 is likely generated as the reactive sulfonating agent.

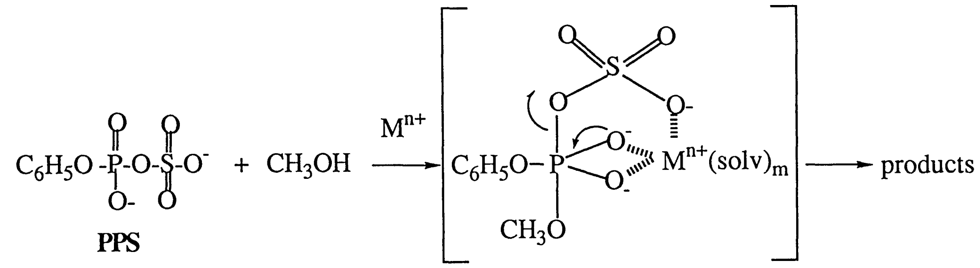
As discussed above, the P–O and S–O bond cleavage appear to occur with different mechanisms. Then, what is the role of a metal ion? The answer seems to be partly obtained by examining the data in Fig. 3 which shows the correlation of k_2 values with the ionic radii of the metal ions. Fig. 3 shows two rate maxima corresponding to the two different groups of metal ions which either catalyze the P–O or promote the S–O bond cleavage of PPS. The ionic radius of the metal ion to give the former rate maximum is in the vicinity of 0.75 Å. Quite interestingly the same ionic radius for the rate maximum was also reported by Benkovic et al. for the divalent metal ion-catalyzed hydrolysis of lactic acid *O*-phenyl phosphate, and it was suggested that the role of metal ion is to facilitate the formation of phosphorus(V)intermediate (**1** or **2**) which then rapidly decomposes to the products.[21]



1



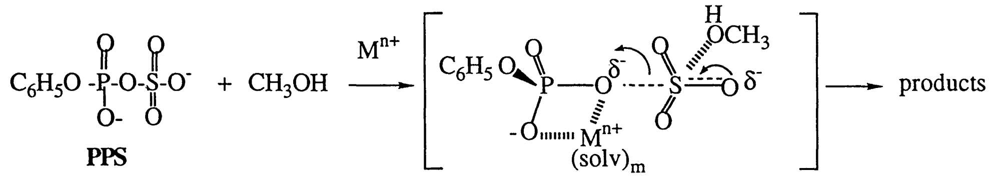
2



3

M^{n+} : Mg^{2+}, Cu^{2+}, Cr^{3+}, Mn^{2+}, Ca^{2+}

Scheme 3. A postulated pentacovalent phosphorus intermediate in a metal ion catalyzed methanolysis of PPS to give P-O bond cleavage.



4

M^{n+} : Be^{2+}, Al^{3+}, Fe^{3+}, Zn^{2+}

Scheme 4. Proposed transition state involving solvated sulfur trioxide.

The correlation with ionic radius suggests the importance of bidentate coordination with strict geometrical requirements as in **1** or **2**. Similar phosphorus(V) intermediate (**3**) can also be considered for the present metal ion-catalyzed P-O bond cleavage as illustrated in Scheme 3. In the structure **3**, a metal ion occupies a basal position and is chelated with two anionic oxygen ligands to form a four-membered cyclic complex. The metal ion is also assumed to form an ionic bond with sulfate oxygen ion. It is important to notice that the bond angle of the chelating $\angle^{-}O\text{-}P\text{-}O^{-}$ is 120°, the same as that of **1** but different from that of 90° in **2**. As discussed below, the relationship between the metal ion radius and the bond angle of a bidentate ligand seems to be of key importance in determining the selectivity in the P-O and S-O bond cleavage.

In the case of the S-O bond cleavage, Fig. 3 shows that the ionic radius of a metal ion to give the rate maximum is in the vicinity of 0.53 Å, much shorter than 0.75 Å in the case of P-O bond cleavage. A shorter ionic radius suggests a smaller bond angle for the bidentate chelation, and it led us to consider **4** in Scheme 4 as a possible transition state structure. In **4** is assumed a solvent assisted unimolecular S-O bond cleavage which is consistent with the above mentioned results (Figs. 4, 5 and Scheme 2). It is important to notice in **4** that a metal ion assists the S-O bond cleavage by chelating with two anionic oxygen ligands of the leaving phosphoryloxy group, in which the $\angle^{-}O\text{-}P\text{-}O^{\delta-}$ bond angle is near 109.5°, much smaller than the above 120° in the P-O bond cleavage.

The Zn^{2+} which promotes the selective S-O bond cleavage appears to be an exception from the consideration of ionic radius alone, since our previous studies demonstrated that in aqueous media Zn^{2+} always catalyzes the P-O bond cleavage, e.g. in the aminolysis of PPS,[10,13] in the 2-pyridinecarbaldehyde oxime-catalyzed hydrolysis of PPS,[10,22] and in the hydrolysis of 2-pyridyl phosphonosulfate.[15] It is known that Be^{2+} and Al^{3+} with smaller ionic radii, or Zn^{2+} and Fe^{3+} with the electron configuration of $3d^{10}$ and $3d^{5}$, respectively favor tetrahedral coordination.[23] On the other hand, metal ions such as Mg^{2+}, Cu^{2+}, Cr^{3+}, Mn^{2+}, and Ca^{2+} favor octahedral coordination. Thus, it is conceivable that the change in selectivity of Zn^{2+} from the P-O in water to the S-O bond cleavage in methanol is due to the change of solvation in the metal ion-substrate complex which affects the type of coordination, i.e. tetrahedral in methanol but octahedral coordination in water.

Finally, it is considered in general that the effective charge of metal ion is important for an electrophilic catalysis as in the present case. For this point, the above mentioned results are not conclusive, since the difference in k_2 values is rather small between Fe^{3+} and Zn^{2+} or between Cr^{3+} and Cu^{2+}. It is conceibable that the solvation to reduce effective charge is stronger for a trivalent metal ion than for a divalent metal ion, resulting in a smaller difference in their reactivities.

Much remains to be clarified as to the mechanism of metal ion catalysis in the reactions of P–O–S linkage, as seen in the dual behavior of Zn^{2+}. Moreover, a mysterious gap between the selectivity of Mg^{2+} in the P–O–S bond cleavage in enzyme and model reactions has still remain unsolved. Nevertheless, it is important to notice that there is a certain correlation between the ionic radii of metal ions and the selectivity in the P–O–S bond cleavage.

We are much indebted to Messrs T. Ogihara and A. Kato, and Miss F. Arai who carried out the early part of this study.

References

1) D. E. Metzler, "Biochemistry," Academic Press, New York (1977), pp. 395, 611—615, and 636.

2) F. Lipmann, *Science,* **128**, 575 (1958).

3) A. B. Roy, *Adv. Enzymol.,* **22**, 205 (1960).

4) H. D. Peck, Jr., in "The Enzymes," 3rd ed, ed by P. D. Boyer, Academic Press, New York (1974), Vol. 10, Chapt. 21, pp. 61.

5) A. B. Roy and P. A. Trudinger, "The Biochemistry of Inorganic Compounds of Sulfur," Cambridge Univ. Press, London and New York (1970).

6) A. B. Roy, "The Enzymes," 3rd ed, ed by P. D. Boyer, Academic Press, New York (1971), Vol. 5, Chapt. 1, pp. 1.

7) R. E. Dickerson and R. Timkovich, in "The Enzymes," 3rd ed, ed by P. D. Boyer, Academic Press, New York (1975), Vol. 11, pp. 397—547.

8) D. V. DerVartanian and J. LeGall, *BBA,* **346**, 79 (1974).

9) H. D. Peck, Jr., R. Bramlett, and D. V. DerVartanian, *Z. Naturforsch., B,* **27**, 1084 (1972).

10) W. Tagaki and T. Eiki, *Adv. Chem. Ser.,* **191**, 409 (1980).

11) W. Tagaki, Y. Asai, and T. Eiki, *J. Am. Chem. Soc.,* **95**, 3037 (1973).

12) T. Eiki, Y. Asai, and W. Tagaki, *Bull. Chem. Soc. Jpn.,* **55**, 1097 (1982).

13) T. Eiki, K. Tomuro, S. Aoshima, M. Suda, and W. Tagaki, *Nippon Kagaku-Kaishi,* **1980**,454.

14) T. Eiki and W. Tagaki, *Bull. Chem. Soc. Jpn.,* **55**, 1102 (1982).

15) T. Eiki, T. Horiguchi, M. Ono, S. Kawada, and W. Tagaki, *J. Am. Chem. Soc.,* **104**, 1986 (1982).

16) T. Eiki and W. Tagaki, *Chem. Lett.,* **1981**, 1465.

17) T. Eiki, T. Ogihara, A. Kato, F. Arai, and W. Tagaki, *J. Chem. Soc., Chem. Commun.,* **1985**, 49.

18) W. Tagaki, T. Eiki, and I. Tanaka, *Bull. Chem. Soc. Jpn.,* **44**, 1139 (1971).

19) S. J. Benkovic and L. Dunikoski, *J. Am. Chem. Soc.,* **93**, 1526 (1971).

20) R. D. Shannon, *Acta Crystallogr., Sect. A,* **32**, 751 (1976).

21) J. J. Steffens, I. J. Siewers, and S. J. Benkovic, *Biochemistry,* **14**, 2431 (1975).

22) K. Tomuro, Thesis, Gunma University, Kiryu, 1976.

23) R. P. Houghton, "Metal Complexes in Organic Chemisty," Cambridge Univ. Press, London, New Yord and Melbourne (1979).