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METAL ION DEPENDENT SELECTIVE P-O VS. S-O BOND FISSION IN THE REACTION OF PHENYL PHOSPHATOSULFATE

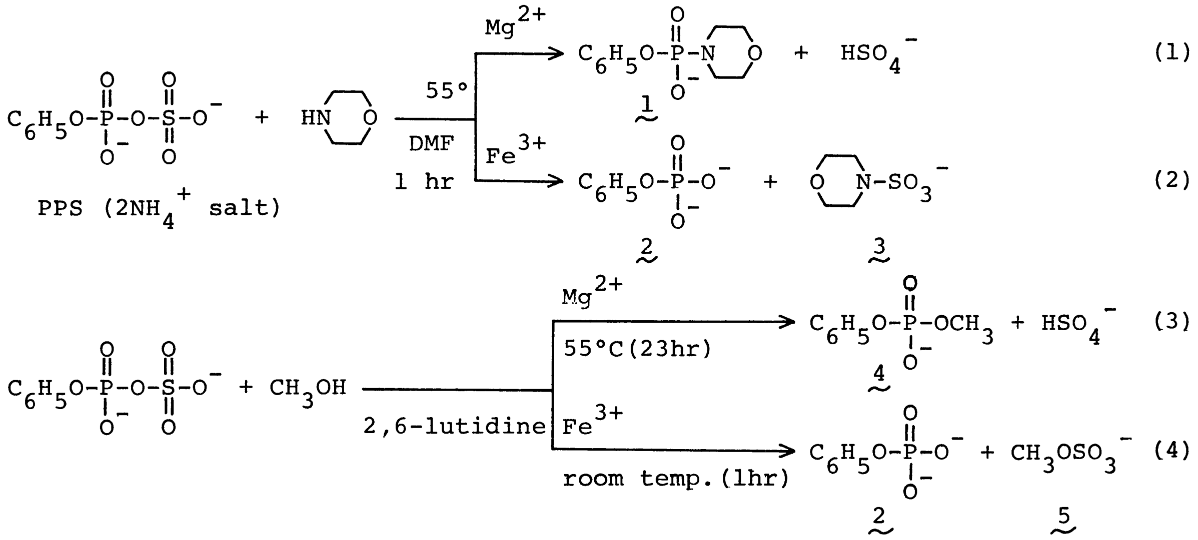
Toshio EIKI and Waichiro TAGAKI*
Department of Chemistry, Faculty of Technology, Gunma University,
Kiryu, Gunma 376: *Department of Applied Chemistry, Faculty of
Engineering, Osaka City University, Sugimoto-cho, Sumiyoshi-ku,
Osaka 558

Metal ion promoted highly selective P-O or S-O bond fission has been found in the aminolysis and the methanolysis of phenyl phosphatosulfate, i.e. magnesium ion promoted the P-O bond fission, while ferric ion promoted the S-O bond fission. The selectivity was almost 100% in each case.

3'-Phosphoadenosine 5'-phosphatosulfate (PAPS) is the key intermediate as the sulfate donor in the formation of various biologically important sulfate esters.1) PAPS and adenosine 5'-phosphatosulfate (APS) have also been known to be intermediates in the reduction of sulfate to sulfite.1) An interesting subject for these biological reactions is the role of metal ions. It was reported that APS reductase contains non-heme iron together with FAD coenzyme.2) Arylamine sulfatase requires Mg^{2+} ion as the cofactor in the sulfate group transfer from PAPS to arylamine.3) In these reactions, the S-O bond of P-O-S linkage must be selectively cleaved. Besides these enzymes, there are a number of other hydrolytic enzymes which are also dependent on divalent metal ions in the hydrolysis of PAPS.4) However, in these hydrolysis reactions, the site of cleavage of the P-O-S linkage has been still unclarified. In the model reactions, we have observed that the S-O bond fission occurs only under acidic conditions both in the absence and presence of divalent metal ions,5,6,7) while under neutral and basic conditions divalent metal ions always promote an exclusive fission of P-O bond.6) We now wish to report that Fe^{3+} ion promotes selective fission of the S-O bond in contrast to the P-O fission by Mg^{2+} ion in the reaction of phenyl phosphatosulfate (PPS).

The reactions were carried out in DMF or methanol.8) In the presence of Mg^{2+} ion, the reaction of PPS with morpholine in DMF resulted in a quantitative formation of phenyl phosphormorpholidate (1) and inorganic sulfate (eq 1), indicating selective fission of P-O bond. On the other hand, under the same conditions, Fe^{3+} ion promoted the selective fission of S-O bond to give phenyl phosphate (2) and 4-morpholinesulfonate (3) in almost quantitative yield (eq 2).

Similar metal ion dependent reversed selectivity was also observed in the methanolysis. In the absence of metal ions, methanolysis of PPS gave 2 and methyl sulfate (5) with a half life of 19 hr under the conditions described in Figure 1. Mg^{2+} ion promoted, on the contrary, the reaction to give methyl phenyl phosphate (4) and inorganic sulfate in quantitative yield in the presence of excess amount of 2,6-lutidine[9] (eq 3). While in the presence of Fe^{3+} ion under the same conditions, the reaction occurred readily at room temperature to give 2 and 5 in quantitative yields as observed in the absence of metal ions (eq 4).



The metal ion effects on the rates of methanolysis are shown in Figure 1. The rates of formation of inorganic sulfate increased, but tended to be

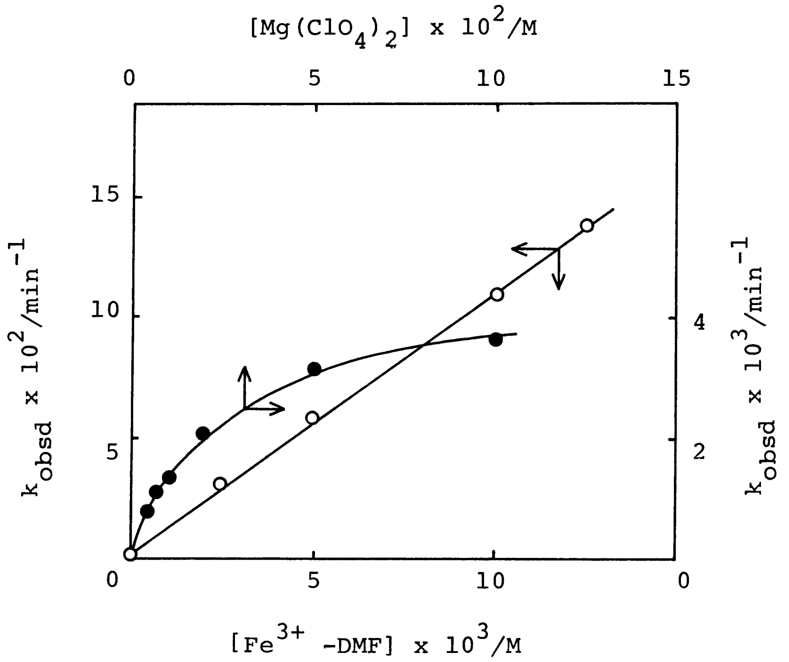
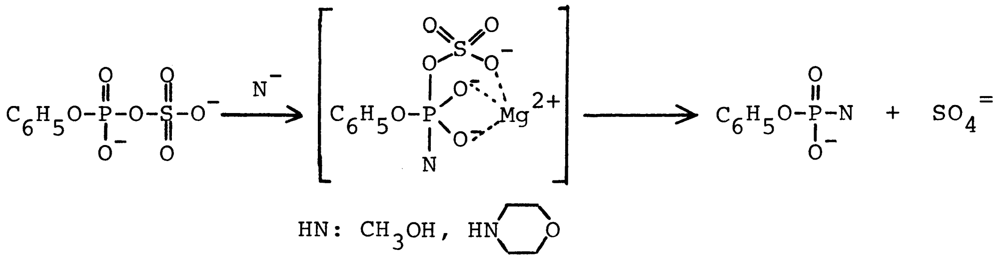


Figure 1. Concentration effect of metal ion on the rates of methanolysis of PPS. The reactions were carried out in N-ethylmorpholine-HCl (4:1, 0.4 M) buffer.
$Mg(ClO_4)_2$ (●); $[PPS]=2.0\times10^{-2}M$ in CH_3OH (55 °C)
$[Fe(DMF)_3Cl_2][FeCl_4]$ (○); $[PPS]=1.0\times10^{-2}M$ in 40% CH_3OH-60% DMF (30 °C).

saturated as increasing the concentration of Mg^{2+} ion which suggests the complexation of PPS and Mg^{2+} ion. In the presence of Fe^{3+} ion, the reaction was carried out in a mixture of DMF-methanol (6:4 v/v) mixed solvent, since the product **2**-Fe^{3+} complex precipitated in pure methanol as the reaction proceeded. The presence of DMF did not affect the selectivity in P-O vs. S-O bond fission. The reaction showed a first-order dependence on the concentration of Fe^{3+} ion.[10] The figure indicates that the rates for Fe^{3+} ion are much larger than those for Mg^{2+} ion.

The above results clearly indicate that under basic conditions Mg^{2+} ion promotes the attack of nucleophiles (morpholine and methanol) on phosphorus atom resulting in the phosphate group transfer reactions. Mg^{2+} ion effect may be accounted for by electrostatic facilitation of nucleophilic attack on phosphorus atom to form a pentacovalent intermediate as shown in eq 5.[6] However, such selective P-O bond fission is in fact contrary to the S-O bond fission in some enzymatic reactions of PAPS.[3] Such opposite selectivity may not be due to the difference in substrate structure, since we also obtained essentially similar results as shown in eq 1 for the reaction of γ-phenylpropyl phosphatosulfate.[6,11]



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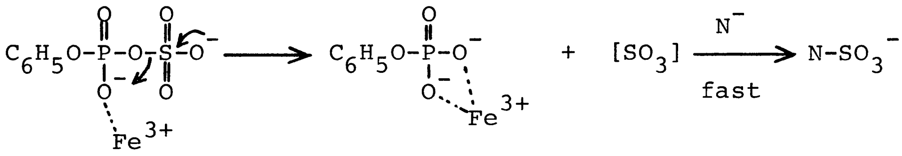
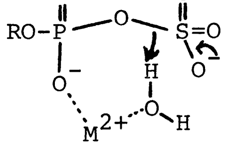


(6)

On the contrary, Fe^{3+} ion promoted S-O bond fission in a specific fashion, even under basic conditions, to result in a selective sulfate group transfer reaction. Beside Mg^{2+} ion, Ca^{2+}, Co^{2+}, Zn^{2+} and Cu^{2+} ions were all observed to effect selective P-O fission under basic conditions. Thus the effect of Fe^{3+} ion is very unusual.

It appears to be necessary to recall our previous observation that Mg^{2+} ion enhances the acid catalyzed hydrolysis or methanolysis with predominant S-O fission.[5,6,7] As an explanation, we have proposed a mechanism of "metal ion assisted proton transfer catalysis" to facilitate unimolecular fission of S-O bond as illustrated in the hypothetical intermediate **6**. The above faster rate and the selective S-O fission by Fe^{3+} ion resemble the reactions under such acidic condition. Therefore, Fe^{3+}-DMF complex might act by a similar mechanism (eq 6) under basic conditions to that in the presence of Mg^{2+} ion under acidic conditions, although the real mechanism including the validity of **6** is entirely obscure at present.



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The present results demonstrated for the first time that the metal ion promoted selective S-O fission of P-O-S linkage could occur under neutral and basic conditions. This finding, although the mechanistic details are the subjects of future study, may be important for the consideration of biological reactions of the compounds having the phosphatosulfate linkage.

References

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(8) Metal ions used were $Mg(ClO_4)_2$ and $FeCl_3$-DMF complex (prepared by the method of S. Tobashi and E. Kotani, J. Am. Chem. Soc., 94, 309 (1973)). Reaction conditions: for eqs 1 and 2, PPS=1 mmole, metal ion 2 mmoles and morpholine 10 mmoles in DMF 10 ml, 55°C; for eqs 3 and 4, PPS=1 mmole, metal ion=2 mmoles and 2,6-lutidine=20 mmoles in methanol 15 ml, either at room temperature or 55°C. The products were identified by UV, ^1H NMR and chromatography, after the reaction mixture being lyophilized. The NMR signals were 3.74 (3H, d) for the CH_3 of **4** and 3.81 (3H, s) for the CH_3 of **5**. Paper chromatography was performed by Toyo Roshi No. 50 paper using n-PrOH-conc.ammonia-water (6:3:1 v/v). R_f values were 0.65(PPS), 0.84(**1**), 0.48(**2**), and 0.80(**4**).

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(10) The rates were determined by following the phosphate **2** based on our previous method in ref 5-7, and 9. The rates were first-order in the presence of an excess of Fe^{3+} ion over PPS. However, under the reversed conditions of excess PPS over Fe^{3+} ion, typical burst kinetics were observed with initially rapid, but later slower production of **2**, suggesting the formation of very tight **2**-Fe^{3+} complex.

(11) PAPS and γ-phenylpropyl phosphatosulfate are the alkyl esters, while PPS is an aryl ester.

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