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Influence of Metal Ions and of Metal Chelates on the Hydrolysis of α-D-Glucose 1-Phosphate*

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The hydrolysis reaction of α-D-glucose 1-phosphate has been studied in the presence of several metal ions and metal chelates at 40.0 and 60.0°C, in the pH range of 1.69—2.71, and at an ionic strength of 0.10 M (KNO_3). The reaction rate was followed by the colorimetric method using a combination of the amidol reagent and 8.3% molybdate solution. Among the metal ions employed in this work, dipositive ions, such as manganese(II), cobalt(II), nickel(II), copper(II), zinc(II) and uranyl ions, were observed to be nearly without effect on the rate of hydrolysis, while iron(III) and thorium(IV) ions demonstrated a negative catalytic effect. On the other hand, all the chelating agents employed tend to restore the reaction rate in reference to the corresponding metal-ion-effected reactions. With the 1:1 thorium(IV)-EDTA and the 1:1 iron(III)-EDTA chelate system, the rate was completely restored. Both iron(III)-kojate and thorium(IV)-Tiron chelate systems restored the reaction rate to a lesser extent. The roles of the chelating agents were discussed. The reaction mechanism in the presence of the catalyst species has been considered, and two possible mechanisms have been put forward for the present reactions.

The hydrolysis of organic orthophosphates are in general complicated by several factors[1] even in the absence of metal ions and of metal chelates. Firstly, an organic phosphate exists in various ionic forms in aqueous media, the composition of which depends on the nature of the medium, the structure of the alcohol substituent, and the acid dissociation constants of the phosphate. Secondly, each ionic species of the substrate undergoes either carbon-oxygen or phosphorus-oxygen bond fission, or both simultaneously in the course of hydrolysis. Thirdly, the reactivity of each ionic species is controlled by the structure and the physical properties of the alcohol substituent, and by the nature of the medium. The reactivity is also affected by a reaction process through which hydrolysis of a substrate proceeds.

Among the organic phosphates of biological interest, α-D-glucose 1-phosphate is known to form as an intermediate in biological system during the course of degradation of sugars. The hydrolysis of this phosphate has been extensively studied by Bunton and co-workers[2] and the neutral species was found to be the most reactive one among the neutral, monoionic and diionic species. A unimolecular mechanism was assigned to the hydrolysis of both the neutral and the monoionic species.

Present research has been carried out to investigate the participation of metal ions and metal chelates in the hydrolysis of α-D-glucose 1-phosphate. Since a series of studies on the hydrolysis of salicyl phosphate analogs have been carried out previously[3-5] to investigate the catalytic roles of metal ions and of metal chelates, this work may provide some additional information on the role of metals in such reactions.

Experimental

Reagents.—The dihydrate form of dipotassium α-D-glucose 1-phosphate was purchased from Sigma Chemical Company, St. Louis, Mo., U. S. A. The purity of this compound, which was established by phosphorus analysis and potentiometric titration, was sufficiently high, so that further purification was not necessary.

The disodium salt of ethylenediaminetetraacetic acid and Tiron (sodium pyrocatechol-3, 5-disulfonate) were the products of the Research Laboratories of Dojindo & Co., Ltd.; both were used without further purification. Kojic acid was purchased from Tokyo Kasei Kogyo Co., Ltd. and was purified by sublimation in vacuo.

The nitrates of divalent metals, $Co(NO_3)_2\cdot 6H_2O$, $Ni(NO_3)_2\cdot 6H_2O$, $Cu(NO_3)_2\cdot 3H_2O$, and $Zn(NO_3)_2\cdot 6H_2O$; and manganese sulfate, $MnSO_4\cdot nH_2O$, of an analytical grade were purchased from Wako Pure Chemical Industries, Ltd. The nitrates of actinide metals, $UO_2(NO_3)_2\cdot 6H_2O$ and $Th(NO_3)_4\cdot 4H_2O$, of an analytical

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[1] See for general review: J. R. Cox, Jr., and O. B. Ramsey, *Chem. Revs.*, **64**, 317 (1964).

[2] C. A. Bunton, D. R. Llewellyn, K. G. Oldham and C. A. Vernon, *J. Chem. Soc.*, **1958**, 3588.

[3] R. Hofstetter, Y. Murakami, G. Mont and A. E. Martell, *J. Am. Chem. Soc.*, **84**, 3031 (1962).

[4] Y. Murakami and A. E. Martell, *J. Phys. Chem.*, **67**, 582 (1963).

[5] Y. Murakami and A. E. Martell, *J. Am. Chem. Soc.*, **86**, 2119 (1964).

grade are the products of the British Drug Houses Ltd. and Wako Pure Chemical Industries, Ltd. respectively. The ferric nitrate nonahydrate was a pure grade reagent of the latter company. Standardization of aqueous stock solutions of these metal salts was established by the usual method of chelatometric titration.

Potentiometric Measurements.—The apparatus and procedures for potentiometric titrations have been mentioned elsewhere.6) Potentiometric titrations of α-D-glucose 1-phosphate were carried out at temperatures of 34.8, 39.8, 45.0 and 60.0°C, and at an ionic strength of 0.10 M with added potassium nitrate. In order to avoid any perturbation of the data which may be caused by hydrolysis of the phosphate during the course of titrations, the reliability of titration data was confirmed by repeated titration runs and by the detection of any liberated inorganic phosphate.

Kinetic Measurements.—The pH value of an experimental solution was maintained constant during each run without adding any base or acid, since each solution was sufficiently acidic that the chemical reactions occurring did not cause any significant change in its acidity. Nevertheless, the pH value of each experimental solution was checked from time to time with an HRL Model P pH meter of the Horiba Instruments Inc. fitted with extension glass and calomel electrodes. The ionic strength of the experimental solutions was maintained at 0.10 M with potassium nitrate. Two reaction temperatures were selected; 40.0±0.1 and 60.0±0.1°C. The rate of hydrolysis was determined by measuring the amount of phosphoric acid liberated in the course of hydrolysis.

The analytical method for the liberated inorganic orthophosphate, employed in this work, essentially followed the procedure established by Allen.7) The two kinds of solutions were prepared: Amidol reagent was prepared by dissolving 2.5 g. of amidol (product of Naniwa Chemical Co., Ltd.) recrystallized from water-methanol and 50 g. of sodium bisulfite (Wako analytical grade) in mineral-free water, and diluting to 250 ml. This mixed solution was allowed to stand overnight in a refrigerator after a small amount of active charcoal being added, and then filtered to remove active charcoal and any other precipitates. The activity of this reagent was checked from time to time to assure the accurate analysis of orthophosphate. The second reagent is the 8.3% molybdate solution. To prepare this solution, 22.035 g. of ammonium molybdate $(NH_4)_6(Mo_7O_{24})\cdot 4H_2O$ (Wako analytical grade) was dissolved in 228 ml. of mineral-free water.

The following analytical procedure was employed in this study. A 2.0 ml. sample was pipetted from the experimental solution and was transferred to a 25 ml. volumetric flask containing 10 ml. of water, which had been cooled in an ice bath. The reaction stopped immediately because of low temperature. Two milliliters of 60% perchloric acid (Wako analytical grade), 2.0 ml. of the amidol reagent, and 1.0 ml. of the 8.3% molybdate solution were added in this order, and the mixture was diluted to 25 ml. with mineral-free water. The volumetric flask was then placed in a constant temperature bath at 15.0±0.5°C for 5 min. to develop the violet color. The absorbance was measured at 720 mμ with a Shimadzu-Bausch & Lomb Spectronic 20 Colorimeter immediately after the development of the color. The amount of inorganic phosphate was determined by reference to a calibration curve obtained with the use of a standard potassium dihydrogen phosphate (Wako analytical grade) solution.

Results

Determination of Acid Dissociation Constants.—The evaluated acid dissociation constants of α-D-glucose 1-phosphate are summarized in Table I. Some different values of pK_{HA} were reported by Ashby and co-workers,8) and the discrepancy can be ascribed to the difference in experimental ionic strength among this work and theirs. Another set of pK values reported by Cori and co-workers9) are in a rather close agreement with the present data.

Kinetics of Spontaneous Hydrolysis. — Hydrolysis of α-D-glucose 1-phosphate has been studied at 40 and 60°C in the lower pH region ranging from 1.72 to 3.38. This hydrolysis reaction was found to follow the apparent first order kinetics, rate constants obtained at various pH's being summarized in Table II. The reaction rate is seen to increase as the pH of the solution decreases.

TABLE I. ACID DISSOCIATION CONSTANTS OF α-D-GLUCOSE 1-PHOSPHATE

Temp., °C	μ	pK_{H_2A}	pK_{HA}	Ref.
34.8±0.1	0.10 (KNO_3)	1.44±0.04	—	This work
39.8±0.1	0.10 (KNO_3)	1.43±0.04	—	This work
45.0±0.1	0.10 (KNO_3)	1.42±0.04	—	This work
60.0±0.1	0.10 (KNO_3)	1.40±0.05	6.09±0.01	This work
35	—	—	6.519*	8
40	—	—	6.531*	8
45	—	—	6.545*	8
50	—	—	6.561*	8
30	—	1.10	6.13	9

* Thermodynamic constant

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6) Y. Murakami, This Bulletin, **35**, 52 (1962).

7) R. J. L. Allen, *Biochem. J.*, **34**, 858 (1940).

8) J. H. Ashby, H. B. Clarke, E. M. Crook and S. P. Datta, ibid., **59**, 203 (1955).

9) C. J. Cori, S. P. Colowick and G. T. Cori, *J. Biol. Chem.*, **121**, 465 (1937).

TABLE II. FIRST ORDER RATE CONSTANTS OF THE HYDROLYSIS OF α-D-GLUCOSE 1-PHOSPHATE
$\mu=0.10$ M (KNO_3); $T_S=1.00\times10^{-3}$ M

	60.0±0.1°C							40.0±0.1°C
$-\log[H^+]$	1.72	1.91	2.04	2.07	2.25	2.71	3.38	1.72
$10^5 k$, sec^{-1}	6.83	4.19	3.08	2.83	1.94	0.617	0.139	0.385

T_S: Initial total concentration of the phosphate species

TABLE III. HYDROLYSIS RATES OF α-D-GLUCOSE 1-PHOSPHATE IN THE PRESENCE OF METAL IONS
$\mu=0.10$ M (KNO_3); $T_M/T_S=1.0$; $T_S=1.00\times10^{-3}$ M

	$k\times10^6$, sec^{-1}			
	60.0±0.1°C			40.0±0.1°C
Metal ion	$-\log[H^+]=1.72$	$-\log[H^+]=2.04$	$-\log[H^+]=2.71$	$-\log[H^+]=1.72$
none	68.3	30.8	6.17	3.85
Mn(II)	67.2	29.7	4.49	3.13
Co(II)	68.6	31.8	—	3.43
Ni(II)	69.3	31.8	6.81	3.32
Cu(II)	67.9	32.5	6.17	3.20
Zn(II)	68.5	32.2	—	3.73
Fe(III)	32.0	13.9	5.28	1.69
UO_2(VI)	60.3	28.9	—	—
Th(IV)	10.9	—[a]	—[a]	—

T_M: Total concentration of the metal ion
T_S: Total concentration of the phosphate species
a) Not first order

Kinetics of Hydrolysis in the Presence of Metal Ions.—In order to investigate effects and roles of metal ions in the hydrolysis reaction of α-D-glucose 1-phosphate, several metal ions of the first transition series and of the actinide series have been employed in this study. The hydrolysis reaction has been studied at a 1:1 molar ratio of substrate to metal ion and the reaction was observed to proceed homogeneously in all cases. The experimental data obtained at 60.0 and 40.0°C indicate an apparent first order rate with respect to the total concentration of unreacted organic phosphate species in most instances. All the rate data obtained for the metal-ion-effected hydrolysis reactions are listed in Table III. In most cases where the hydrolysis reaction followed first order kinetics, the divalent metal ions and uranyl ion did not cause any significant change in reaction rate in reference to the spontaneous rate. As can be seen, those metal ions which caused a significant change in reaction rate acted as negative catalysts. Although the thorium ion provided a first order rate constant at $-\log[H^+]=1.72$ and 60°C, the hydrolysis did not follow first order kinetics under other experimental conditions with this metal ion present. Moreover, the apparent reaction rate increases as pH value is raised in the presence of the thorium ion, while the rate of spontaneous hydrolysis and of other metal ion-effected reactions decreases. The rate profiles at various pH's for the thorium-effected reaction are shown in Fig. 1 along with those for the spontaneous reaction.

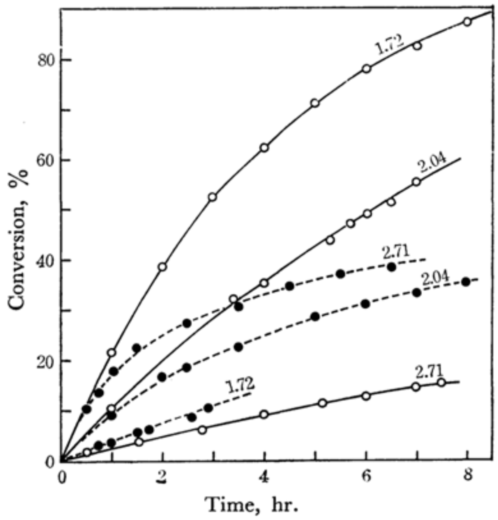


Fig. 1. Hydrolysis of α-D-glucose 1-phosphate at 60.0°C in 0.10 M KNO_3, with the total substrate concentration of 1.00×10^{-3} M; ○, spontaneous reaction; ●, thorium(IV)-ion-effected reaction ($T_S/T_M=1.0$). Numbers refer to the hydrogen ion concentration in $-\log[H^+]$. T_M=total concentration of the thorium ion; T_S=total concentration of the phosphate species.

Kinetics of Hydrolysis in the Presence of Metal Chelates.—Four different chelate systems have been employed in the present work to investigate the interaction of each chelate system with α-D-glucose 1-phosphate in the course of hydrolysis reaction of the latter. These chelate systems are iron(III)-kojate, iron(III)-EDTA, thorium(IV)-Tiron, and thorium(IV)-EDTA. The hydrolysis reaction was studied at 60.0°C and was observed to follow first order kinetics in all instances; all the experimental solutions contained a 1:1:1 molar ratio of metal ion, ligand, and substrate. The specific rate constants determined experimentally are listed in Table IV.

TABLE IV. HYDROLYSIS RATES OF α-D-GLUCOSE 1-PHOSPHATE IN THE PRESENCE OF METAL CHELATES
60.0±0.1°C; $\mu=0.10$ M (KNO_3); $T_M/T_S=1.0$; $T_S=1.00\times10^{-3}$ M; $T_M/T_L=1.0$

Metal chelate system		$k\times10^5$, sec^{-1}	
Metal ion	Ligand	$-\log[H^+]=1.69\pm0.02$	$-\log[H^+]=2.00\pm0.02$
none	none	6.83a)	3.08b)
Fe(III)	none	3.20a)	1.39b)
Fe(III)	kojate	4.87	2.35
Fe(III)	EDTA	7.47	3.78
Th(IV)	none	1.09a)	—c)
Th(IV)	Tiron	1.52	1.18
Th(IV)	EDTA	7.33	3.80

T_M: Total concentration of the metal species
T_S: Total concentration of the phosphate species
T_L: Total concentration of the ligand species
$-\log[H^+]$ values: a, 1.72; b, 2.04
c) Not first order.

As shown in Table IV, at both pH's the interaction of metal ion alone with the phosphate resulted in a much slower rate of hydrolysis than the spontaneous hydrolysis rate. The presence of a ligand capable of coordinating to metal ion, however, tends to restore the rate to the spontaneous one. With the two chelate systems, Fe(III)-EDTA and Th(IV)-EDTA, the rate is recovered completely. The concentration effect of the Fe(III)-kojate chelate system was studied at a selected hydrogen ion concentration ($-\log[H^+]=2.07$), and the rate-concentration profile is shown in Fig. 2. The reaction rate is seen to decrease with an increase of the chelate concentration.

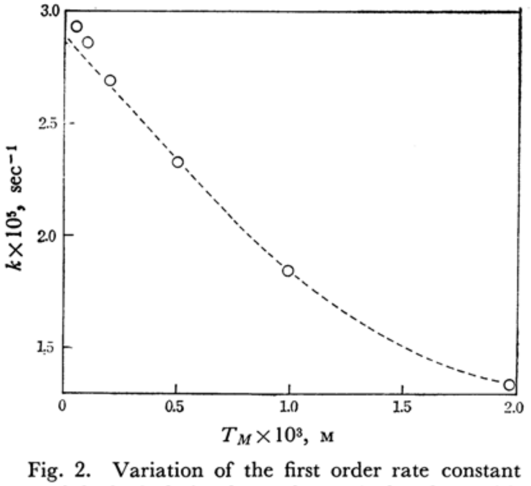
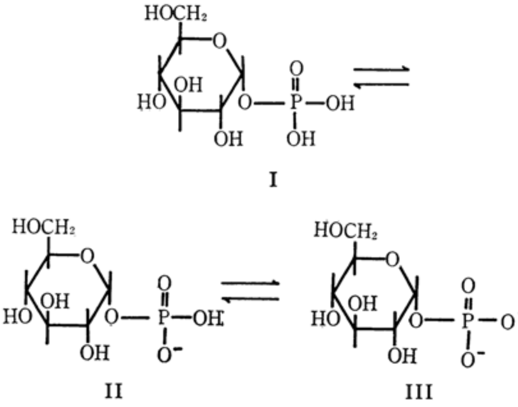


Fig. 2. Variation of the first order rate constant of the hydrolysis of α-D-glucose 1-phosphate with the concentration of Fe(III)-kojate chelate species at $-\log[H^+]=2.07$; $\mu=0.10$ M (KNO_3); 60.0°C; T_M=total concentration of the Fe(III) species (free metal ion and chelate species).

Discussion

Spontaneous Hydrolysis.—The substrate, α-D-glucose 1-phosphate, is considered as a dibasic acid and exists in three different forms, I, II, and III, the composition of which is dependent on the pH value of a solution.


I
II
III

The mole fractions of these three substrate species at various hydrogen ion concentrations are shown in Table V together with the hydrolysis rate constant at each hydrogen ion concentration. A plot of

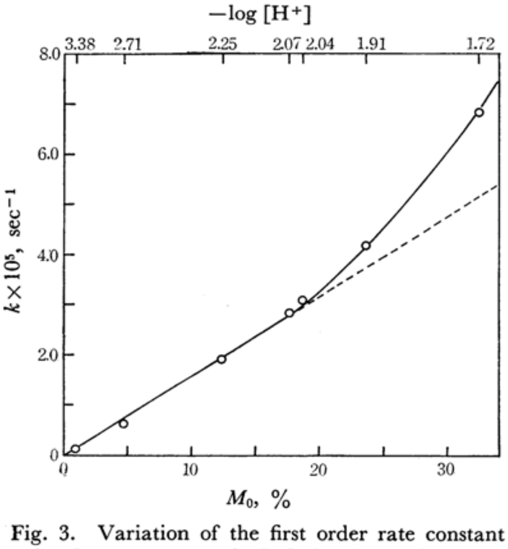


Fig. 3. Variation of the first order rate constant for the spontaneous hydrolysis of α-D-glucose 1-phosphate with the mole fraction of the neutral phosphate species at 60.0°C; $\mu=0.10$ M (KNO_3). Dotted line indicates an extrapolated straight line.

TABLE V. CORRELATION OF MOLE FRACTIONS OF THE SUBSTRATE SPECIES WITH HYDROLYSIS RATES AT VARIOUS HYDROGEN ION CONCENTRATIONS

60.0±0.1°C; $\mu=0.10$ M (KNO_3)

$-\log[H^+]$	$[H^+]$, M	M_0	M_I	M_{II}	$k\times10^5$, sec^{-1}
1.72	1.91×10^{-2}	3.24×10^{-1}	6.76×10^{-1}	2.89×10^{-5}	6.83
1.91	1.23×10^{-2}	2.36×10^{-1}	7.64×10^{-1}	5.05×10^{-5}	4.19
2.04	9.13×10^{-3}	1.87×10^{-1}	8.13×10^{-1}	7.25×10^{-5}	3.08
2.07	8.51×10^{-3}	1.76×10^{-1}	8.24×10^{-1}	7.87×10^{-5}	2.83
2.25	5.63×10^{-3}	1.24×10^{-1}	8.76×10^{-1}	1.26×10^{-4}	1.94
2.71	1.95×10^{-3}	4.67×10^{-2}	9.53×10^{-1}	3.97×10^{-4}	0.617
3.38	4.17×10^{-4}	1.04×10^{-2}	9.90×10^{-1}	1.76×10^{-3}	0.139

M_0: Mole fraction of the neutral species
M_I: Mole fraction of the monoionic species
M_{II}: Mole fraction of the diionic species

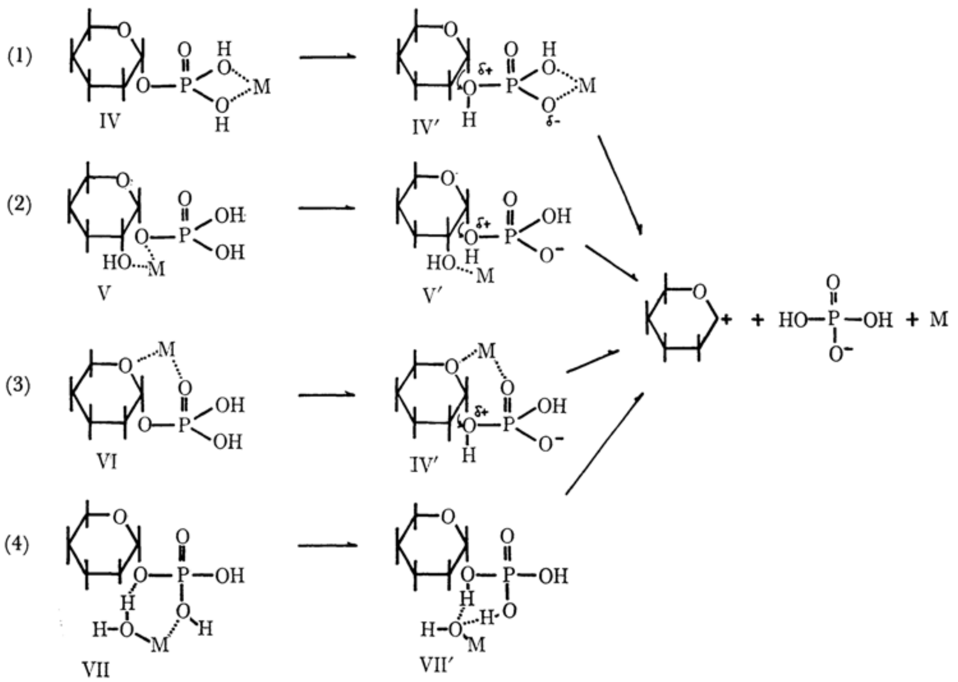


Chart 1. Probable mechanisms for the hydrolysis of α-D-glucose 1-phosphate in the presence of metal species.

a mole fraction of the neutral species against corresponding spontaneous rate constant resulted in a straight line in the region lying below a mole fraction 0.18, as is seen in Fig. 3.
Therefore, the main active species existing in this pH region may be assumed to be the neutral phosphate species. In the higher mole fraction range, the specific rate constant plot deviates from linearity. Thus, an acid catalyzed reaction is considered to be superimposed upon the spontaneous hydrolysis of the neutral species. These considerations are in agreement with and in support of the results obtained by Bunton et al.[2]

Effects of Metal Ions.—In this investigation the pH value of experimental solutions was maintained in the range, 1.72—2.71. Therefore, the acid catalyzed reaction may not be significant under present experimental conditions. The effect of metal ions on the hydrolysis reaction is obviously dependent on the positive charge that these metal ions possess, as illustrated in Table III. Thus, all the divalent metal ions and even the uranyl ion do not cause any significant change in reaction rate. As the mechanism in which metal ions can show any profound effect in the hydrolysis reaction, four cases illustrated in Chart 1 may be considered probable. The four mechanisms proposed are based upon two principal concepts: firstly, that a proton-transfer process plays a vital role in the transition state, and secondly, that the spontaneous hydrolysis mechanism, which results in the formation of a carbonium cation, is not disturbed to a significant extent so that an S_N1 process still holds in the presence of metal ions. Mechanisms 1 and 4 would be favored if the reaction were accelerated in the presence of metal ions. A mechanism similar to the first one involving metal ion attack at the site of the phosphate group was given previously for the hydrolysis of salicyl phosphate and its derivatives.[3-5] In the activated complex of structure IV, the metal ion may act to accelerate the transfer of a phosphate proton to the ester oxygen. This proton-transfer results in the formation of structure IV′, which undergoes degradation to yield the carbonium cation. On the other hand, a proton of a hydrated water in structure VII may be transferred to the ester oxygen by the help of the electron-withdrawing effect of the metal ion, with the C–O bond being subsequently cleaved to give the carbonium ion.

A different situation is expected for mechanisms 2 and 3. The proton-accepting capacity of the ester oxygen would be reduced by the presence of metal ion where the metal ion acts on the ester oxygen as shown in structure V. The coordination of the metal ion to the ether oxygen of the glycoside ring and to the phosphate oxygen, as illustrated in structure VI, acts to reduce the electron-donating power of the ether oxygen on one hand, and to diminish the proton accepting tendency of the ester oxygen on the other. The former effect would be unfavorable for the formation of the carbonium ion and the latter unfavorable for C–O bond cleavage. Therefore, from the above consideration, mechanisms 2 and 3 may be reasonable for the cases where a diminished rate of hydrolysis was observed.

Effects of Metal Chelates.—With the thorium-EDTA chelate system, the hydrolysis rate was found to be comparable with the spontaneous one. The predominant species present in the experimental solutions is the 1:1 chelate species even in the low pH region used in this investigation due to an extremely high stability of the chelate.[10] Since only two coordination sites are left on the nuclear metal of the Th(IV)-EDTA chelate[11] and the thorium ion is well-shielded with the ligand from the substrate species, the steric interaction of the substrate with this thorium chelate may prohibit the formation of the activated complexes mentioned above with the exception of IV. A similar explanation can be given for the effect of the Fe(III)-EDTA chelate system on the hydrolysis reaction. The presence of both Fe(III)-kojate and Th(IV)-Tiron chelate systems resulted in an increased rate of hydrolysis in comparison with the corresponding free metal ions. However, there still remains a negative catalytic effect with these chelates. Under these conditions of hydrogen ion concentration, the 1:1 Fe(III)-kojate[12] and Th(IV)-Tiron[13] chelate systems consist primarily of two components, free metal ion and the 1:1 chelate species. Therefore, these two chelating agents seem to demonstrate their roles in two aspects in the course of hydrolysis:

(1) the reduction of the concentration of the free metal ion, which is the most active negative catalyst;

(2) the reduction of the affinity of metal toward the substrate species through the chelation.

In conclusion, the catalytic hydrolysis reaction of organic phosphates may be explained in terms of the proton-transfer mechanism, whenever the substrate species involve any active protons. When metal ions and metal chelates showed any catalytic effect in this work, they acted to reduce the reaction rate in contrast to the hydrolysis of salicyl phosphate analogs.[3-5] Thus, whether the catalytic effect is positive or negative seems to be controlled by the three factors; ionic forms of a substrate, structure of an alcoholic residue, and hydrolytic reaction mechanism. Some further studies on the roles of metal ions and of metal chelates in the hydrolysis of organic phosphates are under progress in these laboratories.

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