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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, CLARK UNIVERSITY, WORCESTER, MASSACHUSETTS]

Metal Chelates of Adenosinediphosphoric and Adenosinemonophosphoric[1] Acids

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Stability constants of the 1:1 chelates of ADP and of adenosine-3- and 5-phosphoric acids with divalent metal ions, Cu(II), Ni(II), Co(II), Mn(II), Zn(II), Mg(II), Ca(II), Sr(II) and Ba(II) are reported at 25° and 0.1 ionic strength. The stabilities increase in the sequence: Ba < Sr < Ca < Mg < Mn < Co < Zn < Ni < Cu. The first and second hydrolysis constants, and the dimerization constant of the copper (II)-ADP and of the zinc (II)-ADP chelates, are reported.

In an earlier paper[3] the stability constants of some transition metal chelates of adenosinetriphosphate (ATP) have been reported. The present investigation describes similar studies with adenosinediphosphate (ADP), adenosine-3-phosphoric acid and adenosine-5-phosphoric acid.

Metal complexes of adenosine phosphates have been the subject of several investigations.[4–11] The presence of normal and protonic complexes of Ca(II) and Mg(II) were reported by Martell and Schwarzenbach[10] and by Smith and Alberty.[11] The mathematical technique developed in the previous paper[3] is used in the treatment of the data for ADP, and the measurements are extended to metal ions less basic than those of the alkaline earths.

Experimental

The experimental method consisted of potentiometric titration of the monosodium salt of ADP, and titration of adenosine-3 and 5-phosphoric acids, in the absence and presence of the metal ion being investigated. The ionic strength was maintained approximately constant in a 1:1 titration by use of a medium containing 0.1 *M* potassium nitrate and relatively low concentrations of ligand and metal ion. The electrode system was calibrated by direct titration of acetic acid, the observed pH meter reading being compared with the actual hydrogen ion concentration calculated from data tabulated by Harned and Owen.[12] The pH regions below 3.5 and above 10.5 were calibrated by measurements in HCl and KOH solutions, respectively.

Reagents.—Chromatographically pure samples of ADP and adenosine-3- and 5-phosphoric acids, prepared by the Mann Research Laboratory, were employed in this work. For every titration, fresh solid ligand was weighed out to avoid the possibility of hydrolysis which might take place if a stock solution were employed. The metal salt solutions were standardized by titration with the disodium salt of EDTA as described by Schwarzenbach.[13] Carbonate-free KOH was prepared by the method of Schwarzenbach and Biedermann[14] and was standardized by titration with potassium acid phthalate.

Calculations.—The acid dissociation constants, Ka and K_2a, for the monosodium salt of ADP (H_2L^-) and Ka and K_2a of adenosine-3-, and 5-phosphoric acids (H_2L) were calculated by a direct algebraic method. The equilibria involved in the formation of a 1:1 complex of ADP and a divalent metal ion may be expressed as

$$M^{2+} + HL^{2-} \rightleftharpoons MHL \quad K_1 = \frac{[MHL]}{[M^{2+}][HL^{2-}]} \quad (1)$$

$$M^{2+} + L^{3-} \rightleftharpoons ML^- \quad K_2 = \frac{[ML^-]}{[M^{2+}][L^{3-}]} \quad (2)$$

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[3] M. M. Taqui Khan and A. E. Martell, *J. Phys. Chem.*, **66**, 10 (1962).

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[10] A. E. Martell and G. Schwarzenbach, *Helv. Chim. Acta*, **39**, 653 (1956).

[11] R. M. Smith and R. A. Alberty, *J. Am. Chem. Soc.*, **78**, 2376 (1956).

[12] H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," 2nd Ed., Reinhold Publishing Corp., New York, N. Y., 1950, pp. 485 and 578.

[13] G. Schwarzenbach, "Complexometric Titrations," Interscience Publishers, New York, N. Y., 1957, pp. 77 and 82.

[14] G. Schwarzenbach and W. Biedermann, *Helv. Chim. Acta*, **31**, 331 (1948).

Related equilibria may be defined as:

$$M^{2+} + H_2L^- \rightleftarrows MHL + H^+$$

$$K_1' = \frac{[MHL][H^+]}{[M^{2+}][H_2L^-]} \quad (3)$$

$$MHL \rightleftarrows ML^- + H^+ \quad K_2' = \frac{[ML^-][H^+]}{[MHL]} \quad (4)$$

The method of calculating the formation constants for adenosine-3- and 5-phosphoric acids is relatively simple, no protonic complexes being involved in both these cases. The equilibria involved in metal chelate formation in a 1:1 titration of ligand to metal ion may be expressed as

$$M^{2+} + L^{2-} \rightleftarrows ML \quad K_1 = \frac{[ML]}{[M^{2+}][L^{2-}]} \quad (5)$$

The constants K_1 and K_2 were calculated from the potentiometric data with the help of the procedure previously described.[3] Where protonated complexes were encountered, it was not possible to calculate the constants K_1 and K_2 from a single experimental point, and graphical solution[3] was necessary to determine the best values.

Solution Equilibria for Cu(II) and Zn(II)-ADP Complexes.—In the study of the copper(II) and zinc(II) ADP systems, it was found that titration beyond two equivalents of base produces concentration-dependent buffer regions characteristic of polymerization through hydroxo bridges (olation) to give polynuclear complexes. The data were treated mathematically on the basis that four species are present: a normal 1:1 chelate, a monohydroxo complex $M(OH)L\cdot H_2O^{2-}$, a dihydroxo species $M(OH)_2L^{3-}$ and a dimer $[M(OH)L]_2^{4-}$. The corresponding solution equilibria may be represented by the equations

$$ML^{-1} + H_2O \rightleftarrows M(OH)L^{2-} + H^+$$

$$K_{M(OH)L} = \frac{[M(OH)L^{2-}][H^+]}{[ML^{-1}]} \quad (6)$$

$$ML^{-1} + 2H_2O \rightleftarrows M(OH)_2L^{3-} + 2H^+$$

$$K_{M(OH)_2L} = \frac{[M(OH)_2L^{3-}][H^+]^2}{[ML^{1-}]} \quad (7)$$

$$2ML^{1-} + 2H_2O \rightleftarrows [M(OH)L]_2^{4-} + 2H^+$$

$$K_{[M(OH)L]_2} = \frac{[(M(OH)L)_2^{4-}][H^+]^2}{[ML^{1-}]^2} \quad (8)$$

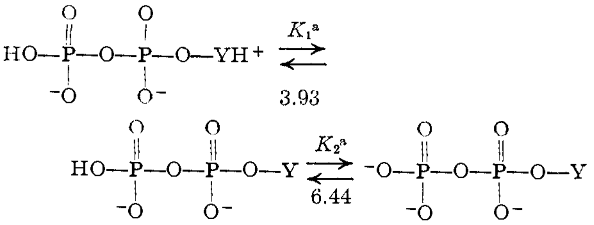
$$2M(OH)L^{2-} \rightleftarrows [M(OH)L]_2^{4-} + 2H_2O$$

$$K_d = \frac{[(M(OH)L)_2^{4-}]}{[M(OH)L^{2-}]^2} \quad (9)$$

The amount of various chelate species present under varying conditions of *p*H and total concentration may be calculated from the above equilibria with the relationships outlined in a previous paper.[3]

Discussion of Results

ADP.—The potentiometric titration of ADP (Fig. 1a) produces two sharp inflections which probably correspond to the separate neutralization reactions



$$HO-\overset{\overset{O}{\|}}{\underset{\underset{^-O}{|}}{P}}-O-\overset{\overset{O}{|}}{\underset{\underset{O^-}{|}}{P}}-O-YH^+ \underset{3.93}{\overset{K_1^a}{\rightleftarrows}}$$

$$HO-\overset{\overset{O}{\|}}{\underset{\underset{^-O}{|}}{P}}-O-\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}-O-Y \underset{6.44}{\overset{K_2^a}{\rightleftarrows}} {}^-O-\overset{\overset{O}{\|}}{\underset{\underset{^-O}{|}}{P}}-O-\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}-O-Y$$

where Y = adenosine residue.

The pK values 3.93 and 6.44 at 25° are close to the corresponding values 3.99 and 6.35 obtained at 20° by Martell and Schwarzenbach.[10] The pK_1 value of ADP is lower than that of ATP (pK_1 = 4.06) and considerably lower than the adenine[15] (pK_1 = 4.24). The lower pK of the $-YH^+$ group in ADP compared to ATP may be considered evidence for binding the proton by an electron donor remote from the phosphate residue (probably to the amino or a ring nitrogen). If the second dissociable proton (K_1a, above) were bound to a phosphate oxygen, the reverse effect would have been observed. The lower pK value of ADP may be due in part to the greater ability of the triphosphate oxygens to accept the negative charge of the dipolar ion, and the possibility of greater intramolecular association with the positive (onium) group by the triphosphate residue than by the diphosphate. The effect is even more pronounced in the adenosinemonophosphoric acids, for which pK_1 is reduced still further.

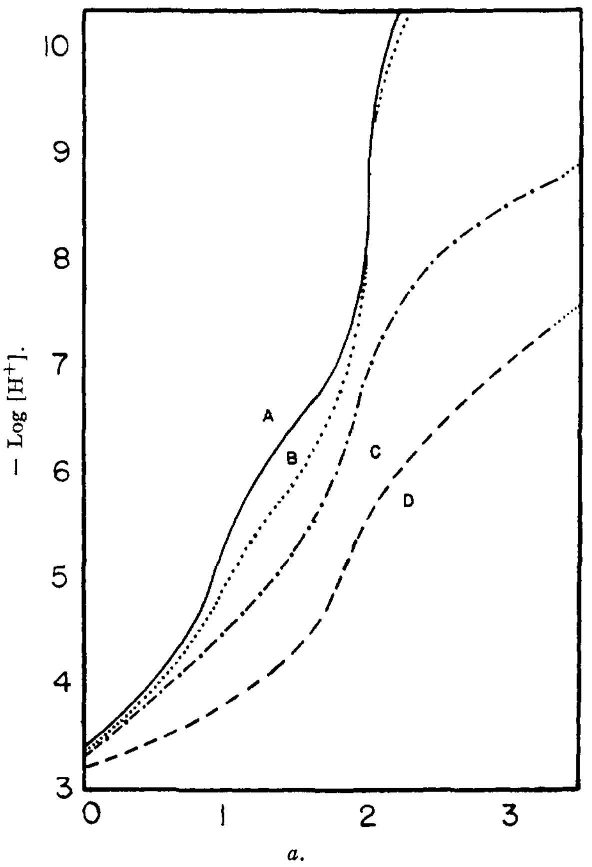


Fig. 1.—Potentiometric titration of Mg(II), Zn(II) and Cu(II) chelates of ADP in 0.1 *M* KNO_3 at 25°, with the following molar ratios of ligand to metal ion: A, ligand alone, 1.927 × 10^{-3} *M*; B, Mg(1:1), 1.928 × 10^{-3} *M*; C, Zn(1:1), 2 × 10^{-3} *M*; D, Cu(1:1), 2 × 10^{-3} *M*; *a* = moles of base per mole of ligand.

The titration curves of adenosine-3- and 5-phosphoric acids (Figs. 2a and 3a) are similar to those of ADP, with two sharp inflections separated by a buffer region. The value of pK_1 of adenosinemonophosphoric acids seems to be influenced by the position of the phosphate group in the ribose ring. The pK_1 of adenosine-3-phosphoric acid (3.63) is lower than that of the 5-phosphoric acid derivative (pK_1 = 3.81). This

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(15) Results obtained in this Laboratory under the same experimental conditions.

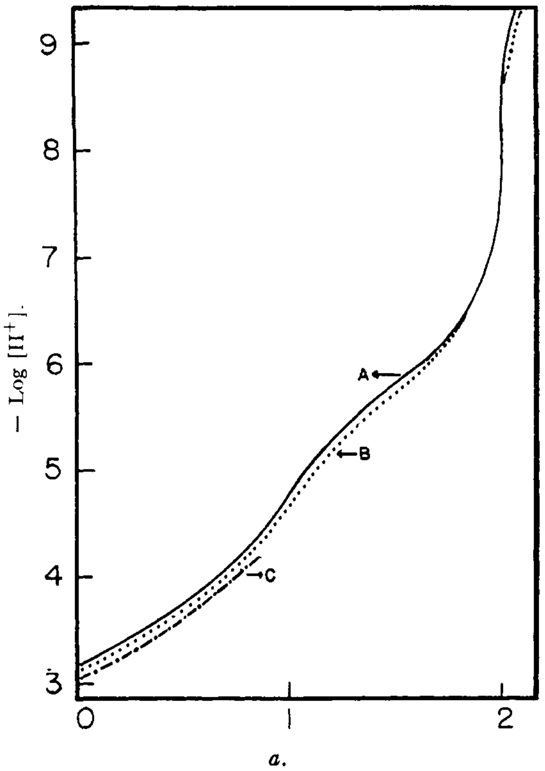


Fig. 2.—Potentiometric titration of Mg(II) and Cu(II) chelates of adenosine-3-phosphoric acid in 0.1 *M* KNO_3 at 25°, with the following molar ratios of ligand to metal ion: A, ligand alone, 3.202×10^{-3} *M*; B, Mg (1:1), 3.202×10^{-3} *M*; C, Cu(1:1), 3.202×10^{-3} *M*, *a* = moles of base per mole of ligand.

difference can hardly be due to a difference in the inductive effects on the phosphate group at the 3- and 5-positions but may be related to the differences in geometric configurations of these groups and hence to differences in proximity to adjacent polar groups.

Potentiometric curves are illustrated in Fig. 1 (B, C and D) for ADP chelates of Mg(II), Cu(II) and Zn(II), respectively, in systems containing a 1:1 ratio of ligand to metal(II) salt. In the case of Ca(II), Sr(II), Ba(II), Mn(II), Co(II) and Ni(II) ions, the results obtained are similar to those shown for Mg(II). In Figs. 2 (B and C) and 3 (B and C), potentiometric curves for Mg(II) and Cu(II) chelates of adenosine-3-phosphoric acid and adenosine-5-phosphoric acids, respectively, are shown. The complexes of Ca(II), Sr(II) and Ba(II) ions give the same results as that shown for Mg(II); and the Mn(II), Co(II), Ni(II) and Zn(II) gave curves nearly superimposable on that of the copper(II) ion, in the case of both of the monacids.

Mg(II), Ca(II), Sr(II), Ba(II), Mn(II), Co(II) and Ni(II) Complexes.—Titration of equimolar amounts of ADP and magnesium(II) nitrate, illustrated in Fig. 1(B), gave an inflection at *a* = 2, corresponding to the formation of a 1:1 chelate. The other ions indicated above gave the same result as did Mg(II), with inflections at *a* = 2. A steep inflection at *a* = 2 was also observed in a 1:1 titration of Mg(II), Ca(II), Sr(II) and Ba(II) with

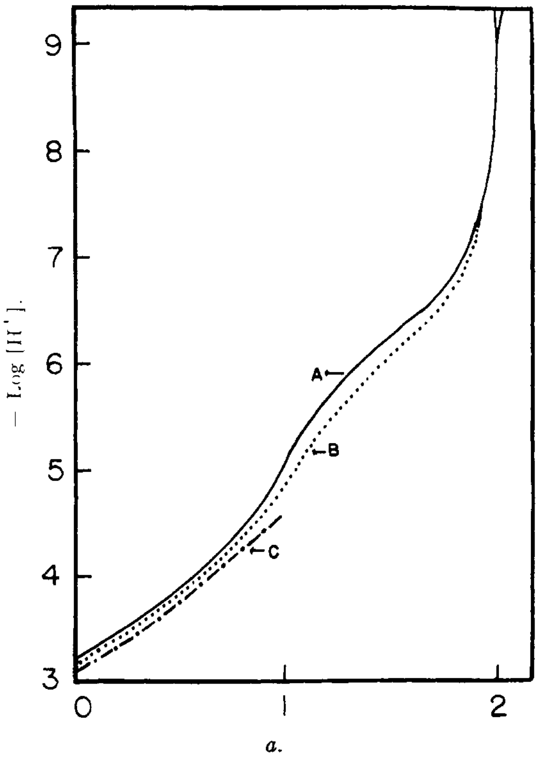


Fig. 3.—Potentiometric titration of Mg(II) and Cu(II) chelates of adenosine-5-phosphoric acid in 0.1 *M* KNO_3 at 25°, with the following ratios of ligand to metal ion: A, ligand alone, 3.040×10^{-3} *M*; B, Mg(1:1), 3.040×10^{-3} *M*; C, Cu(1:1), 3.645×10^{-3} *M*; *a* = moles of base per mole of ligand.

adenosine-3- and 5-phosphoric acids, as is indicated for Mg(II) in Figs. 2(B) and 3(B). A precipitate was invariably obtained in the 1:1 titrations of Cu(II), Zn(II), Ni(II), Co(II) and Mn(II) ions with $a \geqq 1$ for adenosine-3- and 5-phosphoric acids. For these ions, stabilities were calculated in a pH range far below the precipitation point. The stability constants of ADP complexes and those of adenosine-3- and 5-phosphoric acids are given in Table I. The stabilities listed in Table I increase in the order Ba<Sr<Ca<Mg<Mn<Co<Zn<Ni<Cu. The same order is also followed in the case of the acido complexes of ADP. The higher stabilities of ADP chelates, as compared to the monophosphoric acids, are due to the higher basicity and greater number of donor groups of the former ligand. Accordingly, the stabilities increase with the chain length in the order, adenosinemonophosphoric acids < ADP < ATP. As shown in the case of Ca(II) and Mg(II) complexes of ATP,[-3] the sites at which coördination probably occurs are the phosphate chain and not the adenosine residue. This conclusion is supported by the facts that adenosine itself shows little or no interaction with the metal ions studied[10] (*i.e.*, those of Ca(II) and Mg(II)) and also by the considerable increase in stability observed when one goes from monophosphate to diphosphate and from diphosphate to triphosphate. Recently Schwarzenbach,[16] in com-

(16) G. Schwarzenbach and G. Anderegg, *Helv. Chim. Acta.*, **40**, 1229 (1957).

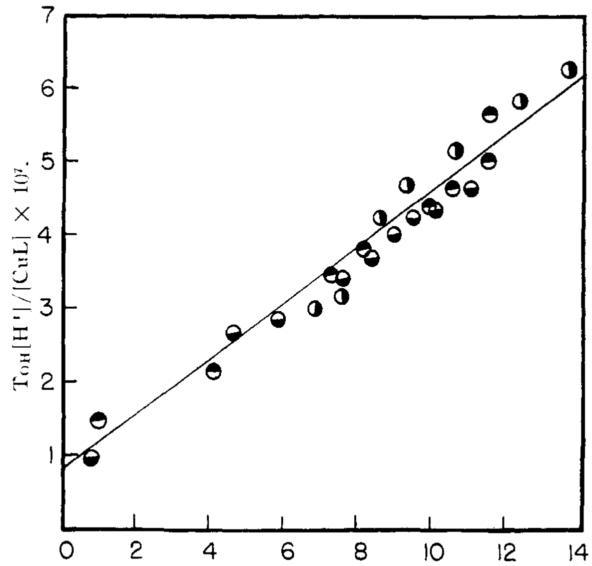


Fig. 4.—Graphical demonstration of dimer of monohydroxocopper(II)-ADP chelate: ◓, 1.000 × 10^{-3} *M*; ◒, 2.000 × 10^{-3} *M*; ◑, 3.000 × 10^{-3} *M*.

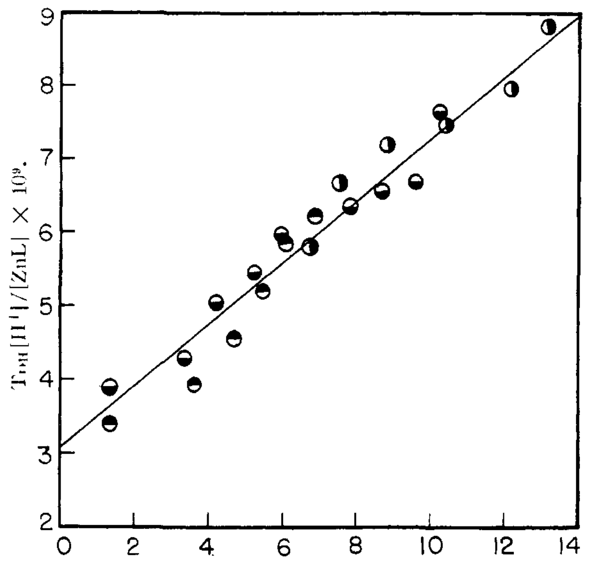


Fig. 5.—Graphical demonstration of dimer of monohydroxozinc(II)-ADP chelate: ◓, 1.009 × 10^{-3} *M*; ◒, 2.000 × 10^{-3} *M*; ◑, 3.000 × 10^{-3} *M*.

paring the similarities in the stabilities of metal complexes of glycerophosphate, fructosephosphate and adenosinemonophosphate, concluded that the metal ion is held only through the phosphate group and that the adenine residue does not participate.

It is interesting that the complexes of adenosine-3-phosphoric acid are less stable than those of the 5-phosphoric acid. This fact correlates with the greater acidity of the 3-phosphoric acid. This difference in basicity toward both metal ions and hydrogen ions must be due to a steric effect involving differences in proximity to the weakly-coördinating hydroxyl groups of the adenosine residue.

TABLE I

EQUILIBRIUM CONSTANTS FOR THE INTERACTION OF ADP AND ADENOSINE-3- AND 5-PHOSPHORIC ACIDS WITH VARIOUS METAL IONS

$t = 25°, \mu = 0.1$

Metal ion	Adenosinediphosphate ($pK_1 = 3.93$, $pK_2 = 6.44$) Log K_1	Log K_2	Adenosine-3-phosphoric acid ($pK_1 = 3.63$, $pK_2 = 5.80$) Log K_{ML}[a]	Adenosine-5-phosphoric acid ($pK_1 = 3.81$) $pK_2 = 6.21$, Log K_{ML}
Ba(II)		2.36 ± 0.03	1.69 ± 0.02	1.73 ± 0.01
Sr(II)		2.54 ± .02	1.71 ± .01	1.79 ± .01
Ca(II)	1.58 ± 0.04	2.86 ± .02	1.80 ± .01	1.85 ± .02
Mg(II)	1.64 ± .02	3.17 ± .01	1.89 ± .01	1.97 ± .01
Co(II)	2.01 ± .02	4.20 ± .01	2.24 ± .01	2.64 ± .02
Mn(II)	1.89 ± .02	4.16 ± .01	2.28 ± .01	2.40 ± .02
Zn(II)	2.04 ± .02	4.28 ± .01	2.60 ± .02	2.72 ± .02
Ni(II)	2.30 ± .02	4.50 ± .02	2.79 ± .01	2.84 ± .01
Cu(II)	2.63 ± .01	5.90 ± .01	2.96 ± .01	3.18 ± .01

[a] K_{ML} is the formation constant of the 1:1 chelate compound.

ADP Chelates of Cu(II) and Zn(II).—The 1:1 titration curves illustrated in Fig. 1 (C and D) show a steep inflection at $a = 2$, corresponding to the formation of a 1:1 chelate compound, followed by an additional concentration-dependent buffer region of the type characteristic of polynuclear metal complexes formation. A precipitate was obtained after $a = 3.5$ for both the Cu(II) and Zn(II) complexes. The monohydroxo and dimerization constants were calculated throughout the range $a = 2$ to $a = 3.3$. A reasonably constant value for the dihydroxo constant could not be calculated. It must be concluded, therefore, that there is no evidence for the formation of dihydroxo derivatives of these complexes, although the lack of constancy may be related to the appearance of the solid phase beyond $a = 3.5$. A summary of the equilibrium constants obtained is given in Table II.

TABLE II

EQUILIBRIUM CONSTANTS FOR Cu(II) AND Zn(II) CHELATES OF ADP

	Log K_1	$pK_{M(OH)L}$	$pK_{[M(OH)L]_2}$	Log K_d
Cu(II)	5.90	7.08	10.73	3.42
Zn(II)	4.28	8.51	13.68	3.34

It is of interest to compare the formation of dimers and hydroxo chelates in the copper(II) and zinc(II)–ADP with the corresponding metal chelates of ATP. In the case of Cu(II), the tendencies of hydrolyze and to form a binuclear hydroxo chelate are greater than those of ATP. This difference in the dimerization tendencies of the Cu(II)–ADP and Cu(II)–ATP chelates correlates with the values of the acid dissociation constants of the ligands. The chelates of the less basic ligand ADP dimerize more than those of the more basic ligand ATP. This may be due to the formation of weaker coördinate bonds by the less basic ligand, in which case the metal ion would have greater affinity for an additional donor group such as a hydroxyl ion. The monohydroxo chelate thus formed would have more affinity for an additional metal ion (or metal complexes of the same kind). Dimer formation thus takes place more readily than in the case of Cu(II) complexes of more basic ligands, which have stronger coordinate bonds and hydrolyze less readily.

PLATE I

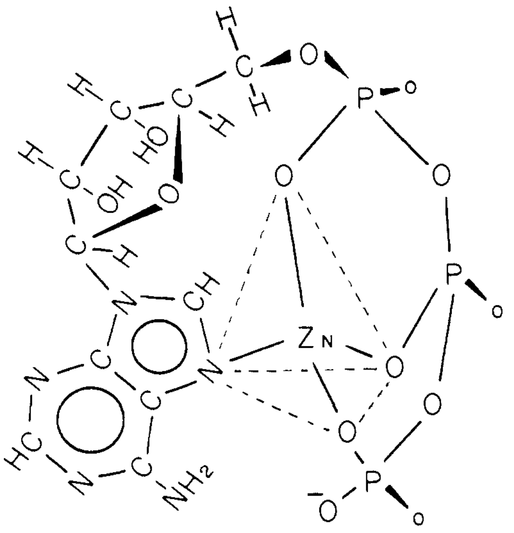
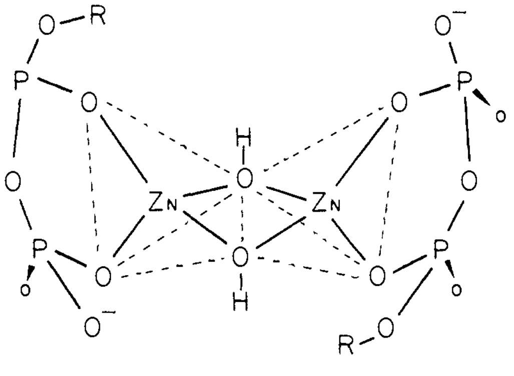


PLATE II



R = ADENOSINE RESIDUE

Similar reasoning would seem to apply to the analogous Zn(II) complexes, although the corresponding Zn(II) complex of ATP does not form a binuclear species. The Zn(II)–ADP chelate, like the Zn(II)–ATP chelate, seems to have enough stability to keep the metal ion from precipitating in the weakly basic pH range, but unlike the latter system, the stability apparently is not enough to prevent hydrolysis and dimerization reactions. This behavior of the zinc(II)–ADP chelate at high pH may be interpreted on the basis of the difference in the donor capacity of ADP and ATP with octahedral and tetrahedral models of the hydrated metal ions. With the tetrahedral zinc(II) ion, ADP and ATP seem to give terdentate and quadridentate structures, respectively, in which the nitrogen at position 7 of the purine ring may take part in metal coördination by folding of the ligand molecule. A model of such a structure for the zinc(II)–ATP chelate is illustrated in Plate I. The additional stability that one would expect from such a quadridentate Zn(II)–ATP chelate correlates with the observed lack of hydrolysis at higher pH.[3]

With respect to the relative dimerization tendencies of Cu(II)–ADP and Zn(II)–ADP chelates, the dimerization constant of Cu(II)–ADP is slightly higher. The small difference may be due to a number of factors, such as the difference in the stereochemistry of the two ions, square planar copper being more favorable for dimer formation than tetrahedral zinc, as well as the fact that Cu(II) generally has stronger coördinating tendencies than does Zn(II). The probable structure of the Zn(II)–ADP chelate dimer is shown in Plate II.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, CLARK UNIVERSITY, WORCESTER, MASSACHUSETTS]

Influence of Metal Ions and of Metal Complexes on the Hydrolysis of Salicyl Phosphate[1]

BY ROBERT HOFSTETTER,[2a] YUKITO MURAKAMI,[2b] GEORGE MONT[2c] AND ARTHUR E. MARTELL[2d]

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The first order hydrolysis of salicyl phosphate was studied at 30.3° at 0.10 *M* ionic strength in the presence of, and in the absence of various salts and metal chelate compounds. The order of increasing catalytic activity for metal salts is Cu(II) < UO_2(VI) < VO(IV) < ZrO(IV), Fe(III), while salts of Ni(II), Co(II), Zn(II) and Cd(II) were found to be inactive. In general it was found that the reaction rates in the presence of metal chelates are considerably lower than the rates for the corresponding aquo metal ions. In the absence of metal ions, the variation of rate of hydrolysis with hydrogen ion concentration shows first order dependence on the individual ionic species of the substrate.

Introduction

Earlier work in these Laboratories on the catalytic effects of metal ions and of metal chelates on solvolysis reactions[3,4] led to studies of the influence of metal compounds on solvolysis of a variety of esters. Since it was found that the hydrolysis of salicyl acetate is not metal-catalyzed, the discovery that salicyl phosphate hydrolysis is metal-catalyzed seemed worthy of detailed investigation. In this paper are reported our studies on the first order hydrolysis of salicyl phosphate under conditions of varying *p*H and in the presence of various metal salts and metal chelate compounds.

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(3) R. C. Courtney, R. L. Gustafson, S. J. Westerback, H. Hyytiainen, S. C. Chaberek and A. E. Martell, *J. Am. Chem. Soc.*, **79**, 3030 (1957).

(4) A. E. Martell, R. L. Gustafson and S. C. Chaberek, 'Metal Chelate Compounds in Homogeneous Aqueous Catalysis." in Advances in Catalysis and Related Subjects, **9** (1957).