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The Apparent Stability Constants of Ionic Complexes of Various Adenosine Phosphates with Divalent Cations[1,2]

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Equations have been derived for the calculation from *p*H measurements of apparent stability constants of complexes for cases in which more than one complex may be formed. The formation of complexes of adenosine-5-mono, di and triphosphate, orthophosphate and creatine phosphate with Ca^{++}, Mg^{++}, Sr^{++} and Mn^{++} has been studied at 0.2 ionic strength and 25°. The stability constants of the complexes were calculated on the basis of the assumption that $(CH_3)_4N^+$, $(C_2H_5)_4N^+$ and $(n\text{-}C_3H_7)_4N^+$ do not form complexes with the phosphate anions. Since the highest pK_a' values were obtained in solutions containing $(n\text{-}C_3H_7)_4N^+$, it is considered that the most accurate values of the stability constants are obtained on the basis of the assumption that $(n\text{-}C_3H_7)_4N^+$ ions do not form complexes. The low solubility of the $(n\text{-}C_4H_9)_4N^+$ halides did not permit the extension of the data to larger tetraalkylammonium ions. Only a single complex is detected in the case of orthophosphate, adenosine monophosphate[4] and creatine phosphate while in the case of adenosine di- and triphosphate two complexes are important, and there is indication of a third. The relative values of the stability constants are in the order expected from electrostatic theory; $ATP^{-4} > ADP^{-3} > P^{-2}$, AMP^{-2} and $ATP^{-3} > ADP^{-2}$ and $Mg^{++} > Ca^{++} > Sr^{++}$. Creatine phosphate forms weaker complexes than any of the other phosphate compounds, and Mn^{++} forms complexes which are considerably more stable than those containing the alkaline earth cations. The ionization constants of the complexes of ATP and ADP with Ca^{++}, Mg^{++}, Sr^{++} and Mn^{++} have been calculated.

Introduction

Many reactions of biochemical importance involve organic phosphate compounds, and it is characteristic of these reactions that their equilibria and kinetics are markedly affected by the *p*H and metal ion concentration. In order to understand such effects, it is necessary to have knowledge of the ionization constants of the phosphate compounds and of the stability constants of complexes which are formed. The variation with *p*H of the free energy of hydrolysis of some organic phosphate compounds has been calculated by Dixon,[5] and Hill and Morales[6] have discussed the magnitude of the free energy of hydrolysis of a number of compounds and the effect of *p*H.

The adenosine phosphates AMP, ADP and ATP[4] have 1, 2 and 3 strongly ionized groups, respectively. The apparent ionization constants in 0.15 *M* sodium chloride for the amino and the last phosphate ionizations of these compounds were determined by Alberty, Smith and Bock[7] at 25 and 38°. These apparent ionization constants were used to calculate the *p*H variation of the apparent free energy and the number of equivalents of acid produced or consumed in the reactions ATP = ADP + P, ADP = AMP + P and AMP = A + P. Melchior[8] showed that lower values of the apparent ionization constants are obtained in solutions containing tetraalkylammonium halides rather than alkali halides. He showed that this indicated the formation of complexes of Na^+ and K^+ with ATP and ADP and determined the stability constants for these complexes. This work has been extended in the preceding article in this series.[9]

The effects of Mg^{++} and Ca^{++} on many biochemical reactions have been investigated. It is found that the number of equivalents of acid produced, the apparent equilibrium constant for the reaction and the kinetics may be markedly changed. These various types of effects are illustrated by the studies of myosin ATPase by Green and Mommaerts[10] and of ATP-creatine transphosphorylase by Kuby, Noda and Lardy.[11,12]

The stability of the complex formed between Mg^{++} and $HPO_4^{=}$ has been studied by a number of workers whose results will be summarized in the Discussion section. Evidence for the formation of a complex with ATP, other than that given by the effect of Ca^{++} and Mg^{++} on the equilibria and kinetics of chemical reactions, has been given by Hers[13] who has reported that the titration curve for ATP is displaced to lower *p*H values by the addition of magnesium chloride and by Spicer[14] who has measured the quantity of acid produced when calcium or magnesium salts were added to solutions of ATP. Burton and Krebs[15] have made preliminary measurements of the stability constants for the magnesium complexes of ATP and ADP and used these values to estimate the effect of magnesium concentration on the free energy of hydrolysis of ATP.

Treatment of Data.—The pK_a' value of a weak acid HA is defined by

$$pH = pK_a + \log\left[\gamma_{A^-}/\gamma_{HA}\right] + \log\left[a/(1-a)\right] = pK_a' + \log\left[a/(1-a)\right] \quad (1)$$

where a is the fraction in the conjugate basic form, and γ_{A^-} and γ_{HA} are the activity coefficients for the conjugate basic and acidic forms, respectively. The apparent ionization constant K_a' may be considered to be equal to the product of the thermody-

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(2) This paper is based upon a dissertation presented by Rex M. Smith to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1955.

(3) Pure Oil Fellow, 1953–1955.

(4) Adenosine mono-, di- and triphosphate are represented by AMP, ADP and ATP, respectively, orthophosphate by P and creatine phosphate by CrP.

(5) M. Dixon, "Multi-enzyme Systems," Cambridge University Press, 1949.

(6) T. L. Hill and M. F. Morales, THIS JOURNAL, **73**, 1656 (1951).

(7) R. A. Alberty, R. M. Smith and R. M. Bock, *J. Biol. Chem.*, **193**, 425 (1951).

(8) N. C. Melchior, *ibid.*, **208**, 615 (1954).

(9) R. M. Smith and R. A Alberty, *J. Phys. Chem.*, **60**, 180 (1956).

(10) I. Green and W. F. H. M. Mommaerts, *J. Biol. Chem.*, **202**, 541 (1953).

(11) S. Kuby, L. Noda and H. A. Lardy, *ibid.*, **210**, 65 (1954).

(12) L. Noda, S. Kuby and H. A. Lardy, *ibid.*, **210**, 83 (1954).

(13) H. G. Hers, *Biochim. Biophys. Acta*, **8**, 424 (1952).

(14) S. S. Spicer, *J. Biol. Chem.*, **199**, 301 (1952).

(15) K. Burton and H. A. Krebs, *Biochem. J.*, **55**, 94 (1953).

namic dissociation constant K_a and the ratio of the activity coefficients for the acidic and conjugate basic forms of the weak acid. As expected from the Debye–Hückel theory[16] the values of pK'_a for phosphate compounds decrease as the ionic strength increases in dilute solution. For example, pK_2 for orthophosphate at 25° is 7.20,[17] while the pK'_2 values at 0.10, 0.15 and 0.20 ionic strength contributed by $(CH_3)_4NCl$ are 6.96 ± 0.02, 6.90 ± 0.02 and 6.86 ± 0.02, respectively.

It has been shown earlier[9] that different pK'_a values are obtained when orthophosphate and the adenosine phosphates are titrated in solutions of LiCl, NaCl, KCl and $(CH_3)_4NCl$. The fact that the highest pK'_a values are obtained with $(CH_3)_4NCl$, in each case indicates that $(CH_3)_4N^+$ is bound to a smaller extent than the other cations. Upon testing $(C_2H_5)_4N^+$ and $(n\text{-}C_3H_7)_4N^+$ it was found that the same pK'_a values were obtained for orthophosphate, AMP and ADP in the presence of these cations and that there was a difference in pK'_a values of only 0.04 for ATP. Since an asymptote seemed to have been closely approximated, the following calculations are based upon the assumption that the pK'_a values obtained with solutions containing $(n\text{-}C_3H_7)_4N^+$ are characteristic of the weak acid in the absence of complexing. The $(n\text{-}C_4H_9)_4N^+$ halides are not soluble to the extent of 0.2 N at 25°.

The assumption that activity coefficients are constant at constant ionic strength when a singly charged cation is replaced by a doubly charged one is fundamental to the method used here. While this is only approximately true,[18] the effects of complexing are quite large in the cases discussed here.

In the following derivation provision will be made for the formation of four complexes. A derivation has been given earlier[9] for the case that a single complex is formed between the more basic phosphate anion and a metal ion. The apparent stability constants are defined as follows where the parentheses represent molar concentrations, A^{-z} represents the phosphate anion with charge $-z$ and M^{+2} the divalent metal ion.

$$K'_1 = (MA^{2-z})/(M^{+2})(A^{-z}) \tag{2}$$

$$K'_2 = (MHA^{3-z})/(M^{+2})(HA^{1-z}) = K'_a(MHA^{3-z})/(M^{+2})(A^{-z})(H^+)\gamma_{H^+} \tag{3}$$

$$K'_3 = (M_2A^{4-z})/(M^{+2})(MA^{2-z}) = (M_2A^{4-z})/K'_1(M^{+2})^2(A^{-z}) \tag{4}$$

$$K'_4 = (MA_2^{2-2z})/(MA^{2-z})(A^{-z}) = (MA_2^{2-2z})/(K'_1(M^{+2})(A^{-z})^2 \tag{5}$$

It is assumed in the derivation that the K' values defined above are constant at constant ionic strength. This assumption, which implies that the activity coefficients of all the ions are constant at constant ionic strength, may be considerably in error at the relatively high ionic strength used in this work. In view of the relatively high charges on the ATP^{-4}, ATP^{-3}, ADP^{-3}, etc., anions, specific ionic interactions with the divalent cations other than complex formation cannot be discounted. However, it will be seen that the data are satisfactorily represented by assuming that the specific ionic interactions are due to various complexes.

The pH of the phosphate solution containing cations which do not form complexes with the phosphate anions is given by equation 1, and this pH will be referred to as pH_1. For convenience let antilog $(pK'_a - pH_1) = y$ so that

$$y = (H^+)_1\gamma_{H^+}/K'_a = (1 - a)/a \tag{6}$$

where it is assumed that the measured pH is $-\log (H^+)\gamma_{H^+}$. The problems in the measurement of $(H^+)\gamma_{H^+}$ have been discussed by Bates.[19]

If $K'_1 > K'_2$, the pH will be lowered when metal ions which can form complexes are added. At constant ionic strength the pH of the phosphate solution containing metal ions is given by the equation

$$pH_2 = pK'_a + \log\left[\frac{a(A)_T - (MA) - (M_2A) - 2(MA_2) + \delta(H^+)}{(1 - a)(A)_T - (MHA) - \delta(H^+)}\right] \tag{7}$$

where $(A)_T$ is the total concentration of weak acid and $\delta(H^+)$ is the change in hydrogen ion concentration resulting from the shift in the dissociation equilibrium caused by the addition of M^{+2}. Since the K'_a values of the terminal phosphate groups of the adenosine phosphates and the K'_2 value for orthophosphate are of the order of 10^{-7} and $(A)_T$ is about 10^{-3} M, the $\delta(H^+)$ term is negligible for a shift in pH of as much as 2 units around pH 7. For larger shifts in (H^+), the $\delta(H^+)$ term must be considered. In the case of creatine phosphate the $\delta(H^+)$ term is not negligible because the secondary phosphate ionization occurs at lower pH values. Assuming the activity coefficient of hydrogen ion to have a value of unity at constant ionic strength, $\delta(H^+)$ was calculated from the change in pH. For convenience $\delta(H^+)$ will be abbreviated to δ in the remainder of the derivation.

Let antilog $(pK'_a - pH_2) = x$ so that

$$x = (H^+)_2\gamma_{H^+}/K'_a \tag{8}$$

where the activity coefficient is assumed to have the same value as in equation (6) because the ionic strength is the same. The total concentration $(A)_T$ of weak acid constituent in solutions containing metal ions is given by

$$(A)_T = (A) + (HA) + (MA) + (MHA) + (M_2A) + 2(MA_2) \tag{9}$$

If equations 8 and 9 are substituted into equation 7, the result is

$$\frac{1}{x} = \frac{a(A) + a(HA) - (1 - a)(MA) + a(MHA) - (1 - a)(M_2A) - 2(1 - a)(MA_2) + \delta}{(1 - a)(A) + (1 - a)(HA) + (1 - a)(MA) - a\,(MHA) + (1 - a)(M_2A) + 2(1 - a)(MA_2) - \delta} \tag{10}$$

Then if the terms in the right-hand member of equation 10 are divided through by (A) and the equations defining the apparent stability constants of the complexes and the apparent equilibrium constant for the acid dissociation are introduced into equation 10, the result is

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(16) P. Debye and E. Hückel, *Phys. Z.*, **24**, 185 (1923).

(17) R. G. Bates, *J. Research Natl. Bur. Standards*, **39**, 411 (1947).

(18) H. S. Harned and R. Gary, THIS JOURNAL, **76**, 5924 (1954); **77**, 1994, 4695 (1955).

(19) R. G. Bates, "Electrometric pH Determinations," John Wiley and Sons, Inc., New York, N. Y., 1954.

$$x = \frac{(1 - a) + (1 - a)(\mathrm{H}^+)_2\gamma_{\mathrm{H}^+}/K_a' + (1 - a)(\mathrm{M})K_1' - a(\mathrm{H}^+)_2\gamma_{\mathrm{H}^+}(\mathrm{M})K_2'/K_a' + (1 - a)(\mathrm{M})^2K_1'K_3' + 2(1 - a)(\mathrm{M})(\mathrm{A})K_1'K_4' - \delta/(\mathrm{A})}{a + a(\mathrm{H}^+)_2\gamma_{\mathrm{H}^+}/K_a' - (1 - a)(\mathrm{M})K_1' + a(\mathrm{H}^+)_2\gamma_{\mathrm{H}^+}(\mathrm{M})K_2'/K_a' - (1 - a)(\mathrm{M})^2K_1'K_3' - 2(1 - a)(\mathrm{M})(\mathrm{A})K_1'K_1' + \delta/(\mathrm{A})} \qquad (11)$$

If the terms in the right-hand member of equation 11 are divided through by $(1 - a)$ and equation 8 and $y = (1 - a)/a$ are introduced, equation 11 becomes

$$x = \frac{1 + x + K_1'(\mathrm{M}) - xK_2'(\mathrm{M})/y + K_1'K_3'(\mathrm{M})^2 + 2K_1'K_4'(\mathrm{M})(\mathrm{A}) - \delta(y + 1)/y(\mathrm{A})}{(1 + x)/y - K_1'(\mathrm{M}) + xK_2'(\mathrm{M})/y - K_1'K_3'(\mathrm{M})^2 - 2K_1'K_4'(\mathrm{M})(\mathrm{A}) + \delta(y + 1)/y(\mathrm{A})} \qquad (12)$$

Equation 12 may be multiplied out and the terms grouped to give

$$x(1 + x)/y - (1 + x) = (1 + x)K_1'(\mathrm{M}) - (1 + x)\,xK_2'(\mathrm{M})/y + (1 + x)K_1'K_3'(\mathrm{M})^2 + 2(1 + x)K_1'K_4'(\mathrm{M})(\mathrm{A}) - (1 + x)(1 + y)\delta/y(\mathrm{A}) \qquad (13)$$

cancelling out the quantity $(1 + x)$ from each term and dividing through by (M) gives

$$\frac{x - y}{(\mathrm{M})y} = K_1' - xK_2'/y + K_1'K_3'(\mathrm{M}) + 2K_1'K_4'(\mathrm{A}) - \delta(y + 1)/y(\mathrm{A})(\mathrm{M}) \qquad (14)$$

Equation 14 is the equation used to calculate the apparent stability constants from *p*H measurements. In the experiments to be reported the concentration of metal ion is much greater than that of the weak acid, so that (M) in equation 14 may be taken to be equal to the total metal ion concentration. If x and y values are determined at a sufficient number of metal ion concentrations the various stability constants may be calculated.

Experimental

The *p*H measurements were made with a glass electrode and a saturated calomel electrode. The apparatus and techniques have been described in an earlier publication.[9] The y values were calculated from *p*H measurements on solutions containing only tetraalkylammonium cations, and the x values were calculated from *p*H measurements on solutions in which part or all of the ionic strength was contributed by a 2-1 electrolyte.

For the titrations of creatine phosphate and for the titrations of the adenosine phosphates in the presence of salts of divalent metal ions, the *p*H is sufficiently low so that account must be taken of the concentration of hydrogen ions. Since the ionic strength and volume of reagent added were kept relatively small, the correction of Taque[20] and Parke and Davies[21] was found to be adequate. In this method an aqueous blank of the same ionic strength and volume is titrated, and the amount of acid or base necessary to titrate to the same *p*H as the sample solution is subtracted from the amount of titrant added to the sample solution.

Results

The stability constants were first calculated on the basis of the assumption that $(CH_3)_4N^+$ is not bound. It was found that for orthophosphate and AMP the values of $(x - y)/(M^{+2})y$ were independent of the percentage of the ionic strength replaced by $CaCl_2$ and $MgCl_2$. The fact that a constant value is obtained indicates that a single complex is formed and thus only the first term in equation 14 would be involved. On the basis of the assumption that $(CH_3)_4N^+$ is not bound, these values would be interpreted as the stability constant K_1' of the complex of the divalent metal ion with the more basic form of the phosphate compound.

At 25° and a total ionic strength of 0.2 (with from 25 to 100% of the ionic strength due to MCl_2) the following average values are obtained for the stability constants K_1' (in M^{-1}) for the complexes: CaP, 43 ± 4; MgP, 71 ± 5; CaAMP, 25 ± 2; MgAMP, 34 ± 3. The indicated errors are maximum errors calculated on the assumption that individual *p*H measurements were reproducible to ±0.01 with the Cambridge research model *p*H meter. The quantities $(x - y)/y$ and x/y depend only on the difference $(pH_1 - pH_2)$. Thus any errors in pK_a' values or errors in standardizing the *p*H meter do not affect the calculation of the apparent stability constants if the last term in equation 14 is negligible. The values of the apparent stability constants are dependent upon the ionic strength. As is expected from the Debye–Hückel[16] theory, the values of the apparent stability constants decrease with increasing ionic strength. This is illustrated by the stability constants for $CaHPO_4$ at 0.10, 0.15 and 0.20 ionic strengths at 25° which are 67 ± 8, 50 ± 5 and 43 ± 3 M^{-1} based on the assumption that $(CH_3)_4N^+$ is not bound by $HPO_4^{=}$.

In the case of ADP and ATP experiments with $(CH_3)_4N^+$ salts yield $(x - y)/(M^{+2})y$ values which show a definite trend so that more than one term in equation 14 must be considered. Assuming that complexes are formed with both the more basic anion and the conjugate acid, the values of K_1' and K_2' may be calculated using equation 14. The values of these constants calculated from the data at 50 and 100% replacement of the ionic strength by $CaCl_2$ and $MgCl_2$ represent the data within the experimental error. At 25° and 0.2 ionic strength the following values are obtained for the stability constants (in M^{-1}) for the complexes: CaADP, 16 ± 5; $CaADP^{-1}$, 450 ± 70; $CaATP^{-1}$, 12 ± 5; $CaATP^{-2}$, 1240 ± 180; MgADP, 12 ± 4; $MgADP^{-1}$, 1220 ± 170; $MgATP^{-1}$, 14 ± 5; $MgATP^{-2}$, 2800 ± 400.

As described in an earlier article,[9] there is some binding of $(CH_3)_4N^+$ ions by the phosphate anions because the pK_a' values obtained for solutions of $(C_2H_5)_4N^+$ and $(n\text{-}C_3H_7)_4N^+$ salts are somewhat higher. Since the highest values of pK_a' for ATP were obtained with $(n\text{-}C_3H_7)_4N^+$, it is assumed that the best values of the stability constants are those obtained from the data in Tables I and II on the basis of the assumption that $(n\text{-}C_3H_7)_4N^+$ is not bound. The values of these stability constants are summarized in Table III. The existence of only one complex with P and AMP is indicated by the data of Table I, but the data for ADP and ATP indicate the existence of complexes with both the basic and conjugate acid forms.

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[20] E. L. Taque, THIS JOURNAL, **42**, 173 (1920).

[21] T. V. Parke and W. W. Davies, *Anal. Chem.*, **26**, 642 (1954).

TABLE I

VALUES OF $(x - y)/(\mathrm{M}^{+2})y$ (IN M^{-1}) AT 25° AND 0.2 IONIC STRENGTH ASSUMING $(n\text{-C}_3\text{H}_7)_4\text{N}^+$ IS NOT BOUND

Weak acid	P				AMP			
Salt % Ionic strength[a]	$SrCl_2$	$CaCl_2$	$MgCl_2$	$MnCl_2$	$SrCl_2$	$CaCl_2$	$MgCl_2$	$MnCl_2$
12.5		50 ± 6	76 ± 8	398 ± 26				
25		50 ± 4	79 ± 5	395 ± 22		27 ± 4	42 ± 4	176 ± 13
50	34 ± 3	51 ± 3	80 ± 4	353 ± 17		27 ± 3	42 ± 3	150 ± 11
75	34 ± 2	50 ± 2	72 ± 3			27 ± 2	39 ± 3	
100	31 ± 2	49 ± 2	74 ± 3		21 ± 2	26 ± 2	38 ± 2	141 ± 9
Average	33 ± 2	50 ± 2	76 ± 3	382 ± 29	21 ± 2	27 ± 2	40 ± 2	156 ± 20

[a] Percentage of the total ionic strength of 0.2 which is contributed by the 1–2 electrolyte.

TABLE II

VALUES OF $(x - y)(\mathrm{M}^{+2})y$ (IN M^{-1}) AT 25° AND 0.2 IONIC STRENGTH ASSUMING $(n\text{-C}_3\text{H}_7)_4\text{N}^+$ IS NOT BOUND

Weak acid	ADP				ATP			
Salt % Ionic strength	$SrCl_2$	$CaCl_2$	$MgCl_2$	$MnCl_2$	$SrCl_2$	$CaCl_2$	$MgCl_2$	$MnCl_2$
25	214 ± 13	395 ± 22	661 ± 34	2270 ± 110				
50	155 ± 9	291 ± 16	491 ± 25	1619 ± 77	529 ± 26	816 ± 39	1440 ± 68	4300 ± 200
75					428 ± 20	642 ± 31	1185 ± 57	3220 ± 150
100	119 ± 6	192 ± 8	337 ± 16	1123 ± 53	353 ± 18	517 ± 25	954 ± 45	2780 ± 130

TABLE III

APPARENT STABILITY CONSTANTS (IN M^{-1}) AT 25° AND 0.2 IONIC STRENGTH ASSUMING $(n\text{-C}_3\text{H}_7)_4\text{N}^+$ IS NOT BOUND

	Sr^{++}	Ca^{++}	Mg^{++}	Mn^{++}
CrP^{-2}	12 ± 2	14 ± 2	20 ± 2	110 ± 30
AMP^{-2}	21 ± 2	27 ± 2	49 ± 2	156 ± 20
P^{-2}	33 ± 2	50 ± 2	76 ± 3	382 ± 29
ADP^{-2}	22 ± 4	33 ± 5	28 ± 4	31 ± 4
ADP^{-3}	314 ± 33	642 ± 58	1002 ± 87	3480 ± 280
ATP^{-3}	30 ± 7	41 ± 9	31 ± 7	37 ± 8
ATP^{-4}	1080 ± 160	1970 ± 280	2970 ± 230	9600 ± 1300
(P^{-2} at 0°	18 ± 2	23 ± 2	32 ± 2	187 ± 9)

It will be noted that Table III also gives the stability constants for the complexes with creatine phosphate. The pK'_a value for the final phosphate ionization as determined in 0.2 ionic strength tetra-n-propyl and tetraethylammonium halides at 25° is 4.52 ± 0.03, which is quite close to earlier values.[22,23]

Discussion

The value of the apparent stability constant of the $MgHPO_4$ complex at 0.2 ionic strength given in Tables I and III is appreciably higher than that obtained by earlier workers on the basis of the assumption that Na^+ and K^+ are not bound by $HPO_4^=$. Greenwald, Redish and Kibrick[24] obtained a value of 35 M^{-1} at 0.2 ionic strength by a titration method, and Trevelyan, Mann and Harrison[25] obtained 33 M^{-1} by a similar method. Tabor and Hastings[26] obtained a value of 27 M^{-1} by a conductance method in which the bulk of the ionic strength was made up of sodium chloride.

Clarke, Cusworth and Datta[27] have studied the dissociation of magnesium salts of phosphoric acid, glucose-1-phosphoric acid and glycerol-2-phosphoric acid by use of cells without liquid junction. The values for the thermodynamic dissociation constants of these salts which they obtain at 25° are 520, 310 and 320 M^{-1}, respectively. As expected the thermodynamic value of the stability constant is much higher than the apparent value at 0.20 ionic strength (76 M^{-1} for MgP). It does not appear practical to make a direct comparison between the thermodynamic and apparent values because of lack of adequate data on activity coefficients.

The values of the stability constants for the ATP and ADP complexes given in Table III are quite different from previous values given in the literature. Burton and Krebs[15] obtained apparent stability constants of 830 and 370 M^{-1} for MgATP and MgADP at 0.2 ionic strength on the basis of the assumption that a single complex is formed. DiStefano and Neuman[28] obtained apparent stability constants for CaATP and CaADP of 1.2×10^4 M^{-1} and 5.5×10^3 M^{-1} at 0.1 ionic strength by use of an ion exchange method.

The fact that the stability constants for complexes containing Mn^{++} are so much greater than those for complexes containing the alkaline earth cations is undoubtedly connected with the possibility of forming a bond with some covalent character. Cohn and Townsend[29] using the paramagnetic resonance absorption method set a lower limit for the stability constants of the complexes of ADP and ATP with Mn^{++} at 5×10^4 M^{-1}. This estimate is based upon the failure to observe the lines due to Mn^{++} for a solution containing known total concentrations of Mn^{++} and ATP or ADP. The ionic strength of the medium and the nature of additional electrolyte present was not specified. The values obtained by the titration method and presented in Table III are believed to be more accurate values of the stability constants.

As in the case of the alkali metal ions[9] the relative magnitudes of the stability constants of the complexes with the alkaline earth cations are in the opposite order from that which would be expected from the activity coefficients of the corresponding

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(22) O. Meyerhoff and K. Lohmann, *Biochem. Z.*, **196**, 49 (1928).

(23) W. D. Kumler and J. J. Eiler, THIS JOURNAL, **65**, 2355 (1943).

(24) I. Greenwald, J. Redish and A. C. Kibrick, *J. Biol. Chem.*, **135**, 65 (1940).

(25) W. E. Trevelyan, P. F. E. Mann and J. S. Harrison, *Arch. Biochem. Biophys.*, **39**, 440 (1952).

(26) H. Tabor and A. B. Hastings, *J. Biol. Chem.*, **148**, 627 (1943).

(27) H. B. Clarke, D. C. Cusworth and S. P. Datta, *Biochem. J.*, **58**, 146 (1954).

(28) V. DiStefano and W. F. Neuman, *J. Biol. Chem.*, **200**, 759 (1953).

(29) M. Cohn and J. Townsend, *Nature*, **163**, 1090 (1954).

halides.[30] The fact that the magnitudes of the stability constants correlate with the crystallographic radii of the metal ions rather than their hydrated radii may not mean that the unhydrated ions are actually bound in the complexes. The experimental results are explicable in terms of the concept of localized hydrolysis discussed by Robinson and Harned.[31] If a cation M^+ polarizes the solvent to a sufficient extent a kind of ion pair $M^+ \ldots OH^- - H^+ \ldots A^-$ may be formed with an anion A^- which is a proton acceptor. According to this concept the stability of the complex would be greater for smaller M^+ and stronger bases A^-. This latter point is borne out by the fact that the binding of the various alkali metal cations[9] and of the various alkaline earth cations by P^{-2} and AMP^{-2} correlates with the acid strengths of the corresponding acids. Similarly the weak binding by CrP^{-3} correlates with the low pK_a' of the phosphate group.

The relative values of the stability constants for the more basic forms of the anions are in the order to be expected from the magnitude of the charge: $ATP^{-4} > ADP^{-3} > P^{-2}, AMP^{-2}$. It is of interest to note that the complexes of ATP^{-3} are much less stable than the complexes of ADP^{-3}. This probably results from the greater localization of charge in ADP^{-3}.

The values of the apparent stability constants for the orthophosphate complexes at 0° are also given in Table III. If it is assumed that ΔH for the formation of these complexes is constant from 0 to 25°, the values of ΔH and ΔS may be calculated. The values of ΔH are each about +5 kcal. mole^{-1}, and the values of ΔS are each about 25 kcal. deg.$^{-1}$ mole^{-1}. Thus these complexes are formed because of the favorable entropy change which reflects the relatively greater freedom of the solvent molecules in the presence of the products than in the presence of the reactants.[32]

The fact that the stability constants for the complexes of the divalent cations with ADP^{-2} and ATP^{-3} and their conjugate basic forms have been determined makes it possible to calculate the pK_a' values for the acidic forms of the various complex ions. The values for the apparent dissociation constants of the complexes are given by the expression $K_a'K_1'/K_2'$. The calculated pK_a' values are summarized in Table IV.

TABLE IV

pK_a' VALUES AT 0.2 IONIC STRENGTH AND 25°

ADP^{-2}	6.68	ATP^{-3}	6.95
ADPSr	5.5	$ATPSr^{-1}$	5.4
ADPCa	5.4	$ATPCa^{-1}$	5.3
ADPMg	5.1	$ATPMg^{-1}$	5.0
ADPMn	4.6	$ATPMn^{-1}$	4.5

Whereas the uncertainties in the pK_a' values for ADP^{-2} and ATP^{-3} are ±0.02, the uncertainties in the pK_a' values for the complexes are ±0.1 for the ADP complexes and ±0.2 for the ATP complexes. It is of interest to note that for both ADP and ATP the acid strengths of the complexes increase in the order of increasing affinity of the more basic form of the anion for the metal ion, that is, $Sr^{++} < Ca^{++} < Mg^{++} < Mn^{++}$.

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A Centrifugal Light Scattering Cell for the Ultraclarification of Liquids

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A glass light scattering cell capable of being centrifuged at high speeds (centrifugal cell) is described. The use of this cell makes it possible to clarify liquids to a very high degree and then, without transfer, to make scattering measurements upon them. In addition, the cell has a small volume (6 ml.) and exhibits extremely low stray light down to 26° from the incident beam. It is found that this method of clarifying liquids is superior to all other methods tried, such as high speed centrifugation followed by transfer to a light scattering cell, filtration through plastic filters or filtration through ultrafine sintered glass.

Introduction

Although light scattering is widely used for the determination of size, shape and activity coefficient of macromolecules in solution,[2] the problem of clarification of liquids has continued to present serious obstacles to reliable and accurate measurements. The variety of methods which have been applied to clarifying liquids utilize separations based upon distillation, filtration or centrifugation. Filtration procedures include the use of bacterial filters such as the Seitz asbestos type after impregnation with bakelite,[3] "ultrafine" sintered glass filters,[2] plastic filters of the Millipore type[4] and graded cellulose nitrate membranes.[5] Centrifuga-

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