ADENOSINE AND THE ADENINE NUCLEOTIDES. IONIZATION, METAL COMPLEX FORMATION, AND CONFORMATION IN SOLUTION

ROGER PHILLIPS, S.J.

Fairfield University, Fairfield, Connecticut

Received April 4, 1966

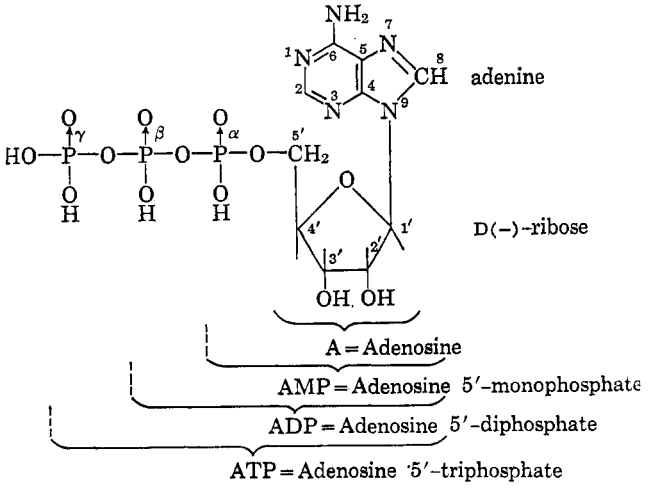
CONTENTS

I. INTRODUCTION

Some of the most biologically important low molecular weight compounds are the adenine nucleotides. Since ATP (adenosine 5′-triphosphate) was isolated in 1929, it has been found that it participates in almost countless biochemical reactions. As early as 1935, the primary chemical structure of the adenine nucleotides was established and has since been confirmed by synthesis (5, 47, 79, 80, 83). Nevertheless, it has only been within the last 15 years that practical recognition has been given to the fact that ATP and its analogs exist in solution and especially in biological fluids as a mixture of variously ionized, metal-complexed, and structurally conformed species (54, 94, 121). Literally dozens of such forms would be present in ordinary biological fluids. These three related factors have already been recognized as playing an important role in

a variety of chemical and biological effects, *e.g.*, the thermodynamics of the hydrolysis of adenine nucleotides (100), the rate of hydrolysis of ATP and adenosine diphosphate (ADP) (55, 77), enzyme activation (16), enzyme inhibition (65, 86), enzyme kinetics (14, 66, 87, 90, 104, 122), nonenzymatic activation of acetate (81), nucleic acid structure (138–140), muscle contraction (53), and the shock-producing ability of ATP (6, 46). It is important, therefore, that the biochemist or biologist concerned with adenine nucleotides know the percentages and structures of the various species present. Although this information is far from complete at the present, it is hoped that the survey presented here will be helpful in interpreting chemical and biological studies involving adenine nucleotides, and will indicate areas for future work in the ionization–complex formation–conformation of ATP and its analogs. Up to the present, published material has been roughly divided among the various aspects of the problem as follows: equilibrium constants, 50%; physical studies, 30%; thermodynamic data, 10%; MO calculations, 5%; kinetics, 5%. These results have been collected here up until Dec 1965. Many useful comparisons can be made with corresponding studies of the inorganic phosphates; a few will be made in this paper. For a collection of ionization, complex formation, and structural data on the inorganic phosphates up until 1958, the review of Van Wazer and Callis (127) and the book by Van Wazer (128) may be consulted. For later developments, references 3, 60, 68, and 108 are pertinent. Also helpful are compendia of stability constants and thermodynamic data (27, 116, 136).

Nomenclature, Symbols, and Abbreviations.—The structure and numbering system for adenosine 5′-triphosphate, ATP; adenosine 5′-diphosphate, ADP; adenosine 5′-monophosphate, AMP; and adenosine, A; are shown by the following



The phosphate groups of the chains are denoted by the Greek letters α, β, and γ beginning with the phosphate group bonded to the D-(−)-ribose moiety. The symbols $atpH_4$, $adpH_3$, $ampH_2$, and a, are used for ATP, ADP, AMP, and adenosine when there is need to specify various stages of ionization. The abbreviations, tma^+, tea^+, and tpa^+ are used for the tetramethylammonium ion, tetraethylammonium ion, and tetrapropylammonium ion, respectively.

II. Equilibrium and Thermodynamic Studies

A. Equilibrium Methods

A variety of methods have been used to determine the ionization and complex formation constants for ATP and its analogs. In this section, a short description of each method will be given along with a reference to the original paper on the method, where possible. References to subsequent papers describing developments, limitations, or sources of error in the method will also be given.

1. pH Titration

Ionization constants are, in general, directly and simply measured by pH titration. Ionization constants of metal phosphate complexes may also be determined directly by pH titration where metal complex formation is virtually complete, *e.g.*, titration of ATP and ADP in the presence of 30 m*M* $MgCl_2$ yields directly the ionization constants of $atpMgH^-$ and adpMgH (101). The pH titration method has the limitation that in itself it gives no information as to where the ionizing site is on the molecule.

pH titration in the absence and presence of complexing metal ion has been extensively used for the determination of complex formation constants. This method seems to have been devised by Bjerrum and was published in 1941 (7). Since then, this method has been applied by several investigators to the adenine nucleotide–metal complexes (2, 15, 18, 49, 56, 63, 64, 67, 84, 88, 91, 92, 98, 99, 113, 114, 117, 118, 126, 134).

In 1964, as a result of infrared and pH titrimetric studies of ATP–M^{2+} complex formation it was stated that "pH titrimetric data cannot be employed in calculating reliable values of the formation constants for the divalent metal ions" (67). However, it seems that this statement must be qualified. It is true with respect to the experimental conditions of one metal ion concentration and one pH. It is not true with respect to studies such as those mentioned above in which several metal ion concentrations or several pH values were used as a basis for calculation.

2. Resin Competition

The resin competition method is one in which metal ion competes with a fixed amount of a suitable ion exchange resin in solution for the ligand. If the resin concentration and the ligand concentration are held constant and the metal ion concentration is varied, the

metal–ligand binding constant can be obtained by the proper plots of metal concentration and ligand distribution. This method seems to have been devised by Schubert (112).

This method has been employed by several investigators in the study of metal–adenine phosphate complexes (31, 91, 92, 97, 101, 129). One source of error that has been reported is a resin saturation effect caused by too high a concentration of ligand (129).

3. *Spectrophotometric Metal Ion Indicator Competition*

In this method, a spectrophotometric metal ion indicator is in competition with the ligand for the metal ion. Optical density measurements of the concentration of metal bound by the indicator and the known indicator equilibrium allow calculation of the metal–ligand binding constant. Relatively few such studies have been made on the adenine nucleotide–metal complexes (17, 98, 99).

4. *Ultraviolet Spectrophotometry*

This method has been used for the study of ring perturbation as an indication of ring–metal and ring–metal–chain interaction (110, 111).

5. *Infrared Spectrophotometry*

The possibilities of employing infrared spectra as a method for the determination of ionization and divalent metal ion complex formation constants of ATP have been explored by Khalil and Brown (67). It is reported that: (1) the pD dependence of the ATP spectrum is markedly affected by divalent metal ions; (2) metal ion coordination does not produce any significant frequency shifts in the ATP spectrum other than those associated with the loss or gain of a proton; (3) infrared spectral observations are clearly capable of yielding information regarding proton dissociations of ATP, which is in reasonable agreement with the more precise titrimetric values; (4) pD shifts measured in the infrared cannot be employed in calculating reliable values of the formation constants for the divalent metal ions.

However, as indicated above, with regard to the attempted calculations of complex formation constants by Khalil and Brown, one metal ion concentration and one ratio of conjugate acid:conjugate base (1:1) were used (67). From this it was correctly concluded that the data were insufficient to yield complex formation constants. However, variation of the conjugate acid to conjugate base ratio and metal ion concentration would give the necessary information (63, 64, 117, 118). Given the added structural information provided by infrared studies with regard to the site of ionization and complex formation, this method seems to be a useful one for the study of ionization and metal complex formation of the adenine nucleotides. As yet, however, it has seen only very limited application.

6. *Fluorescence Metal Ion Indicator Competition*

In this method, 8-hydroxyquinoline competes with a ligand for metal ion in solution. From the known equilibrium constant and fluorescence changes for the binding of metal ion by 8-hydroxyquinoline and the experimental data, the metal binding constant of the ligand can be calculated. This method was devised by Watanabe, Franz, and Trottier in 1963 (133). It was later applied to the Mg^{2+} binding of ATP and ADP (131, 132). It has the advantage of requiring only very dilute solutions (0.04–0.10 mM for ATP) of ligand.

7. *Interferometry*

The change in refractive index with extent of reaction is often large in the formation of complexes and, in fact, is so for the formation of metal–adenine nucleotide complexes. Interferometry, therefore, can and has been used to determine the stability constants of metal–adenine nucleotide complexes (4).

8. *Electron Spin Resonance (Esr)*

Electron spin resonance (esr) measurements were first used by Cohn and Townsend in the investigation of metal ion complexes of biochemical interest (26). This method can be applied to complexes of ions or molecules which are paramagnetic and has been applied to manganese–adenine nucleotide complexes (23, 24, 26, 73, 98). Disappearance of the hyperfine esr spectrum of Mn^{2+} on formation of complexes with ATP and ADP made determination of the binding constants of these complexes possible (26). However, an apparatus of high sensitivity is required for quantitative measurements (23).

9. *Nuclear Magnetic Resonance (Nmr) Titration*

The nmr spectra of ATP and ADP as a function of pH have been studied by Cohn and Hughes (24). Although the primary interest of the study was to correlate nmr data with the reactivity of the various phosphorus groups, the ionization constants for the secondary phosphate ionizations of ATP and ADP may be estimated from a plot of the chemical shifts of the phosphorus nuclei of ADP and ATP against pH. Taking the pH at the inflection points of these sigmoid curves as the pK' value for proton ionizing in the given pH range (pH 4–9) gives pK' values of 6.5 and 6.7 for these ionizations of ADP and ATP, respectively. These values are in the range of those reported from other studies (Table I). Further, the nmr spectra show that these protons are leaving the β-phosphate group of ADP and the γ-phosphate group of ATP.

Similar studies carried out by Cohn and Hughes in 1962 in the presence of 0.1 M divalent metal ion indi-

cate the pK' of atpMgH$^-$ to be 5.4, which again is in the range of other values reported in Table I for this ionization.

It seems that such nmr studies employing pH titration and metal ion titration could yield useful, quantitative information concerning ionization, metal complex formation, and the structural changes accompanying shifts in these two equilibria; however, these possibilities have not as yet been fully explored.

10. Enzyme Mechanism Calculation

In studies of the mechanism of adenosine 5′-triphosphate–creatine transphosphorylase, it has been found that atpMg^{2-} is the substrate, atpH^{3-} is a strong inhibitor, and atp^{4-} is a weak inhibitor (95). The kinetic data could therefore be used to estimate the binding constant for atpMg^{2-} (Table I). Since this type of calculation is usually intended to check a postulated mechanism and not to calculate a binding constant, the constants thus obtained should not be regarded as the most reliable.

11. Metal Ion Titration with Metal Ion Sensitive Electrodes

No such studies have as yet been reported with regard to the adenine nucleotides. However, a recent progress report on the development of various cation sensitive electrodes indicates that Na^+ sensitive electrodes are commercially available while others are in the experimental stage (106). With sufficiently stable and accurate electrodes of this type, ionization and metal complex formation of the adenine nucleotides could be simply and directly studied by the use of such electrodes in conjunction with a simultaneous pH measurement.

B. THERMODYNAMIC METHODS

The most widely used means for obtaining thermodynamic data for the reaction under discussion is temperature variation with the equilibrium methods described in A. However, some more direct methods have been used as exemplified by the following .

1. Calorimetry

A calorimetric method for the determination of the enthalpy of slow reactions in aqueous solution was devised by Buzzell and Sturtevant in 1951 (19). This method has been applied to the p$K = \sim 4$, ring ionization of adenosine (105), and it seems it could also be employed for determination of enthalpies of metal complex formation of the adenine nucleotides. Enough equilibrium data are available (Table I) to calculate enthalpy contributions from the ionization of complexed and uncomplexed species for many metal–adenine nucleotide complexes, thereby making possible the assignment of ΔH values to the individual ionization and complex formation reactions.

2. Thermometric Titration

A thermometric or enthalpy titration procedure was first successfully used to determine the enthalpy of a reaction in aqueous solution by Jordan and Alleman in 1957 (61). This method employs a highly sensitive thermistor bridge circuit for temperature measurement, which yields an accuracy of 4% in the enthalpy with solutions as dilute as 0.5 mM. The method was successfully used to determine heats of complex formation of divalent cations with EDTA (61). The method of calculations of enthalpy from the thermometric titration data has been refined and the method has been applied to the p$K = \sim 4$ ring ionization of adenosine, AMP, ADP, and ATP, and to the secondary phosphate ionizations of AMP, ADP, and ATP by Christensen and Izatt (Table II) (20). For a review on thermometric titrations up until 1960, *cf.* ref 137. Given the enthalpy of ionization data of Christensen and Izatt (20) it seems that metal ion thermometric titrations of the adenine nucleotides would easily yield useful enthalpy data concerning enthalpies of complex formation of the variously ionized species.

Recently, a method has been devised for calculating equilibrium constants as well as enthalpies of ionization from thermometric titration data for ionization reactions in which the equilibrium constant is less than 10^3, and which have an appreciable ΔH value (50). This method which is referred to as "entropy titration" has been applied to the p$K = 12.35$ ionization of the ribose moiety of adenosine (58).

C. EQUILIBRIUM AND THERMODYNAMIC DATA

An effort has been made to gather a reasonably complete collection of equilibrium and thermodynamic data for the ionization and metal complex formation of adenosine and the adenine nucleotides. These data are presented in this section under five headings.

1. 1:1 Metal–Nucleotide Complexes Involving Only Ring and Secondary Phosphate Hydrogens

The data under this heading, which constitute a large percentage of the total data, are presented in Tables I and II.

A few observations might be made on the data of Tables I and II as follows.

(a) Very little work has been done on the primary ionizations of ATP and its analogs. pK values for these ionizations were estimated by analogy with similar compounds as late as 1960 (44). It seems that the only published experimental work on these groups was by Levene in 1925 (75, 76).

(b) The complex formation constants for the various metals seem in general to fall into the three expected

TABLE I

EQUILIBRIUM DATA FOR THE IONIZATIONS AND 1:1 METAL COMPLEX FORMATION OF ADENOSINE AND THE ADENINE NUCLEOTIDES

Adenosine (a): $a + H^+ \leftrightarrow {}^+Ha$

M	Method	pH	Temp, °C	Adenosine, m*M*	Supporting electrolyte	μ	Log K^H_{aH}	Ref
	pH titration (H_2)		25	50.0			3.45	75, 76
	pH titration (H_2)		25				3.6 ± 0.1	96
	pH titration		25		NaCl	0.15	3.63	2
			38		NaCl	0.15	3.60	
	pH titration		20	1.0	KCl	0.1	3.55 ± 0.02	84
	pH titration		10				3.61	51
			25				3.51	
			40				3.37	
	Infrared spectra		32	100.0			3.80	67
			32	100.0	KNO_3	~0.4	3.75	
					$CuCl_2$ (0.1 *M*)	~0.4	3.60	

Adenosine (a): $a^- + H^+ \leftrightarrow a$ (ribose ionization)[a]

M	Method	pH	Temp, °C	Adenosine, m*M*	Supporting electrolyte	μ	Log K^H_a	Ref
	pH titration (H_2)		25				12.5	75, 76
	Entropy titration		25	10.0		0	12.35 ± 0.03	58

Adenosine (a): $a + M^{2+} \leftrightarrow aM^{2+}$

M	Method	pH	Temp, °C	Adenosine, m*M*	Supporting electrolyte	μ	Log K^M_{aM}	Ref
Ca^{2+}	Ultraviolet spectra			2.0	$M(ClO_4)_2$			110
Mg^{2+}	Ultraviolet spectra			2.0	$M(ClO_4)_2$			110
Mn^{2+}	Ultraviolet spectra			2.0	$M(ClO_4)_2$	3.0–9.0	−0.82	110
Co^{2+}	Ultraviolet spectra			2.0	$M(ClO_4)_2$	3.0–9.0	−0.30	110
Ni^{2+}	Ultraviolet spectra			2.0	$M(ClO_4)_2$	3.0–9.0	−0.17	110
Cu^{2+}	Ultraviolet spectra			1.0	$M(ClO_4)_2$	0.3–6.0	+0.84	110
Zn^{2+}	Ultraviolet spectra			2.0	$M(ClO_4)_2$	1.5–9.0	−0.28	110

AMP ($ampH_2$): $amp^{2-} + H^+ \leftrightarrow ampH^{1-}$

M	Method	pH	Temp, °C	AMP, m*M*	Supporting electrolyte	μ	Log K^H_{ampH}	Ref
	pH titration (H_2)		25	25.0			6.01	75
	pH titration (H_2)		25	20.0			6.2	130
	pH titration		25		NaCl	0.15	6.05	2
			38		NaCl	0.15	6.08	
	pH titration		20	1.0	KCl	0.1	6.14 ± 0.02	84
	pH titration		25	1.0	tpaBr	0.2	6.45 ± 0.02	117, 118
	pH titration		25	2.0	tmaBr	0.1	6.40	126
			25	2.0	KCl	0.1	6.30	
	pH titration		25	1.0–3.0	KNO_3	0.1	6.21	64
	pH titration		25	0.5	tpaBr	0.1	6.46 ± 0.01	103
			25	0.5	tpaBr	0	6.67 ± 0.01	

AMP ($ampH_2$): $amp^{2-} + M^+ \leftrightarrow ampM^-$

M	Method	pH	Temp, °C	AMP, m*M*	Supporting electrolyte	μ	Log K^M_{ampM}	Ref
Li^+	pH titration		25	1.0	tpaBr	0.2	0.61 ± 0.04	117
Na^+	pH titration		25	1.0	tpaBr	0.2	0.46 ± 0.06	117
Na^+	Resin competition	7.0	25	30.0–100.0	tmaBr	0.25	0.45 ± 0.01	126
K^+	pH titration		25	1.0	tpaBr	0.2	0.20 ± 0.08	117

AMP ($ampH_2$): $amp^{2-} + M^{2+} \leftrightarrow ampM$

M	Method	pH	Temp, °C	AMP, m*M*	Supporting electrolyte	μ	Log K^M_{ampM}	Ref
Mg^{2+}	pH titration		20	1.0	KCl	0.1	1.69 ± 0.02	84
Mg^{2+}	pH titration		25	1.0	tpaBr	0.2	1.69 ± 0.02	118
Mg^{2+}	Resin competition	8.8	25	1.0	NaCl[b]	0.1	2.00	92
Mg^{2+}	pH titration		25	3.0–5.0	KCl	0.1	2.14	134
Mg^{2+}	Resin competition	8.0–8.2	23	0.06	NaCl	0.1	1.95	129
Mg^{2+}	pH titration		25	1.0–3.0	KNO_3	0.1	1.97 ± 0.01	64
Ca^{2+}	pH titration		20	1.0	KCl	0.1	1.41 ± 0.03	84
Ca^{2+}	pH titration		25	1.0	tpaBr	0.2	1.43 ± 0.02	118
Ca^{2+}	Resin competition	8.0–8.2	23	0.06	NaCl	0.1	1.76	129
Ca^{2+}	pH titration		25	1.0–3.0	KNO_3	0.1	1.85 ± 0.02	64
Sr^{2+}	pH titration		25	1.0	tpaBr	0.2	1.32 ± 0.02	118
$Sr^{90\,2+}$	Resin competition	7.4	~25		NaCl	0.15	1.52	97
Sr^{2+}	pH titration		25	1.0–3.0	KNO_3	0.1	1.79 ± 0.01	64
Ba^{2+}	pH titration		25	1.0–3.0	KNO_3	0.1	1.73 ± 0.01	64
Mn^{2+}	pH titration		25	1.0	tpaBr	0.2	2.19 ± 0.06	118
Mn^{2+}	Resin competition	8.0–8.2	23	0.06	NaCl	0.1	2.31	129
Mn^{2+}	Resin competition	6.9	25			0.1	2.19	126
Mn^{2+}	pH titration		25	1.0–3.0	KNO_3	0.1	2.40 ± 0.02	64
Co^{2+}	Resin competition	8.0–8.2	23	0.06	NaCl	0.1	2.58	129

TABLE I (*Continued*)

AMP ($ampH_2$): $amp^{2-} + M^{2+} \leftrightarrow ampM$

M	Method	pH	Temp, °C	AMP, m*M*	Supporting electrolyte	μ	Log K^{M}_{ampM}	Ref
Co^{2+}	pH titration		25	1.0–3.0	KNO_3	0.1	2.64 ± 0.02	64
Ni^{2+}	pH titration		25	1.0–3.0	KNO_3	0.1	2.84 ± 0.01	64
Cu^{2+}	pH titration		25	1.0–3.0	KNO_3	0.1	3.18 ± 0.01	64
Zn^{2+}	pH titration		25	1.0–3.0	KNO_3	0.1	2.72 ± 0.02	64
$Y^{90\,2+}$	Resin competition	7.4	~25		NaCl	0.15	5.70	97

AMP ($ampH_2$): $ampH^{1-} + H^{+} \leftrightarrow {}^{+}HampH^{-}$

M	Method	pH	Temp, °C	AMP, m*M*	Supporting electrolyte	μ	Log K^{H}_{HampH}	Ref
	pH titration (H_2)		25	25.0	None	Varying	3.70	75
	pH titration (H_2)		23	20.0	None	Varying	3.8	130
	pH titration		25		NaCl	0.15	3.74	2
			38		NaCl	0.15	3.71	
	pH titration		20	1.0	KCl	0.1	3.81 ± 0.01	84
	pH titration		25	1.0–3.0	KNO_3	0.1	3.81	64

AMP ($ampH_2$): ${}^{+}HampH^{1-} + H^{+} \leftrightarrow {}^{+}HampH_2$

M	Method	pH	Temp, °C	AMP, m*M*	Supporting electrolyte	μ	Log $K^{H}_{amp^{H2}}$	Ref
	pH titration (H_2)		25	25.0	None	Varying	0.89	75

ADP ($adpH_3$): $adp^{3-} + H^{+} \leftrightarrow adpH^{-}$

M	Method	pH	Temp, °C	ADP, m*M*	Supporting electrolyte	μ	Log K^{H}_{adpH}	Ref
	pH titration		25		NaCl	0.15	6.26	2
			38		NaCl	0.15	6.27	
	pH titration		25	1.8	teaBr	0.2	6.65 ± 0.01	88
	pH titration		20	1.0	KCl	0.1	6.35 ± 0.03	84
	pH titration		25	1.0	tpaBr	0.2	6.68 ± 0.02	117
	pH titration		25	3.0–5.0	KCl	0.1	6.61	134
	Nmr titration			500.0	tmaCl	1.0	6.5 (est)[g]	24
	pH titration		25		tmaBr	0	7.00	59
	pH titration		25	1.0–3.0	KNO_3	0.1	6.44	64
	pH titration		25	0.5	tpaBr	0.1[c]	6.80 ± 0.01	103
			25	0.5	tpaBr	0	7.20 ± 0.01	

ADP ($adpH_3$): $adp^{3-} + M^{+} \leftrightarrow adpM^{2-}$

M	Method	pH	Temp, °C	ADP, m*M*	Supporting electrolyte	μ	Log K^{M}_{adpM}	Ref
Li^{+}	pH titration		25	1.0	tpaBr	0.2	1.15 ± 0.02	117
Na^{+}	pH titration		25	1.8	teaBr	0.2	0.65 ± 0.07	88
Na^{+}	pH titration		25	1.0	tpaBr	0.2	0.83 ± 0.03	117
K^{+}	pH titration		25	1.8	teaBr	0.2	0.68 ± 0.01	88
K^{+}	pH titration		25	1.0	tpaBr	0.2	0.74 ± 0.04	117

ADP ($adpH_3$): $adp^{3-} + M^{2+} \leftrightarrow adpM^{-}$

M	Method	pH	Temp, °C	ADP, m*M*	Supporting electrolyte	μ	Log K^{M}_{adpM}	Ref
Mg^{2+}	pH titration		25	4.0–18.0		0.2	2.57 (est)[d]	18
Mg^{2+}	pH titration		20	1.0	KCl	0.1	3.11 ± 0.05	84
Mg^{2+}	pH titration		25	1.0	tpaBr	0.2	3.00 ± 0.04	118
Mg^{2+}	Resin competition	8.8	23	1.0	NaCl[b]	0.1	3.03[b]	92
Mg^{2+}	Resin competition	8.0–8.2	23	0.06	NaCl	0.1	3.15	129
Mg^{2+}	pH titration		25	3.0–5.0	KCl	0.1	3.23	134
Mg^{2+}	Spectrophotometric Mg^{2+} indicator competition	7.9	25	0.62	tbeaBr	0.11	3.34	17
		7.9	35	0.62	tbeaBr	0.11	3.48	
		8.8	64	0.62	tbeaBr	0.11	3.84	
Mg^{2+}	pH titration			1.0	tmaCl	0.05	3.34	56
Mg^{2+}	pH titration		25	1.0–3.0	KNO_3	0.1	3.17 ± 0.01	64
Mg^{2+}	Calcd extrapolation of Burton's 1959 results	7.9	25	0.62	tbeaBr	0	4.10	43
Mg^{2+}	Spectrophotometric Mg^{2+} indicator competition	8.0	30	1.0–5.0	N-Ethyl morpholine buffer	0.1	3.62	99
Mg^{2+}	Resin competition	8.7	25	0.07	tpaBr	0.1	3.47 ± 0.03	101
		8.7	25	0.07	tpaBr	0	4.27 ± 0.10	
Mg^{2+}	Fluorescence Mg^{2+} indicator competition	8.2	25	0.04–0.10	Tris	0.1	3.54	132
		8.1	25	0.04–0.10	tea Buffer	0.1	3.78	
Ca^{2+}	Resin competition	7.4	37	0.5–4.0	Veronal buffer	0.1	3.74	31
Ca^{2+}	pH titration		20	1.0	KCl	0.1	2.78 ± 0.03	84
Ca^{2+}	pH titration		25	1.0	tpaBr	0.2	2.81 ± 0.04	118
Ca^{2+}	Resin competition	8.0–8.2	23	0.06	NaCl	0.1	2.82	124
Ca^{2+}	Spectrophotometric Ca^{2+} indicator competition	8.8	25	1.23	tbeaBr	0.11	2.89	17
Ca^{2+}	Interferometry	8.0	23	2.0	tmaCl	0.11	2.93	4
Ca^{2+}	pH titration		25	1.0–3.0	KNO_3	0.1	2.86 ± 0.02	64
Ca^{2+}	Spectrophotometric Ca^{2+} indicator competition	8.0	30	1.0–5.0	N-Ethyl morpholine buffer	0.1	3.34	98
Sr^{2+}	pH titration		25	1.0	tpaBr	0.2	2.50 ± 0.04	118
Sr^{2+}	pH titration		25	1.0–3.0	KNO_3	0.1	2.54 ± 0.02	64

TABLE I (*Continued*)

ADP ($adpH_3$): $adp^{3-} + M^{2+} \leftrightarrow adpM^-$

M	Method	pH	Temp, °C	ADP, m*M*	Supporting electrolyte	μ	Log K^M_{adpM}	Ref
Ba^{2+}	pH titration		25	1.0–3.0	KNO_3	0.1	2.36 ± 0.03	64
Mn^{2+}	pH titration		25	1.0	tpaBr	0.2	3.54 ± 0.04	118
Mn^{2+}	Resin competition	8.0–8.2	23	0.06	NaCl	0.1	3.94	129
Mn^{2+}	Esr titration				0.05 *M* Tris–0.1*M* tmaCl	0.15	4.00	22
Mn^{2+}	pH titration		25	1.0–3.0	KNO_3	0.1	4.16 ± 0.01	64
Mn^{2+}	Spectrophotometric Mn^{2+} indicator competition	8.0	30	1.0–5.0	N-Ethyl morpholine buffer	7.1	4.40	98
Mn^{2+}	Esr	8.0	27		N-Ethyl morpholine buffer	0.1	4.48	98
Ca^{2+}	Resin competition	8.0–8.2	23	0.06	NaCl	0.1	3.68	129
Co^{2+}	pH titration		25	1.0–3.0	KNO_3	0.1	4.20 ± 0.01	64
Ni^{2+}	pH titration		25	1.0–3.0	KNO_3	0.1	4.50 ± 0.02	64
Cu^{2+}	pH titration		25	1.0–3.0	KNO_3	0.1	5.90 ± 0.01	64
Zn^{2+}	pH titration		25	3.0–5.0	KCl	0.1	4.13	134
Zn^{2+}	pH titration		25	1.0–3.0	KNO_3	0.1	4.28 ± 0.01	64

ADP ($adpH_3$): $adpH^{2-} + H^+ \leftrightarrow {}^+HadpH^{2-}$

M	Method	pH	Temp, °C	ADP, m*M*	Supporting electrolyte	μ	Log K^H_{HadpH}	Ref
	pH titration		25		NaCl	0.15	3.95	2
			38		NaCl	0.15	3.92	
	pH titration		20	1.0	KCl	0.1	3.99 ± 0.03	84
	pH titration		25	3.0–5.0	KCl	0.1	4.21	134
	pH titration		25		tmaBr	0	4.20	59
	pH titration		25	1.0–3.0	KNO_3	0.1	3.93	64

ADP ($adpH_2$): $adpH^{2-} + M^{2+} \leftrightarrow adpMH$

M	Method	pH	Temp, °C	ADP, m*M*	Supporting electrolyte	μ	Log K^M_{adpMH}	Ref
Mg^{2+}	pH titration		20	1.0	KCl	0.1	1.5 ± 0.2	84
Mg^{2+}	pH titration		25	1.0	tpaBr	0.2	1.45 ± 0.06	118
Mg^{2+}	pH titration		25	3.0–5.0	KCl	0.1	1.58	134
Mg^{2+}	pH titration		25	1.0–3.0	KNO_3	0.1	1.64 ± 0.02	64
Mg^{2+}	Resin competition	4.1	25	0.07	tpaBr	0.1	2.0 ± 0.2	101
Mg^{2+}	Thermodynamic calcn		25		tpaBr	0.1	1.95 ± 0.2	101
Mg^{2+}	Resin competition	4.1	25	0.07	tpaBr	0	2.45 ± 0.20	101
Ca^{2+}	pH titration		25	1.0	tpaBr	0.2	1.52 ± 0.06	118
Ca^{2+}	Interferometry	5.0	23	2.0	tmaCl	11	1.36	4
Ca^{2+}	pH titration		25	1.0–3.0	KNO_3	0.1	1.58 ± 0.04	64
Sr^{2+}	pH titration		25	1.0	tpaBr	0.2	1.34 ± 0.08	118
Mn^{2+}	pH titration		25	1.0	tpaBr	0.2	1.49 ± 0.05	118
Mn^{2+}	pH titration		25	1.0–3.0	KNO_3	0.1	1.89 ± 0.02	64
Co^{2+}	pH titration		25	1.0–3.0	KNO_3	0.1	2.01 ± 0.02	64
Ni^{2+}	pH titration		25	1.0–3.0	KNO_3	0.1	2.30 ± 0.02	64
Cu^{2+}	pH titration		25	1.0–3.0	KNO_3	0.1	2.63 ± 0.01	64
Zn^{2+}	pH titration		25	3.0–5.0	KCl	0.1	2.34	134
Zn^{2+}	pH titration		25	1.0–3.0	KNO_3	0.1	2.04 ± 0.02	64

ADP ($adpH_3$): $adpM^- + H^+ \leftrightarrow adpMH$

M	Method	pH	Temp, °C	ADP, m*M*	Supporting electrolyte	μ	Log K^H_{adpMH}	Ref
Mg^{2+}	pH titration		20	1.0	KCl	0.1	4.7 ± 0.2	84
Mg^{2+}	pH titration		25	1.0	tpaBr	0.2	5.1 ± 0.1	118
Mg^{2+}	pH titration		25	0.5	$MgCl_2$	0.1	5.30 ± 0.03	101
			25	0.5	$MgCl_2$	0	5.38 ± 0.10	
Ca^{2+}	pH titration		25	1.0	tpaBr	0.2	5.4 ± 0.1	118
Sn^{2+}	pH titration		25	1.0	tpaBr	0.2	5.5 ± 0.1	118
Mn^{2+}	pH titration		25	1.0	tpaBr	0.2	4.6 ± 0.1	118

ATP ($atpH_4$): $atp^{4-} + H^+ \leftrightarrow atpH^{3-}$

M	Method	pH	Temp, °C	ATP, m*M*	Supporting electrolyte	μ	Log K^H_{atpH}	Ref
	pH titration		25		NaCl	0.15	6.48	2
			38		NaCl		6.50	
	pH titration		25	0.6	teaBr	0.15	6.98 ± 0.02	88
	pH titration		20	1.0	KCl	0.1	6.50 ± 0.01	84
	pH titration		25	1.0	tpaBr	0.2	6.95 ± 0.02	117
	pH titration		25	3.0–5.0	KCl	0.1	6.73	134
	Nmr titration			500.0	tmaCl	1.0	6.7 (est)[g]	124
	pH titration		25	1.0–6.0	KNO_3	0.1	6.53	63
	pH titration		25	0.5	tpaBr	0.1[c]	7.04 ± 0.01	103
			25	0.5	tpaBr	0	7.68 ± 0.01	
	pH titration		30	0.525	teaBr	0.1	6.97 ± 0.02	98
	Infrared spectra		32	100.0	None	0.3–0.4	6.7[e,f]	67
					KNO_3 (0.3 *M*)	0.6–0.7	6.9[e,f]	

TABLE I (*Continued*)

ATP ($atpH_4$): $atp^{4-} + M^{+} \leftrightarrow atpM^{3-}$

M	Method	pH	Temp, °C	ATP, m*M*	Supporting electrolyte	μ	Log K^{M}_{atpM}	Ref
Li^+	pH titration		25	1.0	tpaBr	0.2	1.57 ± 0.03	117
Na^+	pH titration		25	2.2	teaBr	0.2	0.96 ± 0.04	88
Na^+	pH titration		25	1.0	tpaBr	0.2	1.16 ± 0.02	117
Na^+	Spectrophotometric Mg^{2+} indicator competition	8.0	30		Methyl morpholine buffer	0.1	1.18	98
K^+	pH titration		25	2.2	teaBr	0.2	1.00 ± 0.02	88
K^+	pH titration		25	1.0	tpaBr	0.2	1.06 ± 0.04	117
K^+	Spectrophotometric Mg^{2+} indicator competition	8.0	30		N-Ethyl morpholine buffer	0.1	1.15	98

ATP ($atpH_4$): $atp^{4-} + M^{2+} \leftrightarrow atpM^{2-}$

M	Method	pH	Temp, °C	ATP, m*M*	Supporting electrolyte	μ	Log K^{M}_{atpM}	Ref
Be^{2+}	pH titration		25	10.0	KCl	0.1	5.01 ± 0.02	15
Mg^{2+}	pH titration		20	1.0	KCl	0.1	4.00 ± 0.04	84
Mg^{2+}	pH titration		25	4.0–18.0		0.2	2.92 (est)[d]	18
Mg^{2+}	pH titration		25	1.0	tpaBr	0.2	3.47 ± 0.04	118
Mg^{2+}	Resin competition	8.8	10	1.0	NaCl[b]	0.15	3.06	92
		8.8	23	1.0	NaCl[b]	0.15	3.34	
		8.8	43	1.0	NaCl[b]	0.15	3.50	
Mg^{2+}	Resin competition	8.0–8.2	23	0.06	NaCl	0.1	3.77	129
Mg^{2+}	pH titration		25	3.0–5.0	KCl	0.1	4.04	134
Mg^{2+}	Spectrophotometric Mg^{2+} indicator competition	8.4	25	0.4–3.7	tbeaBr	0.11	4.59	17
		8.8	64	0.4–3.7	tbeaBr	0.11	4.99	
		8.4	25	0.4–3.7	tbeaBr	0.22	4.35	
Mg^{2+}	Enzyme mechanism calcn	5.8–9.0	30	0.5–4.0		2.1	4.95	95
Mg^{2+}	pH titration			1.0	tmaCl	0.05	3.90	56
Mg^{2+}	pH shift		25	0.087	teaBr	0.1	4.43 ± 0.03	91
Mg^{2+}	Resin competition	7.0	25	0.05	teaBr	0.1	4.32 ± 0.04	91
		8.7	25	0.05	teaBr	0.1	4.37 ± 0.05	
Mg^{2+}	Spectrophotometric Mg^{2+}	8.0	30	0.5–3.0	Tris	0.1	4.30 ± 0.04	99
		8.0	30	0.5–3.0	tea buffer	0.1	4.93 ± 0.03	
	indicator competition	8.0	30	0.5–3.0	N-Ethyl morpholine buffer	0.1	4.93 ± 0.03	
Mg^{2+}	pH titration		30	0.5	teaBr	0.1	5.02 ± 0.06	99
Mg^{2+}	Interferometry	9.0	23	2.0	Tris	0.1	3.88 ± 0.08	4
Mg^{2}	pH titration		20	0.8	KCl	0.1	3.84	49
Mg^{2+}	pH titration		25	1.06–6.0	KCl	0.1	4.22 ± 0.01	63
Mg^{2+}	Calcd extrapolation of Burton's results	8.4	25	0.4–3.7	tbeaBr	0	5.70	43
Mg^{2+}	Fluorescence Mg^{2+} indicator competition	8.0–8.3	25	0.04–0.10	Tris	0.1	4.85 ± 0.05	132
		8.0–8.3	25	0.04–0.10	tea buffer	0.1	4.60 ± 0.1	
Mg^{2+}	pH titration		30	0.5	teaBr	0.1	4.94	98
Mg^{2+}	Resin competition	8.7	25	0.07	tpaBr	0.1	4.60 ± 0.03	98
		8.7	25	0.07	tpaBr	0	5.83 ± 0.10	
Ca^{2+}	Resin competition	7.4	37	0.5–4.0	Veronal buffer	0.1	4.06	31
Ca^{2+}	pH titration		20	1.0	KCl	0.1	3.60 ± 0.03	84
Ca^{2+}	pH titration		25	1.0	tpaBr	0.2	3.29 ± 0.08	118
Ca^{2+}	Resin competition	8.8	23	1.0	NaCl[c]	0.15	3.34	92
Ca^{2+}	Resin competition	8.0–8.2	23	0.06	NaCl	0.1	3.77	129
Ca^{2+}	Spectrophotometric Ca^{2+} indicator competition	9.0	25	0.84	tbeaBr	0.11	3.45	17
Ca^{2+}	Spectrophotometric Ca^{2+} indicator competition	7.0	25	0.9–2.0	NaCl[c]	0.1	3.43 ± 0.07	91
Ca^{2+}	pH shift		25	0.087	teaBr	0.1	3.92 ± 0.03	91
Ca^{2+}	Resin competition	7.0	26	~0.05	teaBr	0.1	3.97	91
Ca^{2+}	Interferometry	8.0	23	2.0	tmaCl	0.11	3.89	4
Ca^{2+}	pH titration		25	1.0–6.0	KNO_3	0.1	3.97 ± 0.01	63
Ca^{2+}	pH titration		30	0.525	teaBr	0.1	4.51	98
Ca^{2+}	Spectrophotometric Ca^{2+} indicator competition	8.0	30		N-Ethyl morpholine buffer	0.1	4.49	98
Sr^{2+}	pH titration		25	1.0	tpaBr	0.2	3.03 ± 0.06	118
$Sr^{90\ 2+}$	Resin competition	7.4	25		NaCl	0.15	3.15	97
Sr^{2+}	pH shift		25	0.10	teaBr	0.1	3.60 ± 0.03	91
Sr^{2+}	pH titration		25	1.0–6.0	KNO_3	0.1	3.54 ± 0.01	63
Ba^{2+}	pH shift		25	0.10	teaBr	0.1	3.37 ± 0.05	91
Ba^{2+}	pH titration		25	1.0–6.0	KNO_3	0.1	3.29 ± 0.01	63
Mn^{2+}	pH titration		25	1.0	tpaBr	0.2	3.98 ± 0.06	118
Mn^{2+}	Resin competition	8.0–8.2	23	0.06	NaCl	0.1	4.75	129
Mn^{2+}	pH titration		22	2.7	KCl	0.1	6.78	12, 13
Mn^{2+}	Esr titration				0.05 *M* Tris–1 *M* tmaCl	0.15	4.88	23
Mn^{2+}	pH titration		20	0.8	KCl	0.1	4.52	49
Mn^{2+}	pH titration		25	1.0–6.0	KNO_3	0.1	4.78 ± 0.01	63
Co^{2+}	Resin competition	8.0–8.2	23	0.06	NaCl	0.1	4.62	129
Co^{2+}	pH titration		25	10.0	KCl	0.1	4.53 ± 0.05	15
Co^{2+}	pH titration		22	2.7	KCl	0.1	4.71	12, 13
Co^{2+}	pH titration		25	1.0–6.0	KNO_3	0.1	4.66 ± 0.02	63

TABLE I (*Continued*)

ATP ($atpH_4$): $atp^{4-} + M^{2+} \leftrightarrow atpM^{2-}$

M	Method	pH	Temp, °C	ATP, m*M*	Supporting electrolyte	μ	Log K^{M}_{atpM}	Ref
Ni^{2+}	pH titration		25	10.0	KCl	0.1	4.61 ± 0.04	15
Ni^{2+}	pH titration		22	2.7	KCl	0.1	4.54	12, 13
Ni^{2+}	pH titration		25	1.0–6.0	KNO_3	0.1	5.02 ± 0.02	63
Cu^{2+}	pH titration		25	10.0	KCl	0.1	5.50 ± 0.01	15
Cu^{2+}	pH titration		22	2.7	KCl	0.1	5.77	12, 13
Cu^{2+}	pH titration		20	0.8	KCl	0.1	5.82	49
Cu^{2+}	pH titration		25	1.0–6.0	KNO_3	0.1	6.13 ± 0.01	63
Zn^{2+}	pH titration		25	3.0–5.0	KCl	0.1	4.26	134
Zn^{2+}	pH titration		22	2.7	KCl	0.1	4.80	12, 13
Zn^{2+}	pH titration		20	0.8	KCl	0.1	4.75	49
Zn^{2+}	pH titration		25	1.0–6.0	KNO_3	0.1	4.85 ± 0.02	63
$Y^{90\,2+}$	Resin competition	7.4	25		NaCl	0.15	11.10	97
Cd^{2+}	pH titration		25	10.0	KCl	0.1	4.70 ± 0.05	12, 13

ATP ($atpH_4$): $atpH^{3-} + H^{+} \leftrightarrow {}^{+}HatpH^{3-}$

M	Method	pH	Temp, °C	ATP, m*M*	Supporting electrolyte	μ	Log K^{M}_{HatpH}	Ref
	pH titration		25		NaCl	0.15	4.00	2
			38		NaCl	0.15	4.00	
	pH titration		20	1.0	KCl	0.1	4.05 ± 0.01	84
	pH titration		25	3.0–5.0	KCl	0.1	4.26	134
	pH titration		25	1.0–6.0	KNO_3	0.1	4.06	63
	pH titration		30	0.525	teaBr	0.1	3.93 ± 0.02	98
	Infrared spectra		32	100.0		0.3–0.4	4.05	67

ATP ($atpH_4$): $atpH^{3-} + M^{2+} \leftrightarrow atpHM^{-}$

M	Method	pH	Temp, °C	ATP, m*M*	Supporting electrolyte	μ	Log K^{M}_{atpMH}	Ref
Mg^{2+}	pH titration		20	1.0	KCl	0.1	2.0 ± 0.1	84
Mg^{2+}	pH titration		25	1.0	tpaBr	0.2	1.49 ± 0.09	118
Mg^{2+}	pH titration		25	3.0–5.0	KCl	0.1	2.16	134
Mg^{2+}	pH titration		20	0.8	KCl	0.1	2.09	49
Mg^{2+}	pH titration		25	1.0–6.0	KNO_3	0.1	2.24 ± 0.01	63
Mg^{2+}	pH titration		30	0.525	teaBr	0.1	2.85	98
Mg^{2+}	Resin competition	4.1	25	0.07	tpaBr	0.1	2.86 ± 0.15	101
Mg^{2+}	Thermo cycle calcn		25		tpaBr	0.1	2.70 ± 0.12	101
Mg^{2+}	Resin competition	4.1	25	0.07	tpaBr	0.1	3.59 ± 0.12	101
Ca^{2+}	pH titration		20	1.0	KCl	0.1	1.8 ± 0.1	84
Ca^{2+}	pH titration		25	1.0	tpaBr	0.2	1.61 ± 0.09	118
Ca^{2+}	Interferometry	5.0	23	2.0	tmaCl	0.11	1.45	4
Ca^{2+}	pH titration		25	1.0–6.0	KNO_3	0.1	2.13 ± 0.01	63
Sr^{2+}	pH titration		25	1.0	tpaBr	0.2	1.48 ± 0.09	118
Sr^{2+}	pH titration		25	1.0–6.0	KNO_3	0.1	2.05 ± 0.01	63
Ba^{2+}	pH titration		25	1.0–6.0	KNO_3	0.1	1.85 ± 0.01	63
Mn^{2+}	pH titration		25	1.0	tpaBr	0.2	1.57 ± 0.08	118
Mn^{2+}	pH titration		20	0.8	KCl	0.1	2.61	49
Mn^{2+}	pH titration		25	1.0–6.0	KNO_3	0.1	2.39 ± 0.01	63
Co^{2+}	pH titration		25	1.0–6.0	KNO_3	0.1	2.32 ± 0.02	63
Ni^{2+}	pH titration		25	1.0–6.0	KNO_3	0.1	2.72 ● 0.02	63
Cu^{2+}	pH titration		20	0.8	KCl	0.1	3.25	49
Cu^{2+}	pH titration		25	1.0–6.0	KNO_3	0.1	3.12 ± 0.01	63
Zn^{2+}	pH titration		25	3.0–5.0	KCl	0.1	2.75	134
Zn^{2+}	pH titration		20	0.8	KCl	0.1	2.78	49
Zn^{2+}	pH titration		25	1.0–6.0	KNO_3	0.1	2.67 ± 0.01	63

ATP ($atpH_4$): $atpM^{2-} + H^{+} \leftrightarrow atpMH^{-}$

M	Method	pH	Temp, °C	ATP, m*M*	Supporting electrolyte	μ	Log K^{H}_{atpMH}	Ref
Mg^{2+}	pH titration		20	1.0	KCl	0.1	4.5 ± 0.1	84
Mg^{2+}	pH titration		25	1.0	tpaBr	0.2	5.0 ± 0.2	118
Mg^{2+}	Nmr titration			100.0	$MgCl_2$ (0.1 *M*)	~0.6	5.4 ± 0.2	23
Mg^{2+}	pH titration		20	0.8	KCl	0.1	4.75	49
Mg^{2+}	Infrared spectra		32	100.0	$MgCl_2$ (0.1 *M*)	~0.6	4.5[e]	67
Mg^{2+}	pH titration		25	0.5	$MgCl_2$	0.1	5.21 ± 0.03	101
			25	0.5	$MgCl_2$	0	5.44 ± 0.05	
Ca^{2+}	pH titration		20	1.0	KCl	0.1	4.7 ± 0.1	84
Ca^{2+}	pH titration		25	1.0	tpaBr	0.2	5.3 ± 0.2	118
Ca^{2+}	Infrared spectra		32	100.0	$CaCl_2$ (0.1 *M*)	~0.6	5.1[f]	67
Sr^{2+}	pH titration		32		$SrCl_2$		5.4 ± 0.2	67
Mn^{2+}	pH titration		20	0.8	KCl	0.1	4.59	49
Mn^{2+}	pH titration		25	1.0	tpaBr	0.2	4.5 ± 0.2	118
Ni^{2+}	Infrared spectra		32	100.0	$NiCl_2$ (0.1 *M*)	~0.6	4.1[f]	67
Cu^{2+}	pH titration		20	0.8	KCl	0.1	3.93	49
Cu^{2+}	Infrared spectra		32	100.0	$CuCl_2$ (0.1 *M*)	~0.6	2.9[f]	67
			32	100.0	$CuNO_3$ (0.1 *M*)	~0.6	2.8[f]	
Zn^{2+}	pH titration		20	0.8	KCl	0.1	4.53	49
Zn^{2+}	Infrared spectra		32	100.0	$ZnCl_2$	~0.6	3.7[f]	67

TABLE I (*Continued*)

ATP ($atpH_4$): $^+HatpH^{3-} + M^{2+} \leftrightarrow {}^+HatpHM^-$

M	Method	pH	Temp, °C	ATP, m*M*	Supporting electrolyte	μ	Log K^M_{HatpMH}	Ref
Mg^{2+}	pH titration		20	0.8	KCl	0.1	1.58 ± 0.2	49
Mg^{2+}	pH titration and computer anal		30	0.525	teaBr	0.1	1.3	98
Mn^{2+}	pH titration and computer anal		30	0.525	teaBr	0.1	2.03 ± 0.2	98
Zn^{2+}	pH titration and computer analysis		30	0.525	teaBr	0.1	2.09 ± 0.2	98
Cu^{2+}	pH titration and computer analysis		30	0.525	teaBr	0.1	1.94 ± 0.2	98

ATP ($atpH_4$): $atpMH^- + H^+ \leftrightarrow {}^+HatpMH^-$

M	Method	pH	Temp, °C	ATP, m*M*	Supporting electrolyte	μ	Log K^H_{HatpMH}	Ref
Mg^{2+}	pH titration		20	0.8	KCl	0.1	3.44	49
Mg^{2+}	Infrared spectra		32	100.0	$MgCl_2$ (0.1 *M*)	~0.6	3.80[f]	67
Ca^{2+}	Infrared spectra		32	100.0	$CaCl_2$ (0.1 *M*)	~0.6	3.80[f]	67
Mn^{2+}	pH titration		20	0.8	KCl	0.1	3.37	49
Ni^{2+}	Infrared spectra		32	100.0	$NiCl_2$	~0.6	3.70[f]	67
Zn^{2+}	pH titration		20	0.8	KCl	0.1	3.27	49
Zn^{2+}	Infrared spectra		32	100.0	$ZnCl_2$ (0.1 *M*)	~0.6	3.65[f]	67
Cu^{2+}	pH titration		20	0.8	KCl	0.1	2.68	49
Cu^{2+}	Infrared spectra		32	100.0	$CuCl_2$ (0.1 *M*)	~0.6	3.55[f]	67
			32	100.0	$Cu(NO_3)_2$ (0.1 *M*)	~0.6	3.40[f]	

[a] Since the ionizable proton of the ribose moiety of the nucleotide is not included in the formulas for the description of ionizations, *i.e.*, $atpH_4$, $adpH_3$, and $ampH_2$, it was felt that it would be confusing to do so for adenosine. Therefore, un-ionized adenosine is represented as "a," which symbol includes the ionizable proton of the ribose moiety. [b] A correction was applied for Na^+ competition. [c] In this reference pK' values are expressed as analytic functions of ionic strength which gives pK' = ±0.04 over the ionic strength range 0–0.2 at 10, 25, and 40°. [d] Later revised by the same author. [e] A correction was applied for Na^+ and K^+ competition. [f] pD value of a solution 0.1 *M* in Na_2H_2ATP and 0.1 *M* in MCl_2 or $M(NO_3)_2$ at which the conjugate acid:conjugate base ratio for the group in question = 1. This pD value was also corrected for solvent isotope effect: 0.6 pK unit for ionization of H_2ATP^{2-} (ring), and 0.2 pK unit for $HATP^{3-}$ (secondary phosphate chain). [g] Estimated from the data of this reference.

groups of alkali, alkali earth, and transition metals in the following relation: transition > alkali earth >> alkali.

(c) There are considerable discrepancies in reported values for complex formation constants. This might be attributed to two factors. First, the various methods used are almost all indirect methods, and thus incorporate the uncertainties of another system and its relation to the one being measured into the measurements. Secondly, a variety of supporting electrolytes were used, some of which contain cations, *e.g.*, Na^+, K^+, that form complexes of considerable stability with ADP and ATP.

(d) Very few values given are zero ionic strength. Note the difference that extrapolation to zero ionic strength makes for a reaction with large charge cancellation, *e.g.*, log K^{Mg}_{atpMg} in Table I. On the other hand extrapolation would make little difference for the equilibrium constant of a reaction with little or no change in charge, *e.g.*, for the ring ionization of adenosine.

2. *Complexes Involving Primary Phosphate Hydrogens*

As can be seen from Table I, very little ionization data are available for primary ionizations of the adenine nucleotides. In the absence of such data, these ionization constants have been estimated by analogy with similar compounds. The pK values for AMP, ADP, and ATP have been estimated as follows: AMP (one primary phosphate hydrogen) 1.0; ADP (two primary phosphate hydrogens) 1.0 and 2.0; ATP (three primary phosphate hydrogens) 1.0, 1.0, and 2.0 (44). Given these pK values, at pH 3 roughly 10% of the final primary phosphate hydrogen would be un-ionized for ATP and ADP. At lower pH values even more primary phosphate hydrogen would be un-ionized, including the pK = 1.0 phosphate ionizations. Therefore, the net charge on the phosphate chain of ATP and ADP at pH <3 would be an average value for several differently protonated species. This net charge on the phosphate chain would, of course, vary from 0 at pH −1 to −3 for ATP and −2 for ADP at pH 4. At pH values between −1 and 3, therefore, the following is true: (1) the phosphate chain is a mixture of differently protonated species, making structural interpretations of equilibrium data difficult; (2) even the net charge on the phosphate chain is not well known because of the uncertainties in the primary phosphate ionization pK values.

Keeping these limitations in mind, experimental studies in this pH range may still yield useful information about the structure of metal nucleotide complexes. One such study by Watanabe, Evenson, and Gulz employs a fluorescence technique at pH 2.8 for MgATP binding (131, 132). Apparently, this pH was selected in an effort to have ATP with all primary phosphate hydrogens ionized and the ring hydrogen (pK =~4) un-ionized. Given a pK of 2.0 for the final primary phosphate ionization, this would only be roughly true. Nevertheless, the results are valid and describe Mg^{2+}

TABLE II
THERMODYNAMIC DATA FOR THE IONIZATION AND 1:1 METAL COMPLEX FORMATION OF ADENOSINE AND THE ADENINE NUCLEOTIDES

Adenosine: a

Reaction	Method	pH	Temp, °C	Supporting electrolyte	μ	ΔF, kcal/mole	ΔH, kcal/mole	ΔS, eu	Ref
a + $H^+ \rightleftharpoons {}^+Ha$ (ring ionization)	pH titration		25	NaCl	0.15	−4.95	−1.0	13	2[a]
	pH titration		25		0.005	−4.77	−3.4	5	51
	Calorimetry		25	NaCl	0.1		−3.81 ± 0.11		105
	Thermometric titration		25	tpaBr	0.0251		−3.00 ± 0.1		20
					0		−3.1 ± 0.1		
$a^- + H \rightleftharpoons a$ (ribose ionization)	Entropy titration		25			−16.85 ± 0.04	−9.7 ± 0.1	23.9 ± 0.3	58

AMP: $ampH_2$

Reaction	Method	pH	Temp, °C	AMP, mM	Supporting electrolyte	μ	ΔF, kcal/mole	ΔH, kcal/mole	ΔS, eu	Ref
$amp^{2-} + H^+ \rightleftharpoons ampH^-$	pH titration		25		NaCl	0.15	−8.25	1.0	31	2[a]
	Thermometric titration		25		tpaBr	0.0592		0.36 ± 0.1		20
			25		tpaBr	0		1.8 ± 0.1		
	pH titration		25	0.5	tpaBr	0	−9.10 ± 0.02	0.85 ± 0.3	33.4 ± 1.0	103
$ampH^{1-} + H^+ \rightleftharpoons$	pH titration		25		NaCl	0.15	−5.10	−1.0	14	2[a]
$^+HampH^-$	Thermometric titration		25		tpaBr	0.0152		−3.91 ± 0.1		20
			25		tpaBr	0		−4.2 ± 0.1		

ADP: $adpH_3$

Reaction	Method	pH	Temp, °C	ADP, mM	Supporting electrolyte	μ	ΔF, kcal/mole	ΔH, kcal/mole	ΔS, eu	Ref
$adp^{3-} + H^+ \rightleftharpoons adpH^{2-}$	pH titration		25		NaCl	0.15	−8.55	0.3	30	2[a]
	Thermometric titration		25			0.0556		0.48 ● 0.1		20
						0		1.3 ± 0.1		
	pH titration		25	0.5	tpaBr	0	−9.83 ± 0.02	1.37 ± 0.3	37.5 ± 1.0	103
$adp^{3-} + Mg^{2+} \rightleftharpoons$	Spectrophotometric Mg^{2+} indicator competition	7.9–8.8	25		tbeaBr	0.11	−4.55	5.9	35	17[a]
$adpMg^-$	Calcd extrapolation of Burton's 1959 results	7.9–8.8	25		tbeaBr	0	−5.6	5.8 ± 1.0	38 ± 3	43
	Resin competition	8.7	25	0.07	tpaBr	0	−5.83 ± 0.14	4.3 ± 0.3	33.9 ± 1.5	101
$adpH^{2-} + H^+ \rightleftharpoons$	pH titration		25		NaCl	0.15	−5.39	−1.0	15	2[a]
$^+HadpH^{2-}$	Thermometric titration		25		tpaBr	0.028		−4.83 ± 0.1		20
			25		tpaBr	0		−4.1 ± 0.1		
$adpH^{2-} + Mg^{2+} \rightleftharpoons$ adpMgH	Resin competition	4.1	25	0.07	tpaBr	0	−3.35 ± 0.27	0.9 ± 1.4	14.3 ± 5.0	101
$adpMg^- + H^+ \rightleftharpoons adpMgH$	pH titration		25	0.5	$MgCl_2$	0	−7.35 ± 0.14	−2.02 ± 0.81	17.9 ± 3.0	101

ATP: $(atpH_4)$

Reaction	Method	pH	Temp, °C	ATP, mM	Supporting electrolyte	μ	ΔF, kcal/mole	ΔH, kcal/mole	ΔS, eu	Ref
$atp^{4-} + H^+ \rightleftharpoons atpH^{3-}$	pH titration		25		NaCl	0.15	−8.85	0.7	32	2[a]
	Thermometric titration		25		tpaBr	0.177		1.23 ± 0.1		20
			25		tpaBr	0		1.2 ± 0.1		
	pH titration		25	0.5	tpaBr	0	−10.48 ± 0.02	1.68 ± 0.3	40.7 ± 1.0	103
$atp^{4-} + Mg^{2+} \rightleftharpoons atpMg^{2-}$	Resin competition	8.8	25	1.0	NaCl[b]	0.15	−4.56	4.12	29.1	92
	Resin competition	8.7	25	0.07	tpaBr	0	−7.95 ± 0.10	5.1 ± 0.3	43.7 ± 1.5	101
$atp^{4-} + Ca^{2+} \rightleftharpoons atpCa^{2-}$	Resin competition	8.8	25	1.0	NaCl[b]	0.15	−4.00	4.56	28.7	92
$atpH^{3-} + H^+ \rightleftharpoons {}^+HatpH^{3-}$	pH titration		25		NaCl	0.15	−5.45	0	18	2[a]
	Thermometric titration		25		tpaBr	0.098		−4.5 ± 0.1		20
			25		tpaBr	0		−3.7 ± 0.1		
$atpH^{3-} + Mg^{2+} \rightleftharpoons$ $atpMgH^-$	Resin competition	4.1	25	0.07	tpaBr	0	−4.89 ± 0.16	2.2 ± 1.25	23.8 ± 4.0	101
$atpMg^{2-} + H^+ \rightleftharpoons$	pH titration		25	0.5	$MgCl_2$	0	−7.42 ± 0.07	−1.22 ± 0.55	20.8 ± 2.0	101
$atpMgH^-$	Spectrophotometric Mg^{2+} indicator competition	8.4–8.8	25		tbeaBr	0.11	−6.27	4.6	36	17[a]
	Calcd extrapolation of Burton's 1959 results	8.4–8.8	25		tbeaBr	0	−7.8	4.8 ± 1.0	42	43

[a] Calculated from the data presented in this reference. [b] A correction was applied for Na^+ competition.

binding and proton dissociation of ATP where the phosphate chain has those species and that net charge proper to pH 2.8. These fluorescence data have been interpreted in terms of five proton dissociation and Mg^{2+} complex formation equilibria for which equilibrium constants were determined. These constants and the experimental conditions under which they were measured are given in Table III. It should be

TABLE III[a]

EQUILIBRIUM DATA FOR THE IONIZATION OF ATP AND THE FORMATION AND IONIZATION OF MgATP INVOLVING PRIMARY PHOSPHATE HYDROGENS AT pH 2.8, ROOM TEMPERATURE, IN 0.5 *M* tmaCl (131)

ATP reaction	Log *K*
(1) $NH^+—PH \rightleftharpoons NH^{+}\cdots P^- + H^+$	−1.3
(2) $NH^{+}\cdots P^- \rightleftharpoons N—P^- + H^+$	−4.1
(3) $NH^{+}\cdots P^- + Mg^{2+} \rightleftharpoons NH^+—PMg$	2.0
(4) $NH^+—PMg \rightleftharpoons N—PMg + H^+$	−3.8
(5) $N—P^- + Mg^{2+} \rightleftharpoons N—PMg$	2.3

[a] P^- represents the phosphate chain with the charge it would have at pH 2.8.

noted in these reactions as written in Table III, that the symbol P^- represents a phosphate chain with the negative charge that it would have at pH 2.8. Also in symbols of the type $NH^{+}\cdots P^-$, the dotted line represents some sort of electrostatic effect and not necessarily a bond in the ordinary use of the term. (More will be said about this in the section on structural implications below.)

A low pH study has also been carried out by Gaucher on the binding of Fe^{3+} to ATP and ADP using a spectrophotometer indicator (5-sulfosalicylic acid) competition method (42). The apparent binding constants for Fe^{3+} to ATP and ADP as determined in this study at pH 2.0 are given in Table IV. The reactions are given in Table IV both as they were written by Gaucher and as they would be written in the symbolism of Watanabe, Evenson, and Gulz, for the sake of comparison. It should be noted that, although in this symbolism the ferric ion reactions are written the same as the third reaction in Table III for binding of Mg^{2+} ion to $NH^{+}\cdots P^-$, the net phosphate chain charge for the latter reaction is higher (or primary phosphate H^+ ion competition is less since it is at pH 2.8). Clearly in spite of the greater primary hydrogen ion competition in the Fe^{3+} reaction the binding of ferric ion is much greater than that of Mg^{2+} ion (6.59 for Fe^{3+}) as compared to 2.0 for Mg^{2+}.

TABLE IV[a]

EQUILIBRIUM DATA FOR THE FORMATION OF FeATP AND FeADP INVOLVING PRIMARY PHOSPHATE HYDROGENS AT pH 2.0, 25°, IN 0.1 *M* $NaClO_4$ (42, 131)

ATP reaction	Log *K*
$ATP + Fe \rightleftharpoons ATPFe$	6.59
$NH^{+}\cdots P^- + Fe^{3+} \rightleftharpoons NH^+—PFe$	
$ADP + Fe \rightleftharpoons ADPFe$	5.66
$NH^{+}\cdots P^- + Fe^{3+} \rightleftharpoons NH^+—PFe$	

[a] P^- represents the phosphate chain with the charge it would have at pH 2.0.

The reactions $ATPFe + Fe \leftrightarrow ATPFe_2$, and $ATPFe + ATP \leftrightarrow ATP_2Fe$ (42) are treated under multiple complexes.

3. *Complexes Involving Hydrolysis of the Hydrated Metal Ion*

The pH titration data of Kahn and Martell for the titration of Cu^{2+} and Zn^{2+} complexes of ATP and ADP with base beyond the secondary phosphate ionization of the phosphate chain show a concentration-dependent buffer region (63, 64). The data are interpreted in terms of four equilibria arising first from the formation of a hydroxo metal complex by a hydrolysis reaction of the type

$$atpCu^{2-} + H_2O \rightleftharpoons atpCu(OH)^{3-} + H^+$$

This monohydroxo metal complex can then either dimerize to form $[atpCu(OH)]_2^{6-}$ or hydrolyze further to form the dihydroxo metal complex, $atpCu(OH)_2^{4-}$. Equilibrium constants for several such reactions have been calculated by Kahn and Martell (63, 64) and are listed in Table V along with a combined hydrolysis and dimerization reaction. These constants have been used to calculate the percentage of each species present over the pH range 5–8 for CuATP (63). These calcu-

TABLE V

EQUILIBRIUM DATA FOR THE FORMATION OF HYDROXO, DIHYDROXO, AND DIMER COMPLEXES OF TRANSITION METAL COMPLEXES OF atp^{4-} AND adp^{3-}

ATP

Reaction	Log *K*	Ref[a]
Hydrolysis		
$atpCu^{2-} + H_2O \rightleftharpoons atpCu(OH)^{3-} + H^+$	−6.47	63
	−7.7	13
$atpZn^{2-} + H_2O \rightleftharpoons atpZn(OH)^{3-} + H^+$	−8.5	13
$atpNi^{2-} + H_2O \rightleftharpoons atpNi(OH)^{3-} + H^+$	−9.3	13
$atpCo^{2+} + H_2O \rightleftharpoons atpCo(OH)^{3-} + H^+$	−9.4	13
$atpMn^{2+} + H_2O \rightleftharpoons atpMn(OH)^{3-} + H^+$	−10.4	13
Dimerization		
$2atpCu(OH)^{3-} \rightleftharpoons [atpCu(OH)]_2^{6-}$	2.59	63
Hydrolysis and Dimerization		
$2atpCu^{2-} + 2H_2O \rightleftharpoons [atpCu(OH)]_2^{6-} + 2H^+$	−10.35	63
Double Hydrolysis		
$atpCu^{2-} + 2H_2O \rightleftharpoons atpCu(OH)_2^{4-} + 2H^+$	−13.49 ± 0.05	63

ADP

Reaction	Log *K*	Ref[a]
Hydrolysis		
$adpCu^- + H_2O \rightleftharpoons adpCu(OH)^{2-} + H^+$	−7.08	64
$adpZn^- + H_2O \rightleftharpoons adpZn(OH)^{2-} + H^+$	−8.51	64
Dimerization		
$2adpCu(OH)^{2-} \rightleftharpoons [adpCu(OH)]_2^{4-}$	3.42	64
$2adpZn(OH)^{2-} \rightleftharpoons [adpZn(OH)]_2^{4-}$	3.34	64
Hydrolysis and Dimerization		
$2adpCu^- + 2H_2O \rightleftharpoons [adpCu(OH)]_2^{4-} + 2H^+$	−10.73	64
$2adpZn^- + 2H_2O \rightleftharpoons [adpZn(OH)]_2^{4-} + 2H^+$	−13.68	64

[a] Experimental conditions: ref 64, 25° and 0.1 *M* KNO_3; ref 13, 22° and 0.1 *M* KCl.

lations indicate that the species constituting the largest percentage of the mixture are as follows: at pH 5.0 $atpCu^{2-}$; at pH 6.3–7.1 $atpCu(OH)^{3-}$ and $[atpCu(OH)]_2^{6-}$; at pH 8.0 $atpCu(OH)_2^{4-}$. Postulated structures for these complexes are given in Figure 1.

Equilibrium constants for the hydrolysis of several ATP–transition metal complexes have been reported by Brintzinger as pK's of the hydrated complex for the first ionization beyond the secondary phosphate (Table V) (13). These pK values were taken as the pH values of a CuATP solution after addition of 0.5 equiv of base beyond the end point of the secondary phosphate group. Since such a procedure does not take into account the large percentages of dimer and dihydroxo metal–ATP complexes, these constants should be regarded as estimates. The values obtained by this method can be compared with those given by the analysis of Kahn and Martell for only one reaction: the hydrolysis of $atpCu^{2-}$ which is given as the first entry in Table V. The reported values of log K for this reaction are -7.7 and -6.47, respectively. The latter should be regarded as a better value. Nevertheless, the work of Brintzinger indicates that hydrolysis to hydroxo complexes with subsequent dimerization tends to take place in the order $Cu^{2+} > Zn^{2+} > Ni^{2+} > Co^{2+} > Mn^{2+} > Mg^{2+}$ with Cu^{2+} beginning this behavior at the lowest pH (13). Further indicated in this study is the fact that such hydrolysis takes place at a much higher pH in triphosphate than in ATP. Also, complexes of AMP and ribose 5-phosphate seem to hydrolyze in this region in a way similar to ATP. It might be concluded from these two observations that the ribose group is somehow involved in the hydrolysis of transition metal complexes of ATP in the pH range above the end point of the secondary phosphate hydrogen (13).

4. *Multiple Complexes*

Complexes containing two or more metal ions and/or nucleotide ions have been reported by several investigators (9, 17, 42, 45, 49, 63, 64, 71, 82, 119). However, very little quantitative data are available. Two of these studies assume the presence of small amounts of multiple complexes for the purpose of calculation in pH titration data (9, 119) and spectrophotometric metal ion indicator competition (17). Others are interpretations of enzyme kinetics as indicating mechanisms involving multiple complexes (71, 82). Another reports an Ag–adenosine polymer which occurs as a precipitate (45). The hydroxo metal nucleotide dimers of Kahn and Martell (63, 64) were discussed in the previous section. This leaves two quantitative studies: that of Gaucher (42) on multiple complexes of ferric ion with ATP and ADP and that of Handschin and Brintzinger on 2:1 transition metal–ATP complexes (49). The data from these studies have been collected in Table VI. Gaucher also presents qualitative evidence for the existence of an ATPFeMg complex in solution at pH 2 (42).

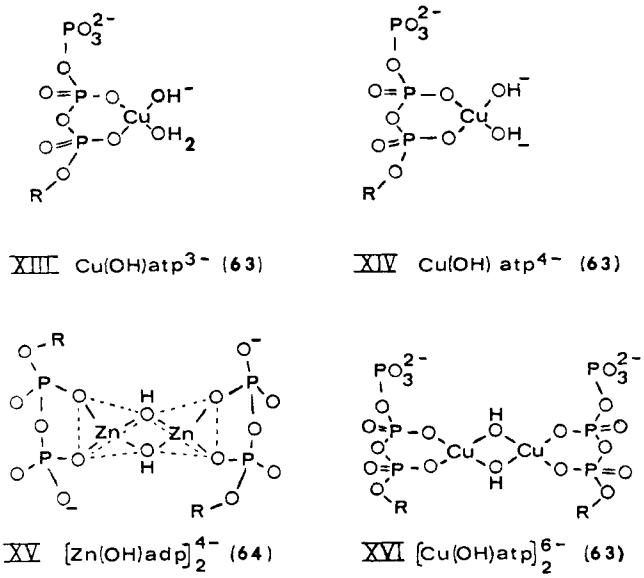


Figure 1.—Suggested structures for the hydroxo, dihydroxo, and dimer complexes of transition metal complexes of atp^{4-} and adp^{3-}.

5. *Folded Complexes*

There have been several physical studies (*cf.* section III) and a great deal of discussion about the folded structure of metal–ATP and –ADP complexes. Apparently, however, the first to study the equilibria between the folded and linear forms of those complexes quantitatively were Schneider and Brintzinger (110). By use of a ultraviolet spectrophotometric technique in which the difference spectra of adenosine–metal complexes against free adenosine were compared to the difference spectra of ATP–metal complexes against free ATP, the folding constant α was determined for several ATP–metal complexes. These constants, which represent the fraction of folded complex, are given in Table VII.

D. STRUCTURAL IMPLICATIONS

1. *The Free Compounds*

a. The Basic Structural Equilibrium

In contrast to the structure of metal–nucleotide complexes, the structure of free nucleotides in solution has received little attention. The few arguments based on equilibrium and thermodynamic data that have been found will be presented here, but clearly the conclusion leaves much to be desired.

It is clear from the use of models that free rotation in the N_9–C_1', C_4'–C_5', and C_5'–O bonds allows the terminal phosphate groups of ATP and ADP to be brought into close proximity to the NH_2 group of the adenine ring (88, 125). This, however, is merely suggestive. A

TABLE VI

EQUILIBRIUM DATA FOR THE FORMATION OF 2:1 METAL-NUCLEOTIDE COMPLEXES AND THE FORMATION OF $(ATP)_2Fe$

ATP ($atpH_4$): $atpM^{2-} + M^{2+} \longleftrightarrow atpM_2$

M	Method	pH	Temp, °C	ATP, mM	Supporting electrolyte	μ	Log $K^{M}_{atpM_2}$	Ref
Mg^{2+}	pH titration		20	0.8	KCl	0.1	None	49
Mn^{2+}	pH titration		20	0.8	KCl	0.1	1.37	49
Zn^{2+}	pH titration		20	0.8	KCl	0.1	1.41	49
Cu^{2+}	pH titration		20	0.8	KCl	0.1	1.88	49

$ATM + M \longleftrightarrow ATPM_2$[a]

M	Method	pH	Temp, °C	ATP, mM	Supporting electrolyte	μ	Log $K^{M}_{ATPM_2}$	Ref
Fe^{3+}	Spectrophotometric Fe^{3+} indicator competition	2.0	0	1.1–1.6	$NaClO_4$	~0.1	3.20[a]	42

$ATPM + ATP \longleftrightarrow (ATP)_2M$[a]

M	Method	pH	Temp, °C	ATP, mM	Supporting electrolyte	μ	Log $K^{ATP}_{(ATP)_2M}$	Ref
Fe^{3+}	Spectrophotometric Fe^{3+} indicator competition	2.0	0	1.1–1.6	$NaClO_4$	~0.1	3.54[a]	42

[a] At pH 2, this reaction would involve a mixture of variously ionized and conplexed species, including primary phosphate ionizations. The given K, therefore, represents a net reaction equilibrium "constant."

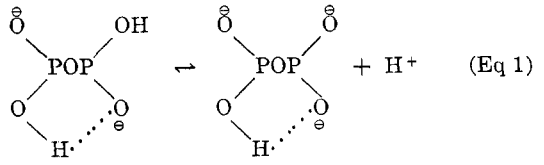
TABLE VII

FRACTION OF FOLDED COMPLEX FOR VARIOUS $ATPM^{2-}$ COMPLEXES AT IONIC STRENGTH 0.15–9.0 WITH $M(ClO_4)_2$ AS THE SUPPORTING ELECTROLYTE AND PRESUMABLY AT pH 5–6 AND ROOM TEMPERATURE (110)[a]

M	α
Mg^{2+}	~0.01–0.03
Ca^{2+}	~0.01–0.03
Mn^{2+}	0.03
Co^{2+}	0.12
Zn^{2+}	0.15
Ni^{2+}	0.20
Cu^{2+}	0.80

[a] α indicates fraction of complex folded, back bound, or in the metal bridge form.

more positive argument for the hydrogen bonded, folded structure is based on the magnitude of the pK values of the final ionization of pyrophosphate, ADP, and ATP as follows (88): the pK of the final ionization of $H_4P_2O_7$ is 9.6 (89) and that of $H_5P_3P_{10}$ is 9.3 (102). It might be expected that the pK values of the final ionizations of ADP and ATP would be of the same order, but they are not. The pK values of the final ionizations of ADP and ATP are as determined by Melchior, 6.7 and 6.9, respectively (88). They are very similar to the next to the final ionizations of $H_4P_2O_7$ and $H_5P_3O_{10}$ which are both 6.6 (89, 102). Calculations of pK values by the method of Branch and Calvin (10) indicate that this difference is due to the fact that, in the next to the final ionization, the final hydrogen is hydrogen bonded to a negative oxygen of the group from which the next to the final hydrogen is dissociating, *i.e.*



(Eq 1)

(This would also explain the extraordinarily high pK of the final ionization.) Melchior concludes that there must be a similar hydrogen bond to a negative oxygen of the phosphate group from which the final proton in ATP and ADP is dissociating. This proton bond, he concludes, must either come from the NH_2 group of the ring, or the C-2′ or C-3′ hydroxyls of the ribose which in any case means that the ATP and ADP molecules must be folded (88).

The possibility of the final hydrogen of ATP and ADP hydrogen bonding to the nitrogen of the NH_2 group is also pointed out. This would tend to make the pK of the last ionization higher. Small percentages of this form might account for the fact that the actual final pK value of ATP and ADP are 0.6 and 0.3 unit higher than the values calculated assuming full hydrogen bonding to a negative oxygen of the ionizing phosphate group (88).

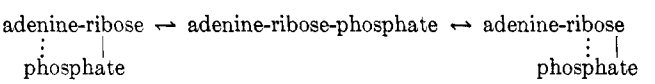
Further evidence for a folded structure of ATP and ADP might be found in the ring pK values of adenosine, AMP, ADP, and ATP, which are 3.6, 3.8, 4.0, and 4.0, respectively (Table I). Melchior argues that if the nucleotides were of the linear form in solution no electrostatic effect from the addition of phosphate groups would be observed in the ring ionization. The conformation of $^{+}HadpH^{2-}$ and $^{+}HatpH^{3-}$, then, must be one in which the phosphate chain is in the neighborhood of the ring ionization, *i.e.*, folded (88). Further confirmation of an electrostatic ring–chain effect in ATP when the ring is positively charged and the chain negatively charged may be found in the fact that addition of a Mg^{2+} ion to the phosphate chain of ATP at pH 2.8 lowers the pK of the ring ionization from 4.1 to 3.8 (131).

Evidence which indicates a lack of ring–chain interaction at higher pH values where the ring carries no positive charge may be found in a recent study of the

thermodynamics of the secondary phosphate ionizations of guanosine, inosine, cytidine, uridine, and adenosine phosphates (102). It was found that the values of $\Delta F°$, $\Delta H°$, and $\Delta S°$ were the same for the five triphosphates as well as the diphosphates and monophosphates in spite of the fact that five different ring structures were present in each set of phosphates. It should be noted that although some of these rings have an H bond forming capability while others do not, none of them have a net charge on the ring in the pH range where the secondary phosphate ionization is operative and was studied. Therefore, this result is compatible with the previous argument in which addition of phosphate groups to the phosphate chain exerted an effect on the ring ionization of the $^{+}$Ha, $^{+}$HampH^{-}, $^{+}$HadpH^{2-}, $^{+}$HatpH^{3-} series.

In Summary.—It seems that the following types of intramolecular interaction are sterically and energetically possible in ADP and ATP: 1. H bond formation between negative oxygens of the phosphate chains and the C-2′ and C-3′ hydroxyls of the ribose moiety (several combinations). 2. H bond formation between negative oxygens of the terminal group of the phosphate chain and the hydrogens of the ring NH_2 group. 3. H bond formation between the secondary phosphate hydrogen of the terminal phosphate group and the nitrogen of the ring NH_2 group. 4. Electrostatic interaction between the positively charged, protonated ring and the negative phosphate chain, with as yet unassessed conformational effects.

It must be admitted then that the general structure of ATP and ADP in solution is described by the following equilibrium

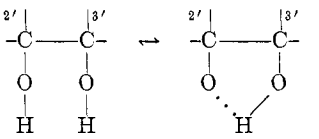


(Eq 2)

where the dotted lines represent hydrogen bonds or electrostatic interaction. Working against the ordering tendency of hydrogen bond formation and electrostatic interactions would be the disruptive effects of hydration and thermal motion (which would be a sizeable factor for such large structural units as adenine, ribose, and the phosphate chain). However, at present it is difficult to say where the balance lies. This remains to be determined by suitable experimental studies.

b. Various Structural Information from Ionization Data

It is of some interest in connection with the above-mentioned possibilities of ribose hydrogen bonding in the adenine nucleotides that a ribose pK has recently been determined in adenosine by an entropy titration method (58). It has been found that both the C-2′ and C-3′ hydroxyls are necessary for the ionization. If either is blocked, the ionization does not occur. It has been postulated that this requirement of both the C-2′ and C-3′ hydroxyls might be due to one or both of the following: 1. The combined inductive effect of the vicinal C-2′- and C-3′-hydroxyl groups. 2. Stabilization of the ribose moiety anion by formation of a hydrogen bonded ring:



(Eq 3)

Further data for this ionization in AMP, ADP, and ATP should provide information with regard to the role of the phosphate chain in C-2′ and C-3′ hydroxyl hydrogen bonding in the nucleotides.

With regard to the relation of the adenine nucleotides in solution to the solution medium, it has been found that these ions have activity coefficient ratios in accordance with the simple Debye-Hückel theory (103). According to this theory, the relation between the apparent pK at a finite ionic strength designated as pK' and the thermodynamic pK designated as pK^0 at 25° for any ionization in aqueous solution is

$$pK' = pK^0 - 0.509(Z_A^2 - Z_{HA}^2)\sqrt{\mu} \quad \text{(Eq 4)}$$

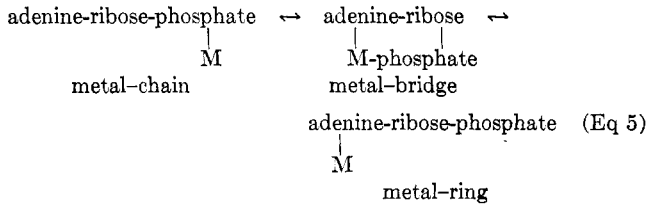
where Z_{HA} = the net charge on the ionizing species, Z_A = the net charge on the conjugate base, μ = the ionic strength of the medium. Thus, for the secondary phosphate ionizations of AMP, ADP, and ATP, the slope of an experimental plot of pK' *vs.* $\sqrt{\mu}$ should be 1.52, 2.54, and 3.56, respectively. These experimental plots of pK' *vs.* $\sqrt{\mu}$ were carried out by Phillips, George, and Rutman (103) and it was found that the slopes of the curves were 1.5, 2.5, and 3.5 at $\mu < 0.01$, where tpaBr was the supporting electrolyte. These studies were later extended to the secondary phosphate ionizations of guanosine, inosine, cytidine, and uridine tri-, di-, and monophosphates (102). It was found that when the pK' *vs.* $\sqrt{\mu}$ curves were expressed in terms of the empirical equation, $pK' = pK^0 - a\sqrt{\mu} + b\mu$, the b constants grouped according to the number of phosphate groups in the phosphate chain as follows at 25°; triphosphates 5.48 ± 0.16, diphosphates 4.19 ± 0.16, and monophosphates 2.93 ± 0.16. No correlation could be made between the variations in the b constants of each phosphate type and ring structure (102).

2. The Metal Complexes

a. The Basic Structural Equilibrium

The many equilibrium and thermodynamic studies of metal–adenine nucleotide complexes have sometimes been in disagreement among themselves and often in disagreement with physical studies as to the structure of metal–nucleotide complexes. These difficulties have been largely removed by recent work, especially that

of Brintzinger. An over-all view of the work to be outlined in the following sections b–d, show that the metal–adenine nucleotide complexes are actually in a variety of forms which are in equilibrium. In general terms and neglecting the formation of multiple complexes, this equilibrium is given as



(Eq 5)

For a given nucleotide, the percentages of each species would vary according to the nature of the metal ion, M, and experimental conditions. For a number of metal ions one or even two of the three general structures might be statistically unimportant to a given analytical method. Nevertheless, depending on the nature of M, any one of the three general types might predominate. This is due to the fact that the adenine nucleotides really have two binding sites, a primary electrostatic site on the phosphate chain and a secondary nitrogen chelating site on the adenine ring. Thus, a metal ion which is electrostatically strong (high net charge and high charge density) and has little nitrogen chelating tendency, like Mg^{2+}, would tend to form virtually all metal–chain complex. A metal ion which is electrostatically strong and also has strong nitrogen chelating tendency, like Cu^{2+}, would tend to form the bridge species. These two cases of Mg^{2+} and Cu^{2+} have been verified by experiment; however, an example of a complex in which the third species (metal–ring) predominates has not been forthcoming from experiment. It would seem logical, however, to predict that Ag^{+} would be an example of a metal ion which would form predominantly the metal–ring species in complexing with the adenine nucleotides, since it has strong nitrogen chelating tendencies and is relatively weak electrostatically.

In the three sections to follow is a sketch of the experimental studies which show the existence of the three general structures of (Eq 5) and in some cases elucidate aspects of structure (Figure 2).

b. The Metal–Chain Complex

In the preceding section, the phosphate chain was referred to as the primary metal binding site of the adenine nucleotides while the ring nitrogens were referred to as the secondary binding site. It might be objected that this is misleading since for *some* metal ions, *e.g.*, Ag^{+}, the ring nitrogens might be the primary binding site and the phosphate chain secondary. Although this is true, it was felt that since for all metals experimentally studied until now, *i.e.*, mainly alkali, alkali earth, and first row transition metals, the phosphate chain was a prime factor in complex formation, proper emphasis demanded that it be referred to as the primary binding site.

For example, in the work of Schneider and Brintzinger (110) it is reported that for $Mg^{2+}$and Ca^{2+} complexes of atp^{4-}, only 1–3% is in the metal–bridge form and an amount that was undetectable by their experimental method in the metal–ring form. Even in the first-row transition metal complexes, according to the data in Table VII, the phosphate chain dominates binding. Therefore, this present section on metal–phosphate chain binding is of central importance.

Treated in this section are the fact of metal–chain binding and several aspects of structure as derived from equilibrium and thermodynamic data.

The Fact of Metal–Chain Binding.—At least four different arguments have been advanced in support of the fact of metal–chain binding: 1. The magnitude of the binding constants of the adenine nucleotides for all metal ions studied are in the order ATP > ADP > AMP (Table I). Several investigators have concluded from this that the phosphate chain is the primary (or the only) metal ion binding site (42, 63, 64, 84, 92, 114, 117, 129, 134). 2. Metal ion binding constants have been determined for guanosine, inosine, cytidine, and uridine phosphates as well as for the adenosine phosphates. These constants have been determined for Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+} by Walaas (129) and for Fe^{3+} by Gaucher (42). It was found that for the given metal ions the complex formation constants were the same within experimental error for all (guanosine, inosine, cytidine, uridine, and adenosine) tri-, di, and monophosphates. The ring structure, therefore, seems to make no difference to the magnitude of the formation constants which indicates chain binding. 3. Various ionization and metal complex formation constants of ATP have been determined by Handschin and Brintzinger (49). As is shown in this reference, the magnitudes of these constants indicate predominantly chain binding as follows: $pK^{M}_{MHL} - pK^{M}_{MH_2L}$ for ATP, where M is Mg^{2+}, Mn^{2+}, Zn^{2+}, and Cu^{2+} is 0.5, 0.6, 0.7, and 1.3 units, respectively. This means that having a positively charged, protonated ring in ATP does not make a great deal of difference to the net binding of M^{2+} as reflected in the binding constant which in turn implies that the binding is taking place at the phosphate chain (the observed change in the binding constant is explained as an electrostatic effect). Further, the quantity, $pK^{H}_{HL} - pK^{H}_{MHL}$ for ATP where M^{2+} is Mg^{2+}, Mn^{2+}, Zn^{2+}, and Cu^{2+} equals 1.7, 1.9, 2.0, and 2.6, respectively. This large effect on the secondary phosphate chain ionization is taken to be evidence that the metal ions are binding to the chain (49). Smith and Alberty develop this argument further by pointing out that for the ionizations of $atpMH^{-}$ and adpMH, which they also have determined, the greater the affinity of the

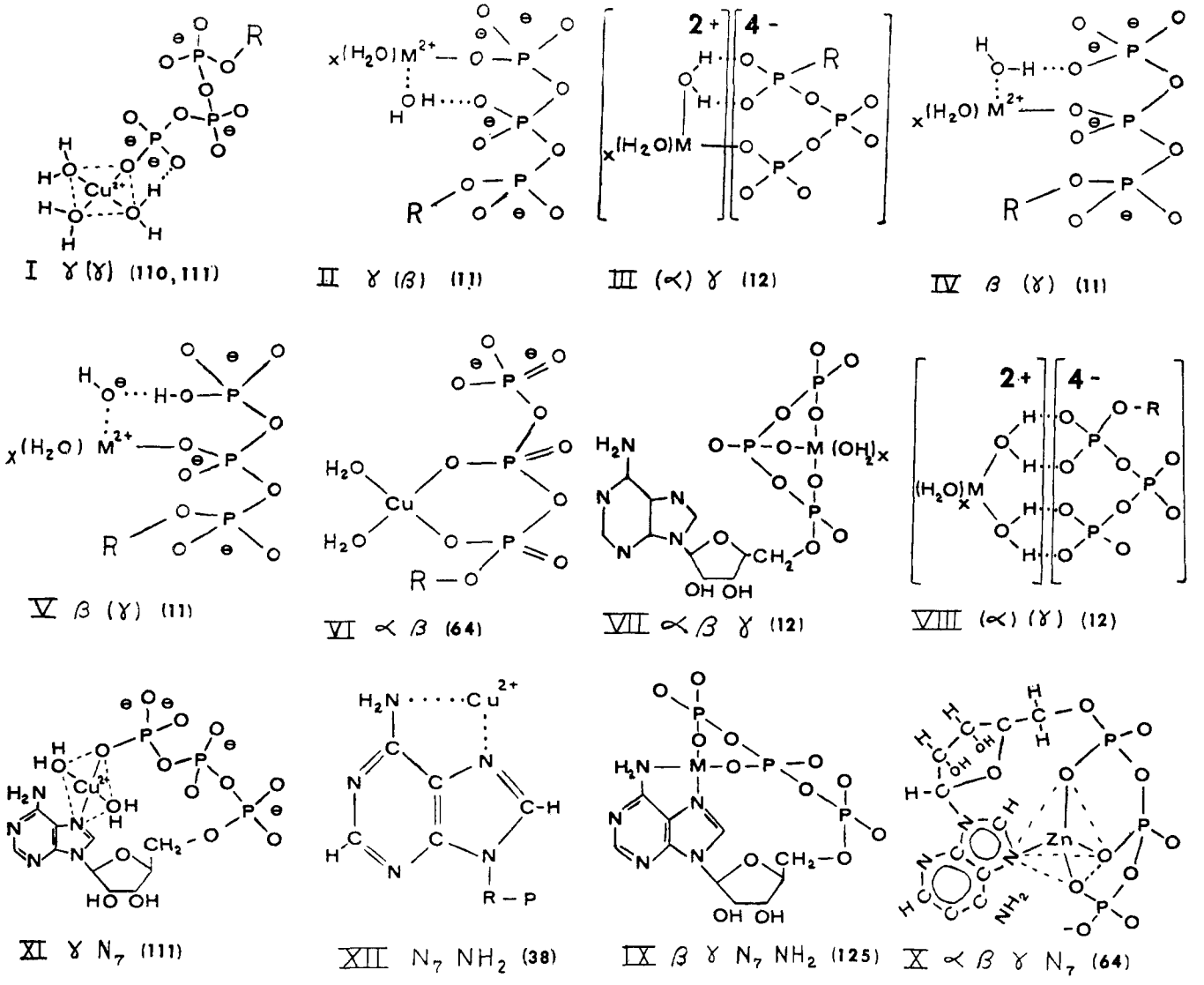


Figure 2.—Suggested structures for the atpM^{2-} complex. Under each structure are listed the sites involved in the binding. Those in parentheses signify binding through a water molecule.

metal ion in the complex for the fully ionized species atp^{4-} and adp^{3-}, the greater is the acidity of the proton remaining on the phosphate chain (117, 118). This would be further evidence that in this complex the metal ion is binding to the phosphate chain. 4. Brintzinger has constructed an argument to show that the binding of Mn^{2+}, Co^{2+}, Ni^{2+}, and Zn^{2+} by ATP is on the phosphate chain by comparing binding constants for these equilibria with those for other complex forming compounds. The compounds chosen were 8-hydroxyquinoline (binding to ring N), oxalate (binding to negative oxygens on adjacent carbons), malonate (binding to negative oxygen atoms separated by a carbon atom), and sulfate (binding to negative oxygen atoms on the same sulfur atom and known to associate as a hydrated ion pair). It was found that the orders of magnitude of the log of the binding constants for the Mn^{2+}, Co^{2+}, Ni^{2+}, and Zn^{2+} complexes of these compounds were as follows: atp^{4-}, 4.5–4.8; 8-hydroxyquinoline, 7.8–9.9; oxalate, 3.9–5.3; malonate, 3.3–4.1; and sulfate, 2.3–2.5. On the basis of the similarity in the magnitude of constants between atp^{4-} and oxalate it was concluded that atp^{4-} was binding these M^{2+} ions through adjacent negatively charged oxygen atoms, *i.e.*, on the phosphate chain (12).

The Metal–Chain Complex Is a Hydrated Ion Pair.—In the previous section it was noted that the complex formation constants for complexes of Mn^{2+}, Co^{2+}, Ni^{2+}, and Zn^{2+} with atp^{4-} and sulfate ion are 4.5–4.8 and 2.3–2.5 (12). Clearly, in both sets of complexes the electronic makeup of the metal ion seems to make little difference to the extent of binding. Rather, the electrostatic nature of the ion seems to be the determining factor, which argues to a mediate bonding through hydrated water molecules. Further, this has actually been shown to be the case for sulfate ion complexes

(120). It is concluded, therefore, that the atp^{4-} complexes are also hydrated ion pairs (12).

Further indication of ion-pair association has been given by distance of nearest approach calculations according to the theory of Fuoss and Kraus (41).

The basic relation is

$$K = A_0 \exp\left(-\frac{Z_1 Z_2 e^2}{DkTa}\right) \qquad \text{(Eq 6)}$$

where K = the empirical association constant; A_0 = a constant which for these calculations has been set equal to 1 (12); D = the macroscopic dielectric constant of the medium; a = distance between centers of positive and negative charge in the ion-pair complex.

Distances between centers of positive and negative charge were calculated both for the Mn^{2+}, Co^{2+}, Ni^{2+}, and Zn^{2+} sulfate and atp^{4-} complexes (12). The sulfate Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+} distances were 5.4, 5.2, 5.3, and 5.3; the atp^{4-} Mn^{2+}, Co^{2+}, Ni^{2+}, and Zn^{2+} distances were 5.2, 5.3, 5.4, and 5.2, respectively. (Although these values are reliable relative values, because of neglect of ionic strength, the macroscopic dielectric constant approximation, and neglect of hydrogen bonding, as absolute values they should be regarded as having an uncertainty of 20%.) Since the distances are reasonable ones for hydrated ion-pair association, and equal to those calculated in a similar way for a known series of ion-pair complexes, this has been taken to be further evidence of hydrated ion-pair binding in the metal-chain complexes (12). (*Cf.* the infrared study in section III for more structural detail with regard to this binding.)

Similar calculations which were carried out by Smith and Alberty (117, 118) for two series of metal ions shows that the relative distances between the centers of positive and negative charge in orthophosphate, AMP, ADP, ATP, and AQP complexes are in the order $Li^+ < Na^+ < K^+$ and $Mn^{2+} < Mg^{2+} < Ca^{2+} < Sr^{2+}$. This order corresponds to crystal radii, not to hydrated radii which would be in the opposite order; however, the ions with smaller crystal radii would be expected to polarize its hydration shell to a greater extent (107) and thus be capable of forming a stronger bond with a smaller distance between the centers of charge (117, 118). This agrees well with the work of Kahn and Martell discussed under the section "Complexes Involving Hydrolysis of the Hydrated Metal Ion," where the transition from polarization to hydrolysis of the hydrated water molecules is described.

$atpMgH^-$ and adpMgH are $\beta\gamma$ and $\alpha\beta$ Mg^{2+}-Chain Complexes, Respectively, with Something Less than a Full Bond to the Terminal Phosphate Group in Each Complex.—The data for the proton dissociation of these complexes are as follows: $atpMgH^-$, $pK^0 = 5.44$, $\Delta H^\circ = 1.22$ kcal/mole, $\Delta S^\circ = -20.8$ eu; and for adpMgH, $pK^0 = 5.38$, $\Delta H^\circ = 2.02$ kcal/mole, $\Delta S^\circ = -17.9$ eu. The similarity in these thermodynamic quantities indicates a similarity in the structural environment which the proton is leaving. Further, the magnitude of the pK^0 values indicates that only a partial charge cancellation is taking place at the negative oxygen of the terminal phosphate group. (Full cancellation of the negative charge, as by a hydrogen ion, would reduce the pK to 2.0 for both complexes.) In the adpMgH complex this would mean that the Mg^{2+} is probably somewhere between the α- and β-phosphate groups, and since the proton is leaving the same kind of structural environment, it is concluded that in the $atpMgH^-$ complex the Mg^{2+} ion would be between the β- and γ-phosphate groups (101). The partial charge cancellation of this negative oxygen of the terminal phosphate group by the Mg^{2+} ion would also be in accord with the hydrated ion theory of the previous section.

In MgATP and MgADP Complexes, the Mg^{2+} Ion Retains Much of Its Positive Character.—Thermodynamic data for the ionization and Mg^{2+} complex formation of ATP and ADP have enabled Phillips, George, and Rutman (101) to calculate relative partial molal entropies for the following two series of ions: $atpH^{3-}$, $atpMgH^-$, $atpMg^{2-}$, atp^{4-}, and $adpH^{2-}$, adpMgH, $adpMg^-$, adp^{3-}. Plotting these relative partial molal entropies against Z^2 should result in a straight line (28, 72). Assuming that Z was unknown for the Mg^{2+} complexes the values of for $atpH^{3-}$, atp^{4-}, $adpH^{2-}$, and adp^{3-} were plotted. The points for $atpMgH^-$, $atpMg^{2-}$, adpMgH, and $adpMg^-$ were placed on the lines according to their relative partial molal entropy values and Z^2 was read off the proper coordinate. This method indicated a $Z_{effective}$ for adpMgH, $adpMg^-$, $atpMgH^-$, and $atpMg^{2-}$ of 2.8, 3.1, 3.5, and 3.9, respectively (101). Since hydration is the major factor in the partial molal entropies of ions in water, this effective charge would refer specifically to effectiveness *in hydrating or fixing water molecules.* In any case, the effective charge values indicate that the Mg^{2+} charge in these complexes is a good distance away from the center of negative charge of the phosphate chain, approaching a zwitterion type of charge distribution.

The Proton in the $atpMH^-$ and adpMH Complexes Is on the Phosphate Chain.—In spite of the fact that a question has been raised in this regard (84), it is well accepted that the proton in the $atpMH^-$ and adpMH complexes is on the phosphate chain. Titrations in the presence and absence of Mg^{2+} and Ca^{2+} ions have shown that the $pK = \sim 4$ ring ionization is only slightly affected by the presence of these metal ions (101, 121). On the other hand, addition of Mg^{2+} or Ca^{2+} ions strongly increases the acidity of the secondary phosphate hydrogen (18, 49, 101, 118, 121). For example, according to Handschin and Brintzinger (49), the quantity $pK^{H}_{HL} - pK^{H}_{MHL}$ for ATP, where M^{2+} is

Mg^{2+}, Mn^{2+}, Zn^{2+}, and Cu^{2+}, equals 1.7, 1.9, 2.0, and 2.6, respectively. Thus, it seems clear that the proton in the $atpMgH^-$ and adpMgH complexes is the secondary phosphate proton of the phosphate chain which has been modified by the presence of M^{2+} on the chain.

Conformation of the Phosphate Chain as a Variable in Metal–Chain Complex Formation.—Intrachain conformational charges with complex formation is a factor which has received little attention in the study of metal–nucleotide complexes. It has, however, been predicted by the use of molecular models that considerable conformational changes in the phosphate chain are to be expected in complex formation. The chain would be expected to remain more or less linear for large cations and fold back on itself or "wrap around" smaller metal cations (88). This explanation has been offered to explain the relative binding abilities of AMP, ADP, and ATP (134). Models show that all cations with radius less than Na^+ would be expected to form a closed-chain structure, while monopositive cations with a radius greater than K^+ would be expected to form an open-chain structure (88).

Recent thermodynamic studies give some indirect, experimental evidence in support of intrachain conformational changes in complex formation. This evidence is given in a comparison of the thermodynamic data for the complex formation of $atpMg^{2-}$, $adpMg^{2-}$, and other Mg^{2+} phosphate complexes. Comparison of this data shows large differences in the thermodynamic quantities (10 eu in $\Delta S°$, 0.8 kcal/mole in $\Delta H°$, and 2.1 kcal/mole in $\Delta F°$). According to the evidence given in a previous section and to evidence presented below under physical studies, these complexes have very similar bonding in the phosphate chain, *i.e.*, to the α- and β-phosphate groups in $adpMg^-$ and the β- and α-phosphate groups in $atpMg^{2-}$. The large observed differences might be explained by the change in charge, which results in a change in chain conformation, both of which result in a change in hydration (101).

c. The Metal–Bridge Complex

If the fact is kept in mind that the adenine nucleotides have a primary (chain) and a secondary (ring) binding site, it will be clear that the arguments presented here are not in opposition to the arguments presented in the previous section to establish the fact of chain binding. In fact, to assert the formation of a metal–bridge species is to assert chain binding, since every bridge must be anchored at both ends. What is asserted in this section is that in *some* molecules the metal ion bound to the chain is also chelated to the nitrogens of the adenine ring in a way which does not greatly disturb primary (chain) site binding. In the two following sections, evidence from equilibrium studies for the existence of a metal–bridge species and some suggested structures for these metal–bridge species will be presented. (Confirmatory evidence may be found in section III.)

The Fact of Metal–Bridge Formation. 1. The a priori Argument.—According to Szent-Gyorgi, energy accepted at the adenine end of the ATP molecule must in some way be transferred to the phosphate end where it is stored and ultimately used. This could be brought about by hydrogen bonding as discussed under structure of the free adenine nucleotides in solution. However, the Mg^{2+} requirement of many phosphorylation reactions implicates the Mg^{2+} ion in this transfer. Atomic models show that free rotation about the N_9–C_1', C_4'–C_5' and C_5'–O bonds allows the negative oxygens of the phosphate chain to be brought into close proximity to the adenine ring. This positioning of the chain and ring allows a metal ion to form a "quadridentate chelate" involving negative oxygens of the β- and γ-phosphate groups of ATP and the N_7 and NH_2 nitrogens of the adenine ring (structure IX, Figure 2) (125). It seems, then, that such a structure is at least sterically and energetically possible.

2. Indirect Proof from Hydrolysis and Dimerization Constants.—Hydrolysis constants for the formation compounds such as $atpM(OH)^{3-}$ and dimerization constants for the formation of complexes such as $[atpM(OH)]_2^{6-}$ have been determined by Kahn and Martell (63, 64) and have been presented in Table V. Zn-ADP, ZnATP, CuADP, and CuATP complexes have been studied with regard to hydrolysis and dimerization. It was found that $adpZn^-$, $adpCu^-$, and $atpCu^{2-}$ form hydroxo complexes and dimers, while $atpZn^{2-}$ does not. This has been explained by Kahn and Martell in terms of metal–bridge formation. It was postulated that $adpZn^-$, $adpCu^-$, and $atpCu^{2-}$ are all α,β-phosphate chain complexes and therefore have metal valences free for the formation of hydroxo complexes and dimers. On the other hand, $atpZn^{2-}$ must be a quadridentate chelate involving α-, β-, and γ-phosphate oxygens and N_7 (structure X, Figure 2). Thus it has no valences free for OH^- groups or dimerization (64).

3. Metal–Bridge Complex Formation Constants.—Using an ultraviolet spectrophotometric technique, Schneider and Brintzinger (110) have quantitatively determined the degree of metal–bridge complex formation for several M^{2+} ions. Essentially, the method follows the ultraviolet spectrum of the adenine ring. The binding of the metal ions to the adenine ring in adenosine was determined in a separate experiment. The effect of direct M^{2+} binding to the adenine ring was then subtracted from the total metal binding on the ring in several $atpM^{2-}$ complexes as determined by ultraviolet spectra. The remaining metal–ring binding is assumed to be by metal already bound to the primary binding site on the phosphate chain. This, therefore, would be a measure of the fraction of metal bound

to both sites, *i.e.*, to the degree of metal–bridge formation. These data have been presented in Table VII. Clearly, sizeable percentages of transition metal–ATP complexes are in the metal–bridge form, especially Cu(II) complexes, which are 80% bridge according to these data. This is to be expected since Cu(II) has strong electrostatic as well as strong nitrogen chelating ability.

A further interesting point made in this study is that although in $atpCu^{2-}$, 80% of the Cu(II) ions are bound to the adenine ring as well as to the phosphate chain, its net binding is only slightly greater than in the Cu(II) methyl triphosphate complex, CH_3tpCu^{2-} (log $K^{Cu}_{CuATP} = 6.30 \pm 0.05$ and log $K^{Cu}_{MTP} = 6.17 \pm 0.05$).

The Structure of Metal–Bridge Complexes.—There is some disagreement about the structural details of metal–bridge complexes. All that is definitely agreed upon is that the negative oxygens of the phosphate chain and the nitrogens of the adenine NH_2 and N_7 are the groups involved. Chemical intuition has guided investigators to several different structures, which have been collected in Figure 2, along with references indicating their source. It is probable that in solution several of these structures are in equilibrium, but which ones are present and to what extent has not as yet been determined.

d. The Metal–Ring Complex

By metal–ring complexes is meant an adenosine or adenine nucleotide–metal ion complex in which the metal ion is bound *only* to the adenine ring. Clearly, for most metal ions the percentage of this species would be extremely small since most ions bind much more strongly to the primary phosphate chain site. However, for metal ions that bind more strongly to the secondary, nitrogen chelating site, *e.g.*, Ag^+, or for the second metal ion in 2:1 metal:nucleotide multiple complexes, this type of bonding would be important.

Evidence for Metal–Ring Complex Formation. 1. pH titration studies have shown that pH titration curves for the titration of adenosine in the presence and absence of M^{2+} ions including Cu(II) and Zn(II) show little or no effect by the metal ion. It has been concluded, therefore, in at least three independent pH titration studies that there is little or no detectable M^{2+}–adenosine complex formation (1, 63, 114). It should be pointed out, however, that pH titration would not be expected to be a sensitive method for determining a M^{2+} complex formation constant for two reasons. First, the largest log formation constant reported for M^{2+} ions and adenosine is for Cu(II) and is only 0.84, while others are far less (Table I). In this method, the M^{2+} ion is competing with H^+ ion which has a binding constant of 4.0. Secondly, the M^{2+} ion might be binding partially or even primarily to the N_7 site (12, 13), making the pH titration method even more indirect.

2. Cu(II) Complex Formation Constants by Effects of Ligands on Cu(II) Catalysis.—A method has been developed for estimating the degree of complex formation of a ligand with Cu(II) by its effect on the rate of oxidation of ascorbic acid, catalyzed by Cu(II) (39). Application of this method to adenine and some of its derivatives at pH 7.2 has indicated the following relative order of Cu(II) complex formation: adenine $\gg$ ATP $>$ AMP $>$ adenosine. N_7-Methylated adenine compounds showed no indication of Cu(II) complex formation. Although no complex formation constants were calculated, this study indicated considerable complex formation of Cu(II) with adenosine and the adenine nucleotides and assigned an essential role to the N_7 nitrogen of the adenine ring in adenosine in the complex formation (38).

3. Metal–Ring Complex Formation Constants by Ultraviolet Spectra.—The complex formation constants of several metal ions with adenosine have been determined as a means of measuring metal–bridge formation as described above (110). These constants are given in Table VII.

E. THE PERCENTAGE OF EACH CHEMICAL SPECIES PRESENT

For a given metal ion and metal ion complexing compound, the percentages of each chemical species present in a system is a simple function of pH and metal ion concentration. This dependence might be illustrated by the quantitative treatment of a simple and most useful general situation of a 1:1 complex with an ionization related to the complex formation

$$\begin{array}{ccc} {}^{y} & {}^{K_1} & {}^{1-x-y-z} \\ LH & \rightleftharpoons & L + H \\ + & & + \\ M & & M \\ \downarrow K_4 & K_3 & \downarrow K_2 \\ LMH & \rightleftharpoons & LM + H \\ {}_{z} & & {}_{x} \end{array} \qquad \text{(Eq 7)}$$

Clearly, there are four different chemical species of the compound present, L, LH, LM, and LMH. The fraction of the total existing in each form has been designated in terms of the parameters x, y, and z. Simultaneous solution of the equilibrium expressions yields the following values for x, y, and z

$$x = \frac{K_1K_2K_3M}{K_1K_2K_3M + K_1K_2MH + K_1K_3 + K_3H} \qquad \text{(Eq 8)}$$

$$y = \frac{K_3H}{K_1K_2K_3M + K_1K_2MH + K_1K_3 + K_3H} \qquad \text{(Eq 9)}$$

$$z = \frac{K_1K_2MH}{K_1K_2K_3M + K_1K_2MH + K_1K_3 + K_3H} \qquad \text{(Eq 10)}$$

where M = concentration of free metal ion; H = hydrogen ion concentration.

In the case of the adenine nucleotides, the species LH and L might be folded by hydrogen bonding as described above by Melchior (88), while the species LMH and LM might be folded by metal–bridge formation as described by Schneider and Brintzinger (110). For the adenine nucleotides the above description, where H is the secondary phosphate ionization, does apply for the pH range 6–9 and for metal nucleotide concentrations where only 1:1 complexes are formed; in general this means in dilute solutions in which there is no large excess of metal ion or nucleotide. Therefore, using the equilibrium constants from Table I, the percentages of each species present at given pH and metal ion concentration can be calculated. In many equilibrium, kinetic, and physical studies it would be expected that plotting concentrations of the various individual species in pH and metal ion concentration variations would give more understandable correlations with experimental results than simply plotting against net nucleotide concentration.

III. Physical Studies

In this section are included several valuable experimental studies which were aimed primarily at the elucidation of molecular structure rather than at the determination of equilibrium or thermodynamic data. It will be seen that these methods are, in general, capable of penetrating to structural detail in far greater degree than the equilibrium methods of the previous section. On the other hand, these methods are, in general, qualitative in their results; *i.e.*, they determine the presence or absence of a given structural feature without specifying how many of the molecules in the system possess this observed structural characteristic. If this is kept in mind, it will become clear that these physical studies are in general agreement with equilibrium and thermodynamic studies and provide valuable structural information with regard to individual chemical species present in varying degrees in the systems studied.

A. X-RAY DIFFRACTION

Several X-ray diffraction studies have been carried out on adenine nucleotides in the solid state. It has been found, for instance, that the adenine ring is planar and that in adenosine the plane of the adenine ring is parallel to the plane of the ribose moiety (52). In adenine hydrochloride, the proton of the HCl is on the N_1 nitrogen of the ring (21). The structure of AMP in the crystalline state has been determined in detail and it has been found that in crystalline AMP the molecules are in a roughly linear form with all H bonding between chain and ring being of the intermolecular type (69, 70, 85).

An X-ray diffraction study of amorphous dibarium ATP has indicated that in this form the extended length of the molecule is 22.5 A and the separation between the two barium atoms is 8.2 A (93).

It must be pointed out, however, with regard to the subject of this review that the above information must be used with extreme caution, since hydration plays a major role in the ionization, metal complex formation, and conformation in solution of adenosine, and especially of the adenine nucleotides.

B. ULTRAVIOLET SPECTROPHOTOMETRY

Ultraviolet spectra have been used to obtain three different types of data with regard to the adenine ring of the adenine nucleotides. They will be treated separately as follows:

1. *Change in the Ultraviolet Spectrum of Adenosine and the Adenine Nucleotides on the Addition of Metal Ions*

It has been found that the ultraviolet spectra of adenosine and the adenine nucleotides change on the addition of metal ions to an extent which depends on the nature of the added metal ions. The change in spectrum is in the following order: $Hg^{2+} \gg Cu^{2+} \gg Ni^{2+} > Co^{2+} > Zn^{2+} > Mn^{2+} > Ca^{2+} > Mg^{2+}$ (32, 110) where the change for Ca^{2+} and Mg^{2+} is so small that one investigator has reported that there is no change (8). This has been interpreted as an indication of nitrogen chelation on the N_7 of the adenine ring with further chelation to the nitrogen of the NH_2 group either possible or present (32, 110).

2. *Changes in Optical Density at 260 mμ on Cu^{2+} Complex Formation for ATP and ADP*

It has been found that there is no change in optical density at 260 mμ on the formation of $atpCu^{2-}$, but that there is a change on the formation of $adpCu^{-}$ (111). This has been interpreted as evidence that the terminal phosphate group in $atpCu^{2-}$ is *trans* to the N_7 atom and in the same plane as the adenine ring, while in $adpCu^{-}$ the terminal phosphate group is to the N_7 atom and not in the same plane as the adenine ring (111).

3. *Changes in $pK = \sim 4$ Ring Ionization by Mg^{2+} and Ca^{2+}*

Using an ultraviolet method for the determination of the $pK = \sim 4$ ring ionization of ATP, ADP, and adenosine, it was found that the apparent pK varies with addition of Ca^{2+} and Mg^{2+} in ATP and ADP but not in adenosine (56). Thus, the presence of the phosphate chain in ATP and ADP enables the Mg^{2+} and Ca^{2+} to influence the ring ionization of these compounds. Since it is known that these ions bind to the phosphate chain, it has been postulated that *part* of the ATP is in a curled or folded form with the Mg^{2+} or Ca^{2+} ions between the chain and the ring (56).

C. INFRARED SPECTROPHOTOMETRY

1. *The pK = ~4 Ionization of ATP*

Infrared studies of ATP in D_2O solution have shown that in the first ionization of $atpH_2^{2-}$ ($pK = \sim 4$) the proton is dissociating from N_1 of the adenine ring (67).

2. *Some Structural Details of Metal–Chain Complex Formation*

Infrared spectra in aqueous solution at pH 8.9 in the range 850–1450 cm^{-1} have been interpreted by Brintzinger (11) as giving evidence for the following. a. Immediate coordination (no intervening water molecule) between M^{2+} and the γ (terminal) phosphate group of ATP occurred to the extent Cu^{2+} 100%; Mn^{2+}, Co^{2+}, Ni^{2+}, and Mg^{2+} 60–40%; Ca^{2+} a few per cent. b. A coordinative interaction between metal ion and the α- or β-phosphate groups occurred in a weak to moderate percentage in the complexes studied (Cu^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Mg^{2+}, Ca^{2+}). c. Correlation of the acidity of the hydrated metal ion with spectral changes accompanying proton dissociation suggests a "localized hydrolysis" as illustrated in structure V of Figure 2. This conclusion is in good agreement with the theory of the section on complexes involving hydrolysis of the hydrated metal ion above. d. Comparison of the spectra of ATP and methyl triphosphate for the region 850–1450 cm^{-1} indicates no ribose or ring involvement in the complex formation. However, as the result of a study of $atpMg^{2-}$ spectra taken at pH 7.0 over an expanded wavelength range, it has been reported by Epp, Ramasarma, and Wetter that on formation of $atpMg^{2-}$ a band appears at 1685 cm^{-1} (36). This is taken to be indicative of a $-C{=}N_7^+$ group which in turn suggests that the Mg^{2+} ion is supplying the positive charge through metal–bridge formation (36). However, a subsequent study of $atpMg^{2-}$ formation by Feldman and Keil has indicated that the band observed by Epp, Ramasarma, and Wetter at 1685 cm^{-1} is largely due to an artifact in the experimental method employed. It was concluded in the subsequent study that solid-state infrared studies show only a small change in the adenine ring spectrum which is probably due to the fact that the freeze drying of samples packs Mg^{2+} ions close to the adenine ring of ATP and that in solution the interaction would be negligible (37).

D. NUCLEAR MAGNETIC RESONANCE (NMR)

1. *The pK = ~7 Ionization of ATP and ADP*

Nmr studies of ATP and ADP as a function of pH indicate that the $pK = \sim 7$ ionization is from the γ-phosphate group in ATP and the β-phosphate group in ADP (24).

2. *Some Structural Details of Metal–Adenine Nucleotide Complexes*

Using an nmr technique the chemical shifts may be followed for all the phosphorus atoms of the phosphate chain and the C_2, C_8, and C_1' protons. This was done for ATP and ADP complex formation reactions with Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+} Ni^{2+}, Zn^{2+}, and Cu^{2+} (23, 48, 123, 124). The data indicate whether or not these groups are involved in the complex bonding, but do not in general give information as to the extent of the interaction. The experimental conditions were such that a small interaction of a few per cent or less might not be detected (24). The results are given in Table VIII. Changes in the C_2, C_8, or C_1' protons indicate ring involvement. An interesting observation was made by Cohn and Hughes (23) to the effect that since Mn^{2+} can replace both Cu^{2+} and Mg^{2+} in some metal specific enzyme reactions, perhaps the ATPMn complex is a mixture of Cu^{2+}-like complex (β, γ, H_8) and Mg^{2+}-like complex (β, γ).

TABLE VIII

SITES INVOLVED IN THE BINDING OF M^{2+} IONS TO atp^{4-} AND adp^{3-} FOR VARIOUS METAL IONS AS DETERMINED BY NMR STUDIES ON THE PHOSPHORUS ATOMS (α, β, γ) OF THE PHOSPHATE CHAIN AND THE H_8, H_2, AND H_1 PROTONS OF THE ADENOSINE MOIETY OF ATP AND ADP

	ATP		ADP	
M	Sites	Ref	Sites	Ref
Mg^{2+}	β, γ	23, 24, 48	α, β	23, 24, 48
Ca^{2+}	β, γ	23, 24, 48		
Mn^{2+}	α, β, γ, H	23, 24, 123, 124	α, β, H	23, 24
Co^{2+}	α, β, γ, H	123, 124		
Ni^{2+}	α, β, γ, H	123, 124		
Zn^{2+}	β, γ, H	23, 24		
Cu^{2+}	β, γ, H	23, 24	α, β, H	23, 24

[a] The symbol H under "sites" indicates that the nmr behavior of one or more of the protons mentioned was altered, indicating some sort of ring involvement in the binding.

Comparison of Table VIII with Table VII shows qualitative agreement between the nmr and ultraviolet results as to the sites involved in complex formation. However, the detailed nmr study of the Mn^{2+}, Co^{2+}, and Ni^{2+} complexes of atp^{4-} by Sternlicht, Shulman, and Anderson has lead to disagreement as to the extent of metal–bridge or "back-bound" complex formation for these three complexes. It has been shown in this nmr study that the times of association of the metal ion with the adenine ring and with the phosphate chain are the same. It was therefore concluded that the metal ion binds to both sites simultaneously, and that since for the $atpMn^{2+}$, $atpCo^{2-}$, and $atpNi^{2-}$ complexes, complex formation is virtually complete, the following might be concluded for these three complexes. (1) There is essentially one species of complex in solution; (2) this species involves close to 100% binding to the adenine ring by the Mn^{2+}, Co^{2+} or Ni^{2+} ion; (3)

the conformation in solution of this complex is probably that suggested by Szent-Syorgyi (structure IX, Figure 2) (123, 124). Clearly, the ultraviolet results of Table VII which give the per cent of folded or backbound complex as 3, 12, and 20% for the Mn^{2+}, Co^{2+}, and Ni^{2+} complexes, respectively, are in considerable disagreement with these conclusions. It seems that neither the nmr nor the ultraviolet evidence is conclusive. The determination of the extent of folding, backbinding, or metal-bridge formation for these compounds therefore remains a problem for another study.

The nmr data of Sternlicht, Shulman, and Anderson are also incompatible with the formation of statistically significant amounts of multiple complexes in the formation of ATPMn, ATPCo, and ATPNi complexes, under the experimental conditions employed (pH 8.5–9.0, large excess of ATP at concentrations of 0.30–0.35 *M*, temperature 0–95°) (123, 124). This is in agreement with the conclusion given in the section on multiple complexes under equilibrium and thermodynamic studies.

E. OPTICAL ROTARY DISPERSION (ORD)

A few optical rotary dispersion studies have indicated some kind of conformational change or electrostatic ring–chain interaction in the free adenine nucleotides in solution as the pH is varied from 3 to 10 (74, 78). These results, however, provide little definite structural information. With regard to the metal–adenine nucleotide complexes it has been found that addition of Ca^{2+} or Mg^{2+} to ATP at pH 7 has no effect on ORD implying no conformational change (86). This is in agreement with the theory that Ca^{2+} and Mg^{2+} complexes of ATP are almost entirely metal–chain complexes. On the other hand, addition of Zn^{2+} brings about changes in ORD indicating conformational change and metal–bridge formation (86).

F. ELECTRON SPIN RESONANCE (ESR)

An early report stemming from esr evidence suggested ring–chain interaction in free ATP in solution (57). It was later found that the commercial ATP used contained 75.2 ± 4 μg of Cu/g of ATP which accounted for the esr signal of ring–chain interaction (109). Further esr study of Cu free ATP did not provide evidence for ring change interaction in free ATP (109).

G. CONDUCTANCE TITRATION

A series of conductance titrations of deoxyribonucleic acid (DNA) with Mg^{2+} and Cu^{2+} ions have been carried out by Zubay under various conditions (138–140). It was found that denaturing DNA greatly increases its ability to bind Mg^{2+} and Cu^{2+}. Also, blocking the NH_2 group of the adenine ring with HCHO cuts the Mg^{2+} ion binding to 50% of what it was before the NH_2 group was blocked. Further, denatured DNA binds 0.71 charge equiv of Mg^{2+}/phosphate group and similarly 0.83 charge equiv of Cu^{2+}/phosphate group. All three of these observations are interpreted by Zubay as indicating moderate to strong Mg^{2+}–NH_2 interaction in DNA–Mg binding. If this conclusion were transferred to the adenine–nucleotides it would be in opposition to the evidence provided by all other studies cited in this paper. However, these observations might well be explained by effects associated with the polymer structure of DNA.

IV. MO CALCULATIONS

In spite of the special difficulties connected with MO calculations on chelate compounds (62), such a set of calculations has been carried out on metal–nucleotide complexes by Fukui, Imamura, and Nagata (40). In considering the results of these calculations it must be kept in mind that they apply to metal–nucleotide chelates *in the gas phase*. Any conclusions drawn from even the most sophisticated MO calculations with regard to the structure or conformation of these compounds in solution must take account of the fact that hydration is a, if not the, dominating factor in these complex formation reactions in solution. This has been shown by thermodynamic studies (101). Therefore, a direct correlation between these very interesting MO calculations and equilibrium thermodynamic and physical studies which have been carried out in solution should not be expected.

Using the simple LCAO MO method, Fukui, Imamura, and Nagata have calculated three parameters for various possible structures of metal–ATP complexes. The four possible structures of M–ATP considered and that of M–ADP are given in Figure 3. The three parameters are: the stabilization energy for complex formation, E_s; superdelocalizability for nucleophilic attack on each of the three phosphorus atoms of the phosphate chain, $S_\alpha^{(N)}$, $S_\beta^{(N)}$, $S_\gamma^{(N)}$; and the total π-electron density at each phosphorus atom q_α, q_β, q_γ. These quantities are listed in Table IX. Experimental studies have provided some information with regard to what the relative values of these quantities should be. First, the regard to stabilization energies: equilibrium studies have provided various $\Delta F°$ values for complex formation reactions. If it were assumed that the entropy term is the same in any two of the given reactions (it would appear that they are not), then the calculated stabilization energy should be a measure of the relative values of $\Delta F°$. Thus, a means would be provided for comparing calculation with experiment. In the present case it is known that $\Delta F°$ for the formation of M–ATP is more favorable than $\Delta F°$ for the formation of M–ADP. It is assumed then that the calculated stabilization energies for any statistically significant structure of M–ATP must be greater

TABLE IX
MO PARAMETERS CALCULATED TO DETERMINE THE CONFORMATION OF atpM^{2-} COMPLEXES IN THE GAS PHASE[a]

Compd	Stabilization energy, E_s	Superdelocalizability for nucleophilic attack on phosphorus atom			π-Electron density for the phosphorus atom		
		$S_{P_\alpha}^{(N)}$	$S_{P_\beta}^{(N)}$	$S_{P_\gamma}^{(N)}$	q_{P_α}	q_{P_β}	q_{P_γ}
adp^{3-} (phosphate part only)	..	1.182	1.099	...	0.270	0.329	...
adpM$^-$	3.705	1.097	1.184	...	0.278	0.270	...
atp$^-$ (phosphate part only)	...	1.183	1.191	1.099	0.269	0.268	0.329
atpM^{2-} model 1	**7.890**	**1.182**	**1.101**	**1.180**	**0.270**	**0.277**	**0.272**
atpM^{2-} model 2	**9.443**	1.096	1.103	1.183	0.278	0.276	0.270
atpM^{2-} model 3	2.538	1.097	1.104	1.099	0.278	0.277	0.329
atpM^{2-} model 4	1.205	**1.182**	**1.105**	**1.184**	**0.269**	**0.272**	**0.270**
atpM^{2-} exptl	atpM^{2-} > adpM$^-$	$S_{P_\alpha}^{(N)}$	$\sim$ $S_{P_\gamma}^{(N)}$	> $S_{P_\beta}^{(N)}$	q_{P_α}	$\sim$ q_{P_γ}	< q_{P_β}

[a] Values in boldface type are in agreement with experimental data given in the bottom row of the table. See Figure 3 for the structures indicated as models 1–4 (40).

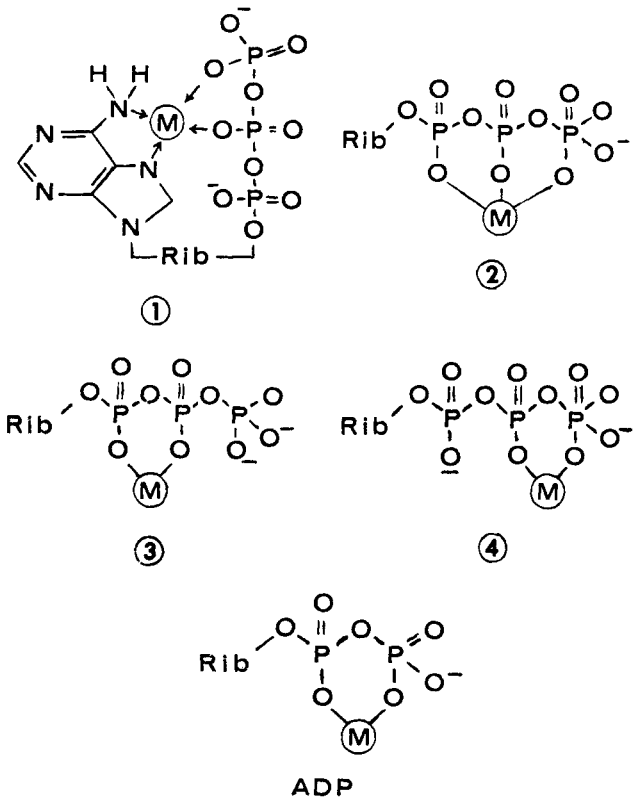


Figure 3.—Models used by Fukui, Imamura, and Nagata for calculating MO parameters to determine the conformation of atpM^{2-} complexes in the gas phase. These parameters are listed in Table IX. It was concluded that model 1 would be most stable (40).

than that of M–ADP. This has been indicated in the bottom row of Table IX as experimental, atpM^{2-} > adpM$^-$ for the stabilization energy. With regard to the values of E_s in column one, it might be noted, in general, that the calculated values of the stabilization energies for model three and especially for model four are significantly smaller than those for models one and two. This seems to be accounted for by the significantly greater possibilities for delocalization in models one and two of the M–ATP complexes.

In order to convert these stabilization energies to $\Delta F°$ values it would be necessary to apply an entropy correction as was mentioned above. It is intuitively clear that such a term would be extremely unfavorable for model one and somewhat unfavorable for model two while it would be much less so for models three and four. In terms of $\Delta F°$ then, even in the gas phase, models three and especially four would be more favorable than would appear from the calculated stabilization energies.

Secondly, with regard to superdelocalizability: experimental evidence on ATP reactivity has shown that phosphate group reactivity for nucleophilic attack is in the following order: α-P $\sim$ γ-P > β-P (25). Since electron superdelocalizability should correlate with reactivity, the calculated values of this parameter for acceptable models should be in the following order: $S_\alpha^{(N)} \sim S_\gamma^{(N)} > S_\beta^{(N)}$. This has been indicated in the bottom row of Table IX.

Thirdly, π-electron density on the phosphorus atoms of ATP: ATP reactivity and nmr studies (25) have shown that π-electron density should be in the following relation: $q_{P_\alpha} \sim q_{P_\gamma} < q_{P_\beta}$. This has been indicated in the bottom row of Table IX, for comparison with the calculated values.

Comparison of the bottom row experimental values with the calculated values for the four possible structures of M–ATP considered shows that only model one, the quadridentate chelate structure, gives proper calculated values; therefore, with the qualifications mentioned above it seems that MO calculations indicate that the quadridentate chelate structure would predominate in the gas phase for M–ATP complexes.

V. KINETIC STUDIES

A. THE TEMPERATURE JUMP METHOD

With the help of short, high voltage electrical impulses, rapid temperature changes can be produced in conducting reaction systems. Reactions having significant enthalpies are displaced from equilibrium by such a temperature jump. The kinetics of return to equilibrium may be then studied by a suitable physical method: in the case described here, change in optical

TABLE X

RATE CONSTANTS FOR THE PROTON-TRANSFER STEPS OF MgATP AND MgADP IONIZATION AND COMPLEX FORMATION (EQ 11) AT 13 ± 2° in 0.1 M KNO_3[a,d]

Reactants[c,d]	Log k_{34}, M^{-1} sec^{-1}	Log k_{43}, M^{-1} sec^{-1}	Log k_{35}, M^{-1} sec^{-1}	Log k_{53}, M^{-1} sec^{-1}	Log k_{43}/k_{34}	
					These data	pH titration[b] data
ADP + PR	7.53	8.85	2.78	10.48	1.32	1.32
ADP + CPR	8.30	8.00	4.28	10.36	−0.30	−0.28
ATP + PR	7.68	8.85	2.78	10.48	1.17	1.18
ATP + CPR	8.30	7.90	4.28	10.36	−0.40	−0.41

[a] Values given are ±30% (30, 33, 35). [b] The values in this column are from the pH titration equilibrium studies of Martell and Schwarzenbach (84). [c] PR = phenol red; CPR = chlor phenol red. [d] The data in this table were first printed with several printing errors (30, 35) but later appeared in the above corrected form (33).

TABLE XI

RATE CONSTANTS FOR THE METAL-COMPLEX FORMATION STEPS OF MgATP AND MgADP IONIZATION AND COMPLEX FORMATION (EQ 11) AT 26 ± 2° IN 0.1 M KNO_3[a,b]

Reactants	Log k_{13}, sec^{-1}	Log k_{31}, M^{-1}, sec^{-1}	Log k_{24}, sec^{-1}	Log k_{42}, M^{-1} sec^{-1}	Log k_{31}/k_{13}, M^{-1}		Log k_{42}/k_{24}, M^{-1}	
					These data	pH titration data[c]	These data	pH titration data[c]
Mg^{2+} + ADP	3.40	6.48	4.48	6.00	3.08	3.1	1.52	1.0
Mg^{2+} + ATP	3.08	7.08	4.48	6.48	4.00	4.0	2.0	2.0
Ca^{2+} + ADP	>5.60	>8.40	...	...	2.80	2.8	...	...
Ca^{2+} + ATP	>5.40	>9.00	...	...	3.60	3.6	...	1.8

[a] Values are ±35% for ADP (30, 33, 35). [b] The data in this table were first printed with several printing errors (30, 35) but later appeared in the above corrected form (33). [c] The values in this column are from the pH titration equilibrium studies of Martell and Schwarzenback (84).

density translated and recorded by an oscillographic system (29).

B. KINETIC DATA FROM THE TEMPERATURE JUMP METHOD

The kinetics of Ca^{2+} and Mg^{2+} complex formation with ATP and ADP have been studied by use of the temperature method by Eigen, Hammes, and Diebler (30, 33, 35). The over-all reaction scheme including the indicators required by the method is as follows

$$\text{(I)}\quad Matp^{2-} + HIn^{-} \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} MatpH^{-} + In^{2-}\quad \text{(II)}$$

$$k_{13}\downarrow\uparrow k_{31} \qquad\qquad k_{24}\downarrow\uparrow k_{42} \qquad\qquad \text{(Eq 11)}$$

$$\text{(III)}\quad M^{2+} + atp^{4-} + HIn^{-} \underset{k_{42}}{\overset{k_{34}}{\rightleftharpoons}} M^{2+} + atpH^{3-} + In^{2-}\quad \text{(IV)}$$

$$\text{III}\quad atp^{4-} + HIn^{-} \underset{k_{43}}{\overset{k_{34}}{\rightleftharpoons}} atpH^{3-} + In^{2-}\quad \text{IV}$$

$$k_{53}\searrow\swarrow k_{35} \qquad\qquad k_{45}\swarrow\nearrow k_{54}$$

$$atp^{4-} + In^{2-} + H^{+}$$

$$\text{(V)}$$

By studying the system in the absence of metal ions, the rate constants for the proton-transfer steps of (Eq 11) were first determined. These have been listed in Table X. Then, direct study of the over-all ionization–complex formation system along with the proton-transfer rate constants yielded the rate constants for the complex formation reaction of the fully ionized and the singly protonated species of ATP and ADP. These are given in Table XI, both for Mg^{2+} and Ca^{2+}. A comparison with equilibrium data was made by Eigen and Hammes by comparing ratios of the rate constants determined by the temperature jump method with equilibrium constants determined by pH titration (30, 33, 35). As can be seen from Tables X and XI the agreement is good.

C. SOME CHEMICAL AND BIOLOGICAL IMPLICATIONS

Inspection of the data in Tables X and XI show the following: 1. Rate constants for the proton transfer reactions of $atpH^{3-}$ and $adpH^{2-}$ are the same. 2. k_{31} (M^{2+} + fully ionized species) is four times greater for ATP than for ADP. k_{42} (M^{2+} + singly protonated species) is three times greater for ATP than for ADP. 3. Rate constants for M^{2+} + atp^{4-}, $atpH^{3-}$, adp^{3-}, or $adpH^{2-}$ are 100 times greater for Ca^{2+} than for Mg^{2+}.

These relations seem to be typical of protonation and Ca^{2+} and Mg^{2+} complex formation reactions in general (34, 135). However, for biochemical systems, these relations have profound implications. Since in biochemical systems reactions often occur by kinetic selection the fact that Ca^{2+} complexes with ATP much more quickly than Mg^{2+} could determine the path of some biological reaction systems. Some of these possibilities with respect to enzyme catalyzed reactions are discussed by Diebler (30).

VI. REFERENCES

(1) Albert, A., *Biochem. J.*, **54**, 646 (1953).
(2) Alberty, R. A., Smith, R. M., and Bock, R. M., *J. Biol. Chem.*, **193**, 425 (1951).

(3) Andress, K. R., and Nachtrab, R., *Z. Anorg. Chem.*, **311**, 1 (1961).
(4) Asai, H., and Morales, M. F., *Arch. Biochem. Biophys.*, **99**, 383 (1962).
(5) Baddiley, J., Michelson, A. M., and Todd, A. R., *Nature*, **161**, 761 (1948).
(6) Bielschowsky, M., and Green, N. H., *Nature*, **153**, 524 (1964).
(7) Bjerrum, J., "Metal Amine Formation in Aqueous Solutions," Haase and Sons, Copenhagen, Denmark, 1941.
(8) Blum, J. J., *Arch. Biochem. Biophys.*, **55**, 486 (1955).
(9) Bock, R. M., *Enzymes*, **2**, 14 (1960).
(10) Branch, G. E. K., and Calvin, M., "The Theory of Organic Chemistry," Prentice-Hall, Inc., New York, N. Y., 1941, p 204.
(11) Brintzinger, H., *Biochem. Biophys. Acta*, **77**, 343 (1963).
(12) Brintzinger, H., *Helv. Chim. Acta*, **44**, 935 (1961).
(13) Brintzinger, H., *Helv. Chim. Acta*, **44**, 1199 (1961).
(14) Brintzinger, H., Prijs, B., and Erlenmeyer, H., *Experientia*, **16**, 468 (1960).
(15) Brintzinger, H., and Fallab, S., *Helv. Chim. Acta*, **43**, 43 (1960).
(16) Bronk, J. R., and Kielley, W. W., *Biochim. Biophys. Acta*, **24**, 440 (1957).
(17) Burton, K., *Biochem. J.*, **71**, 388 (1959).
(18) Burton, K., and Krebs, H. A., *Biochem. J.*, **55**, 94 (1953).
(19) Buzzell, A., and Sturtevant, J. M., *J. Am. Chem. Soc.*, **73**, 2454 (1951).
(20) Christensen, J. J., and Izatt, R. M., *J. Phys. Chem.*, **66**, 1030 (1962).
(21) Cochran, W., *Acta Cryst.*, **4**, 81 (1951).
(22) Cohn, M., and Leigh, J. S., *Nature*, **193**, 1037 (1962).
(23) Cohn, M., and Hughes, T. R., *J. Biol. Chem.*, **237**, 176 (1962).
(24) Cohn, M., and Hughes, T. R., *J. Biol. Chem.*, **235**, 3250 (1960).
(25) Cohn, M., "Mechanisms of Enzymic Cleavage of Some Organic Phosphates," presented at the Symposium on Enzymatic Reaction Mechanisms, Gathinburg, Tenn., April 1959.
(26) Cohn, M., and Townsend, J., *Nature*, **173**, 1090 (1954).
(27) Cooper, W. J., and Scarpiello, D. A., *U. S. At. Energy Comm.*, TID-4500, 58 (1964).
(28) Couture, A. M., and Laidler, K. J., *Can. J. Chem.*, **35**, 202 (1957).
(29) Czerlinski, S., and Eigen, M., *Z. Electrochem.*, **63**, 652 (1959).
(30) Diebler, H., Eigen, M., and Hammes, S. S., *Z. Naturforsch.*, **15b**, 554 (1960).
(31) DiStefano, V., and Neuman, W. F., *J. Biol. Chem.*, **200**, 759 (1953).
(32) Eichhorn, G. L., and Clark, P., *J. Am. Chem. Soc.*, **85**, 4020 (1963).
(33) Eigen, M., and Hammes, G. G., *J. Am. Chem. Soc.*, **83**, 2786 (1961).
(34) Eigen, M., *Z. Electrochem.*, **64**, 115 (1960).
(35) Eigen, M., and Hammes, G. G., *J. Am. Chem. Soc.*, **82**, 5951 (1960).
(36) Epp, A., Ramasarma, T., and Wetter, L. R., *J. Am. Chem. Soc.*, **80**, 724 (1958).
(37) Feldman, I., and Keil, E., *J. Am. Chem. Soc.*, **87**, 3281 (1965).
(38) Frieden, E., and Alles, J., *J. Biol. Chem.*, **230**, 797 (1958).
(39) Frieden, E., and Naile, B., *Arch. Biochem. Biophys.*, **48**, 448 (1954).
(40) Fukui, K., Imamura, A., and Nagata, C., *Bull. Chem. Soc. Japan*, **36**, 1450 (1963).
(41) Fuoss, R. M., and Kraus, C. A., *J. Am. Chem. Soc.*, **79**, 3304 (1957).
(42) Gaucher, C. R., and Taylor, J. F., *J. Biol. Chem.*, **239**, 2251 (1964).
(43) George, P., Phillips, R. C., and Rutman, R., *Biochemistry*, **2**, 508 (1963).
(44) George, P., and Rutman, R., *Progr. Biophys. Biophys. Chem.*, **10**, 1 (1960).
(45) Gillen, K., Jensen, R., and Davidson, N., *J. Am. Chem. Soc.*, **86**, 2792 (1964).
(46) Green, N. H., and Stoner, H. B., *Brit. J. Exptl. Pathol.*, **25**, 150 (1944).
(47) Gulland, M., and Walsh, E., *J. Chem. Soc.*, 169 (1945).
(48) Hamms, S., Maciel, G. E., and Waugh, J. S., *J. Am. Chem. Soc.*, **83**, 2394 (1961).
(49) Handschin, V., and Brintzinger, H., *Helv. Chim. Acta*, **45**, 1037 (1962).
(50) Hansen, L. D., Christensen, J. J., and Izatt, R. M., *Chem. Commun.*, **3**, 36 (1965).
(51) Harkins, T. R., and Freiser, H., *J. Am. Chem. Soc.*, **80**, 1132 (1958).
(52) Hendricks, S. B., *J. Phys. Chem.*, **45**, 65 (1941).
(53) Hermann, V. S., *Hung. Acta Physiol.*, **1**, 21 (1946).
(54) Hers, H. G., *Biochim. Biophys. Acta*, **8**, 424 (1952).
(55) Hock, A., and Huber, G., *Biochem. Z.*, **328**, 44 (1956).
(56) Hotta, K., Brahms, J., and Morales, M. F., *J. Am. Chem. Soc.*, **83**, 997 (1961).
(57) Isenberg, I., and Szent-Gyorgyi, A., *Proc. Natl. Acad. Sci. U. S.*, **45**, 1232 (1959).
(58) Izatt, R. M., Hansen, L. D., Rytting, J. H., and Christensen, J. J., *J. Am. Chem. Soc.*, **87**, 2760 (1965).
(59) Izatt, R. M., and Christensen, J. J., *J. Phys. Chem.*, **66**, 359 (1962).
(60) Johansson, A., and Wanninen, E., *Talanta*, **10**, 769 (1963).
(61) Jordan, J., and Alleman, T. G., *Anal. Chem.*, **29**, 9 (1957).
(62) Jorgensen, C. K., "Absorption Spectra and Chemical Bonding in Complexes," Pergamon Press, London, Addison-Wesley, Inc., Reading, Mass., 1962.
(63) Kahn, T. M. M., and Martell, A. E., *J. Phys. Chem.*, **66**, 10 (1962).
(64) Kahn, T. M. M., and Martell, A. E., *J. Am. Chem. Soc.*, **84**, 3037 (1962).
(65) Kaye, M. A. G., *Biochim. Biophys. Acta*, **18**, 456 (1955).
(66) Kerly, M., and Leaback, D. H., *Biochem. J.*, **67**, 245 (1957).
(67) Khalil, F. L., and Brown, T. L., *J. Am. Chem. Soc.*, **86**, 5113 (1964).
(68) Krasovec, F., and Jan, J., *Croat. Chem. Acta*, **35**, 183 (1963).
(69) Kraut, J., and Jensen, L. H., *Acta Cryst.*, **16**, 79 (1963).
(70) Kraut, J., and Jensen, L. H., *Nature*, **186**, 798 (1960).
(71) Kuby, S. A., Noda, L., and Lardy, H. A., *J. Biol. Chem.*, **210**, 65 (1954).
(72) Laidler, K. J., *Can. J. Chem.*, **34**, 1107 (1956).
(73) Larsson-Raznikiewicz, M., and Malmstrom, B. G., *Arch. Biochem. Biophys.*, **92**, 94 (1961).
(74) Levedahl, B. H., and James, T., *Biochim. Biophys. Acta*, **21**, 298 (1956).
(75) Levene, P. A., Simms, H. S., and Bass, L. W., *J. Biol. Chem.*, **70**, 243 (1926).
(76) Levene, P. A., and Simms, H. S., *J. Biol. Chem.*, **65**, 519 (1925).
(77) Liebecq, C., and Jacquemotte-Louis, M., *Bull. Soc. Chim. Biol.*, **40**, 67 (1958).
(78) Lin, C. Y., Urry, D. W., and Eyring, H., *Biochem. Biophys. Res. Commun.*, **17**, 642 (1964).
(79) Lohman, K., *Biochem. Z.*, **282**, 120 (1935).

(80) Lohman, K., *Biochem. Z.*, **254**, 381 (1932).
(81) Lowenstein, J. M., and Schatz, M. N., *J. Biol. Chem.*, **236**, 305 (1961).
(82) Lowenstein, J. M., *Biochem. J.*, **70**, 222 (1958).
(83) Lythgoe, D., Smith, H., and Todd, A. R., *J. Chem. Soc.*, 355 (1947).
(84) Martell, A. E., and Schwarzenbach, S., *Helv. Chim. Acta*, **39**, 653 (1956).
(85) Mazza, F. P., and Tappi, S., *Arch. Sci. Biol.* (Bologna), **24**, 526 (1938).
(86) McCormick, W. G., and Levedahl, B. H., *Biochim. Biophys. Acta*, **34**, 303 (1959).
(87) Melchior, N. C., and Melchior, J. B., *J. Biol. Chem.*, **231**, 609 (1958).
(88) Melchior, N. C., *J. Biol. Chem.*, **208**, 615 (1954).
(89) Monk, C. B., *J. Chem. Soc.*, **423** (1949).
(90) Morrison, J. F., O'Sullivan, W. J., and Ogston, A. G., *Biochem. Biophys. Acta*, **52**, 82 (1961).
(91) Nanninga, L. B., *Biochim. Biophys. Acta*, **54**, 330 (1961).
(92) Nanninga, L. B., *J. Phys. Chem.*, **61**, 1144 (1957).
(93) Nerheim, A. G., and Shaw, F. H., *Proc. S. Dakota Acad. Sci.*, **30**, 65 (1951).
(94) Neuberg, C., and Mandl, I., *Arch. Biochem.*, **23**, 499 (1949).
(95) Noda, L., Niher, T., and Morales, M. F., *J. Biol. Chem.*, **235**, 2830 (1960).
(96) Ogston, A., *J. Chem. Soc.*, 1713 (1936).
(97) Olivard, J., *Arch. Biochem. Biophys.*, **88**, 382 (1960).
(98) O'Sullivan, W. J., and Perrin, D. D., *Biochemistry*, **3**, 18 (1964).
(99) O'Sullivan, W. J., and Perrin, D. D., *Biochim. Biophys. Acta*, **52**, 612 (1961).
(100) Phillips, R. C., George, P., and Rutman, R., in preparation.
(101) Phillips, R. C., George, P., and Rutman, R., *J. Am. Chem. Soc.*, **88**, 2631 (1966).
(102) Phillips, R. C., Eisenberg, P., George, P., and Rutman, R. J., *J. Biol. Chem.*, **240**, 4393 (1965).
(103) Phillips, R. C., George, P., and Rutman, R., *Biochemistry*, **2**, 501 (1963).
(104) Raaflaub, J., *Helv. Physiol. Pharmacol. Acta*, **12**, C88 (1954).
(105) Rawitscher, M., and Sturtevant, J. M., *J. Am. Chem. Soc.*, **82**, 3739 (1960).
(106) Rechnitz, G., *Anal. Chem.*, **37**, 29A (1965).
(107) Robinson, R. A., and Harned, H. S., *Chem. Rev.*, **28**, 419 (1941).
(108) Roppongi, A., and Kato, T., *Technol. Rept. Tohoku Univ.*, **26**, 1 (1962).
(109) Russell, D. B., and Wyard, S. J., *Nature*, **191**, 65 (1961).
(110) Schneider, P. W., Brintzinger, H., Erlenmeyer, H., *Helv. Chem. Acta*, **47**, 992 (1964).
(111) Schneider, P. W., and Brintzinger, H., *Helv. Chim. Acta*, **47**, 1717 (1964).
(112) Schubert, J., *Methods Biochem. Analy.*, **3**, 247 (1956).
(113) Schwarzenbach, G., and Martell, A. E., *J. Phys. Chem.*, **62**, 886 (1958).
(114) Schwarzenbach, G., and Anderegg, S., *Helv. Chim. Acta*, **40**, 1229 (1957).
(115) Shulman, R. G., and Sternlicht, H., *J. Mol. Biol.*, **13**, 952 (1965).
(116) Sillen, L. G., and Martell, A. E., Ed., "Stability Constants of Metal-ion Complexes," The Chemical Society, London, 1964.
(117) Smith, R. M., and Alberty, R. A., *J. Phys. Chem.*, **60**, 180 (1956).
(118) Smith, R. M., and Alberty, R. A., *J. Am. Chem. Soc.*, **78**, 2376 (1956).
(119) Smith, R. M., Ph.D. Thesis, University of Wisconsin, 1955.
(120) Smithson, J. M., and Williams, J. P., *J. Chem. Soc.*, 457 (1958).
(121) Spicer, S. S., *J. Biol. Chem.*, **199**, 301 (1952).
(122) Squires, R. F., *Biochem. Biophys. Res. Commun.*, **19**, 27 (1965).
(123) Sternlicht, H., Shulman, R. G., and Anderson, E. W., *J. Chem. Phys.*, **43**, 3123 (1965).
(124) Sternlicht, H., Shulman, R. G., and Anderson, E. W., *J. Chem. Phys.*, **43**, 3133 (1965).
(125) Szent-Gyorgyi, A., "Bioenergetics," Academic Press Inc., New York, N. Y., 1957, p 64.
(126) Tucci, E. R., Doody, E., and Li, N. C., *J. Phys. Chem.*, **65**, 1570 (1961).
(127) Van Wazer, J. R., and Callis, C. F., *Chem. Rev.*, **58**, 1011 (1958).
(128) Van Wazer, J. R., "Phosphorus and its Compounds," Vol. I, Interscience Publishers, New York, N. Y., 1958.
(129) Walaas, E., *Acta Chem. Scand.*, **12**, 528 (1958).
(130) Wassermeyer, H., *Z. Physiol. Chem.*, **179**, 238 (1928).
(131) Watanabe, S., Evenson, L., and Gulz, I., *J. Biol. Chem.*, **238**, 324 (1963).
(132) Watanabe, S., Trosper, T., Lynn, M., and Evanson, L., *Biochem. J.*, **54**, 17 (1963).
(133) Watanabe, S., Franz, W., and Trottier, D., *Anal. Biochem.*, **5**, 345 (1963).
(134) Weitzel, S., and Spehr, T., *Z. Physiol. Chem.*, **313**, 212 (1958).
(135) Weller, A., *Z. Electrochem.*, **64**, 55 (1960).
(136) Yatsimirskii, K. B., and Vasil'ev, V. P., "Instability Constants of Complex Compounds," Academy of Sciences, USSR, Moscow, 1959.
(137) Zenchelsky, S. T., *Anal. Chem.*, **32**, 389R (1960).
(138) Zubay, G., *Biochim. Biophys. Acta*, **32**, 233 (1959).
(139) Zubay, G., *Nature*, **182**, 388 (1958).
(140) Zubay, G., and Doty, P., *Biochim. Biophys. Acta*, **29**, 47 (1958).