SITES AND THERMODYNAMIC QUANTITIES ASSOCIATED WITH PROTON AND METAL ION INTERACTION WITH RIBONUCLEIC ACID, DEOXYRIBONUCLEIC ACID, AND THEIR CONSTITUENT BASES, NUCLEOSIDES, AND NUCLEOTIDES

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Received December 28, 1970 (Revised Manuscript Received April 26, 1971)

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I. Introduction, Scope, and Nomenclature

Among the outstanding achievements of this century has been the establishment of the structures of DNA and RNA and of the primary role of nucleic acids and their derivatives as hereditary determinants in biological reproduction and growth. In the course of this work, many questions have arisen concerning the detailed structure and reactions of ribonucleic acid, deoxyribonucleic acid, and their constituent base, sugar, and phosphate units. Central to the answering of these questions is a knowledge of the sites and thermodynamic quantities associated with the interaction of protons and metal ions with these substances, and a considerable body of literature now exists on the subject. In addition to identifying sites of complexation and/or determining the thermodynamic quantities, many investigators have provided interesting accounts of how complexation affects the chemical reactivities of these substances. A large number of potentially reactive sites are present in nucleic acids, and this undoubtedly accounts for the fact that different workers have often assigned a given ionization or complexation step to different sites on the same species. This uncertainty in the assignment of the sites of proton and metal ion attachment to these species is often compounded by the numerous experimental conditions (*i.e.*, ionic strength, presence of competing ions such as Na^+, K^+, etc.) under which the various studies have been performed.

This review covers the literature through August 1970. Sections II and III contain information regarding the sites of interaction of protons and metal ions, respectively, with the heterocyclic bases adenine, guanine, hypoxanthine, xanthine, cytosine, uracil, and thymine; their nucleosides and nucleotides; and DNA and RNA. A summary of the most probable sites of interaction is included at the end of each section. A table containing the available thermodynamic data is included in section IV. All reactions and data throughout the text and in the several tables are, unless otherwise specified, valid in aqueous (or D_2O) solution.

In an excellent review, Phillips[1] has surveyed the literature dealing with proton and metal ion interaction with adenosine and the adenine nucleotides and has discussed the principal techniques and types of instrumentation that have been used

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† Brigham Young University.
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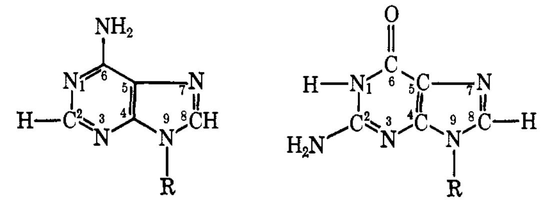
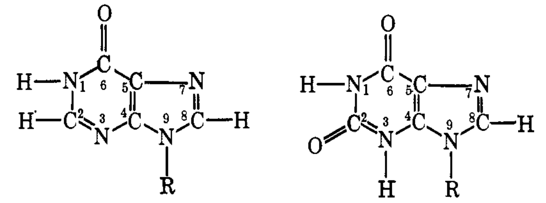
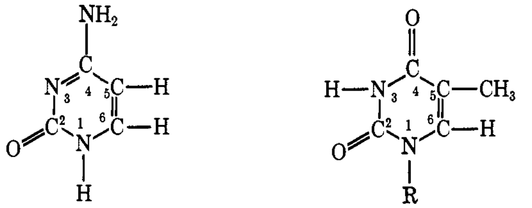
(1) R. Phillips, *Chem. Rev.*, 66, 501 (1966).



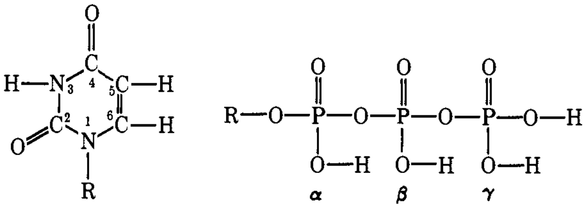
1 (adenine), R = H
2 (adenosine), R = ribose
3 (guanine), R = H
4 (guanosine), R = ribose



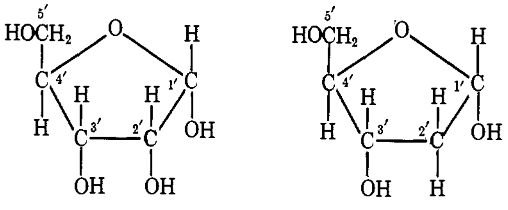
5 (hypoxanthine (H′)), R = H
6 (inosine (I)), R = ribose
7 (xanthine (X′)), R = H
8 (xanthosine (X)), R = ribose



9 (cytosine), R = H
10 (cytidine), R = ribose
11 (thymine (T′)), R = H
12 (thymidine (T)), R = deoxyribose



13 (uracil), R = H
14 (uridine), R = ribose
15



16 (ribose)
17 (deoxyribose)

Figure 1.

in these studies. The information and data given in the review by Phillips will not be repeated here except as required for clarity and continuity, and the reader is referred to it for further information.

Interactions of the purine–purine, purine–pyrimidine, and pyrimidine–pyrimidine base-pairing and base-stacking types will not be considered here. Evidence is mounting, however, that these interactions are often appreciable and in certain cases base pairing and base stacking would be expected to further complicate the analysis and interpretation of the thermodynamic and site data. Unfortunately, most authors have not considered these reactions in interpreting their data.

The structure and numbering systems for the purine and pyrimidine bases, their respective nucleosides, the phosphate chain, ribose, and deoxyribose are given in Figure 1. The abbreviations used for the nucleotides are RMP, RDP, and RTP for the mono-, di-, and triphosphates where R is any one of the purine or pyrimidine bases in Figure 1. Deoxy derivatives are so designated or, in the case of abbreviations, a small d preceeds the abbreviation. Deoxyribonucleic acid and ribonucleic acid are abbreviated DNA and RNA, respectively. The nomenclature used generally follows the 1970 recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature.[2]

II. Sites of Proton Ionization

A. INTRODUCTION

The existence of several basic sites for the nucleosides, nucleotides, and their constituent base, sugar, and phosphate units has frequently led to conflicting literature statements regarding proton ionization sites for these substances. Even in the relatively simple molecule adenine, there are five possible sites for the two observed ionizations from the protonated species, *viz.*, N_1, N_3, N_7, N_8, and C_6NH_2. For the other nucleic acid bases and their respective nucleosides and nucleotides, the possible sites of protonation or ionization are also numerous. A considerable amount of work has been done during the past decade in identifying the sites of proton ionization from these substances and several are now well established. The various investigations carried out concerning the sites of proton ionization will now be presented with a summary of the most probable sites being given in section D.

B. PURINE BASES AND DERIVATIVES

Examination of the purine and purine nucleoside structures in Figure 1 reveals two sites on the imidazole (N_7H^{n+} and N_9H, when present) and one on the ribose moieties (in the nucleosides) whose acidic behavior might be expected to be similar from compound to compound. In contrast, the pyrimidine moieties of these four purines have markedly different substituents, and neighboring groups on these rings might be expected to markedly influence both the site and extent of proton ionization. The available spectroscopic and thermodynamic data generally support these observations although fewer data are available for identification of sites for hypoxanthine and xanthine and their nucleosides than for adenine and guanine and their nucleosides.

1. *Adenine, Adenosine, and Adenosine Nucleotides*

Early workers[3–6] found pK values of 3.5–4.2 to be associated with proton ionization from the protonated forms of adenine, adenosine, AMP, ADP, ATP, and poly(A). By analogy with aniline (protonated aniline, pK = 4.6),[7] it was stated by them and later workers,[8,9] that ionization is from the $C_6NH_3^+$ group. A spectrophotometric study[6] of poly(A) revealed

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[2] *Biochemistry*, **9**, 4022 (1970).

[3] P. A. Levene and H. S. Simms, *J. Biol. Chem.*, **65**, 519 (1925).

[4] H. F. W. Taylor, *J. Chem. Soc.*, 765 (1948).

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

[6] R. F. Beers and R. F. Steiner, *Nature (London)*, **179**, 1076 (1957).

[7] D. L. Levi, W. S. McEwan, and J. H. Wolfenden, *J. Chem. Soc.*, 760 (1949).

[8] G. E. Cheney, H. Freiser, and Q. Fernando, *J. Amer. Chem. Soc.*, **81**, 2611 (1959).

[9] S. Lewin and N. W. Tann, *J. Chem. Soc.*, 1466 (1962).

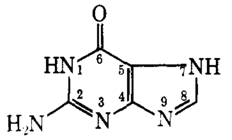
extensive binding of H^+ between pH 6 and 4 which was attributed to binding at the primary amino groups of the adenine bases. This conclusion was based on the additional observation that the binding of H^+ was markedly reduced for formalin-treated AMP with the assumption being made that the action of the formalin was limited to the 6-amino group. The arguments for protonation on N_1H^+ and $C_6NH_3^+$ have been summarized by Lewin[10] who concludes that C_6NH_2 is the protonation site. This conclusion is based on the observation from spectrophotometric and pH variation data that formaldehyde reacts with the amino group of adenine, but is unreactive toward the N_1H^+ group of purine. Although the study leaves no question concerning the site of formaldehyde reaction, the possibility remains that the observed changes in solution pH could result from proton release from N_1H^+ rather than $C_6NH_3^+$. Molecular orbital calculations by Pullman, Pullman, and Berthier[11] indicate that the C_6NH_2 group in adenine has the greatest electron density of any of the nitrogen atoms in the molecule. However, Pullman[12] later concluded that the most basic site is not necessarily determined by the highest electron density, but rather by the conditions in the transition state. This conclusion led him to state that the N_1 position is the most likely site of protonation in adenine. An X-ray crystallographic study of adenine hydrochloride showed the hydrogen atom to be bound to the N_1 position in the crystalline state.[13] In support of ionization from N_1H^+, Zubay[14] pointed out that hydrogen bond breakage in DNA by acid cannot be explained by protonation of the amino group since this protonation would strengthen, not weaken, the hydrogen bond. Recent calorimetric work also provides evidence that proton ionization from protonated adenine and adenosine is from the N_1H^+ group. A characteristic enthalpy change has been found in many cases to accompany proton ionization from a particular donor atom.[15-20] In the present case, the $\Delta H°$ values of 4.81 and 3.91 kcal/mol found[21] for proton dissociation from adenine and adenosine, respectively, resemble more closely the smaller heats found for proton dissociation from protonated compounds where the site of ionization is known to be a nitrogen of the N_1H^+ type (*e.g.*, the protonated forms of cytosine, 5.14,[20] and pyridine, 4.80[22]) than they do the larger heats found for proton dissociation from protonated amino groups of related compounds (*e.g.*, aniline,[7] 7.28 kcal/mol). The assignment of the proton ionization site to the N_1H^+ group in adenosine is further supported by proton nmr data.[23] Although additional work appears warranted to establish unambiguously the ionization site, it is concluded that the N_1H^+ group is the site of proton ionization (p$K \sim 4$) in adenine and adenosine and presumably in the ribonucleotides as well. Support for this conclusion is also found in metal binding studies. For example, proton nmr studies of Cu^{2+}–adenosine and Cu^{2+}–adenosine nucleotide interaction ($((CH_3)_2SO)$) clearly show that Cu^{2+} binds to N_1 and/or N_7, but not to the C_6NH_2 group.[24] Also, it has been pointed out in connection with protonation of guanine and its 9-substituted derivatives that the basicity of heterocyclic amines is very different from that of aromatic amines and amino acids.[25]

There is general agreement that proton ionization from neutral adenine (p$K \sim 10$) is from the N_9H group.[10] As will be pointed out in section II.A.3, calorimetric results indicate a common ionization site for adenine, hypoxanthine, and xanthine which we take to be the N_9H group.

Substitution of a ribose group on the 9 position of adenine to form adenosine creates additional possible sites for proton ionization through the ribose hydroxyl groups. No evidence was found in a pH titration study[26] for proton ionization from the ribose group of adenosine; however, potentiometric[27] and calorimetric titration[19,28] results both indicate dissociation of a proton in very basic solution. The site of this ionization has been shown to be the ribose moiety of adenosine, and *both* the 2′- and 3′-OH groups were found to be necessary for the dissociation.[28] The sites for proton ionization from the phosphate chain of the adenine nucleotides have been discussed.[1]

2. Guanine, Guanosine, and Guanosine Nucleotides

Shapiro[25] in a review of the chemistry of guanine and its derivatives concludes that guanine exists in a mixture of the tautomeric forms **18** and **3**, while guanosine and the several



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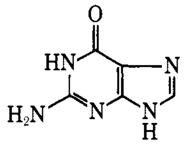

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nucleotides contain the substituent on the 9 position of guanine. He further points out that evidence exists for protonation of guanine on both the N_7 and N_9 positions; however, in the case of 9-substituted guanine derivatives the evidence strongly suggests N_7 as the protonation site. This evidence includes X-ray diffraction studies of guanine hydrochloride,[29] infrared studies of the guanosine cation,[30] the nmr spectrum of guanosine triphosphate in acidic D_2O,[23] and a comparison of the ultraviolet spectra of the cations of 7,9-dimethyl- and 1,7,9-trimethylguanine with those of 9-methylguanine and 1,9-dimethylguanine.[31] Shapiro[25] summarizes additional evidence for protonation on N_7 and points out that earlier studies

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(18) L. Eberson and I. Wadsö, *Acta Chem. Scand.*, **17**, 1552 (1963).

(19) R. M. Izatt, J. H. Rytting, L. D. Hansen, and J. J. Christensen, *J. Amer. Chem. Soc.*, **88**, 2641 (1966).

(20) J. J. Christensen, J. H. Rytting, and R. M. Izatt, *J. Chem. Soc. B*, 1643 (1970).

(21) J. J. Christensen, J. H. Rytting, and R. M. Izatt, *Biochemistry*, **9**, 4907 (1970).

(22) L. Sacconi, P. Paoletti, and M. Ciampolini, *J. Amer. Chem. Soc.*, **82**, 3831 (1960).

(23) C. D. Jardetzky and O. Jardetzky, *ibid.*, 82, 222 (1960).

(24) N. A. Berger and G. L. Eichhorn, *Biochemistry*, **10**, 1847 (1971).

(25) R. Shapiro, *Progr. Nucleic Acid Res.*, **8**, 73 (1968).

(26) T. R. Harkins and H. Freiser, *J. Amer. Chem. Soc.*, **80**, 1132 (1958).

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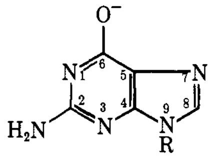
(29) J. Iball and H. R. Wilson, *Proc. Roy. Soc.*, *Ser. A*, **288**, 418 (1965).

(30) H. T. Miles, F. B. Howard, and J. Frazier, *Science*, **142**, 1458 (1963).

(31) W. Pfleiderer, *Justus Liebigs Ann. Chem.*, **647**, 167 (1961).

which cited the C_2NH_2 group as the site of protonation by analogy with protonated aromatic amines erred in not considering the considerable difference between benzenoid and heterocyclic amines.

Ultraviolet and infrared studies support the proposition that ionization from uncharged guanine derivatives is from the N_1H group[25] with **19** being the principal resonance structure of the anion.



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Proton ionization from the ribose group of guanosine has been demonstrated[21] although the site has not been determined.

3. *Hypoxanthine, Inosine, Xanthine, and Xanthosine*

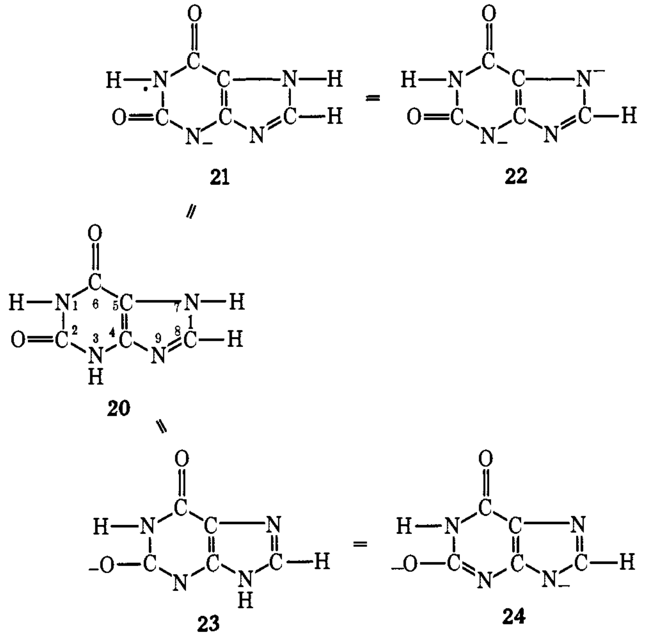
Ionization from protonated hypoxanthine is taken to be from the N_7H^+ group because of its structural similarity to protonated guanosine where ionization is known from nmr and spectroscopic data to be from the N_7H^+ group. The similarity of the $\Delta H°$ values for the p$K \sim 2$ ionization from the protonated forms of guanosine and hypoxanthine as seen in Table IA suggests that ionization is from the same site in these substances. Furthermore, the $\Delta S°$ values are approximately equal, leading to nearly identical pK values for the two species.

Table I

Selected pK, $\Delta H°$, and $\Delta S°$ Values[21] at 25° for the Indicated Reaction Types

Compound	*Probable ionization site*	*pK*	*$\Delta H°$, kcal/mol*	*$\Delta S°$, cal/(deg mol)*
	(A) $H_3A^+ = H_2A + H^+$ (Imidazole)			
Guanosine	N_7H^+	1.9	3.2	2.1
Hypoxanthine	N_7H^+	1.8	2.9	1.7
	(B) $H_2A = HA^- + H^+$ (Pyrimidine) Bases			
Hypoxanthine	N_1H/C_6O	8.91	7.88	−14.4
Xanthine	N_1H/N_3H C_2O/C_6O	7.53	6.33	−13.2
	Nucleosides			
Guanosine	N_1H/C_6O	9.25	7.65	−16.7
Inosine	N_1H/C_6O	8.96	6.50	−19.2
Xanthosine	N_1H/N_3H C_2O/C_6O	5.67	3.74	−13.4
	(C) $HA = A^- + H^+$ (Imidazole)			
Adenine	N_9H	9.87	9.65	−12.9
	$HA^- + A^{2-} + H^+$			
Hypoxanthine	N_9H	12.07	9.53	−23.3
Xanthine	N_9H	11.84	9.61	−22.0

As indicated earlier the N_1H^+ (in adenine and adenosine) and N_1H (in guanine and guanosine) groups have been established as the sites of proton ionization from the pyrimidine moiety in the indicated species. In the case of ionization from the neutral forms of hypoxanthine and xanthine and their nucleosides there are additional possible ionization sites and fewer data available to indicate the site of ionization from the pyrimidine ring. Potentiometric titration data led to the assignment of the 6-hydroxyl group (enol form) as the site of proton ionization in the cases of the neutral forms of hypoxanthine (**5**)[32] (p$K \sim 9$), xanthine (**7**)[33] (p$K \sim 7.5$), and xanthosine (**8**)[32] (p$K \sim 6$). Comparison of pK values derived from spectrophotometric data for xanthine and selected methyl derivatives of xanthine led to the assignment of ionization from neutral xanthine (**20**) to the N_3H group although there is disagreement on whether the monoanion has structure **21**[34] or **23**.[35] In this connection, an X-ray crystallographic study of sodium xanthate shows that the hydrogen atom is not attached to N_3 in crystalline xanthine.[36] In contrast to ionization from the imidazole moiety, a considerable change in the $\Delta H°$ values (kcal/mol) occurs from compound to compound for ionization from the pyrimidine moiety both in the base and the nucleoside series as is seen in Table IB.[21] This change in the $\Delta H°$ values in these series is taken to indicate the probable involvement of neighboring groups in the ionization process probably through the formation of microspecies. Simultaneous ionization of protons from more than one site in neutral xanthine could account for the discrepancies in the observed sites for proton ionization from this molecule.



Two schemes for ionization from the imidazole moiety of xanthine have been proposed based on comparison of ultraviolet absorption spectra and pK values for the monoanion of xanthine and a series of its methylated derivatives.[34,35] These schemes postulate that ionization from **21** and **23** results in the formation of **22**[34] and **24**,[35] respectively. The similar $\Delta H°$ data Table IC indicate that the ionizations from neutral

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(32) A. Albert, *Biochem. J.*, 54, 646 (1953).

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adenine and from the anions of hypoxanthine and xanthine are from similar nitrogen atoms; however, the calorimetric data do not eliminate ionization from either N_7 or N_9. The data in Table IC also show that the much larger pK values for this ionization in the cases of hypoxanthine and xanthine compared to adenine are a result of the larger negative $\Delta S°$ values which, in turn, reflect the different charge types involved in the reaction in these cases.

Two ionization steps have been reported for xanthosine.[34] Since the spectra for 1,7-dimethylxanthine and xanthosine are similar, the first ionization was attributed to the N_3H group. Calorimetric data confirm a second ionization from xanthosine as well as inosine.[21] By analogy with other nucleosides the second ionization from these substances would be expected to occur from the sugar moiety.

C. PYRIMIDINE BASES AND DERIVATIVES

1. Cytosine and Cytidine

Spectrophotometric evidence is reported[37] for proton dissociation from cationic cytosine (9) and its derivatives (H_3L^{2+}) in 1–12 M HCl. Although not positively identified, the site for this dissociation is most likely the $C_4NH_3^+$ group since there is good evidence that the other possible site, N_3H^+, is associated with ionization from the monocation.

The site of proton ionization from the monocation of cytosine and cytidine has been subject to controversy in much the same manner as have the sites for adenine and guanine and their nucleosides. Most early workers either without supporting evidence or based on the similarity of the pK values to those of protonated aromatic amines assigned the p$K \sim 4$ ionization from the protonated forms of cytosine,[38,39] cytidine,[38] and their nucleotides[40] to the protonated $C_4NH_3^+$ group.

In a potentiometric and spectrophotometric study of the reaction of formaldehyde with cytosine in the acid pH range, a pH depression was found which was considered incompatible with a displacement reaction involving the ionization of a ternary positively charged nitrogen such as N_3H^+ because such a reaction should result in pH elevation.[41] This result led Lewin and Humphreys[41] to the conclusion that under their experimental conditions the main site of proton ionization is from the $C_4NH_3^+$ rather than from the N_3H^+ group.

Jardetzky and coworkers[23,42] report nmr data valid in aqueous and trifluoroacetic acid solutions showing proton ionization from protonated cytosine to be from the N_3H^+ group. This finding has been independently supported by others in aqueous and $(CH_3)_2SO$ solutions using nuclear magnetic resonance[43,44] and absorption[45,46] spectroscopy. A calorimetric study[20] favors N_3 protonation. Using reasoning similar to that presented earlier for adenine, the $\Delta H°$ values (kcal/mol) of 5.14 (cytosine) and 5.11 (cytidine) indicate that proton ionization from a ring nitrogen is more likely than from the $C_4NH_3^+$ group. It is possible, however, that a tautomeric equilibrium involving microspecies exists which could account for the slightly higher $\Delta H°$ value for cytosine compared with those for adenine, adenosine, and cytidine. Such a tautomeric equilibrium could also account for the findings of Lewin and Humphreys[41] if the formaldehyde reaction shifted the equilibrium toward the amino protonation reaction. It is of interest that proton nmr studies of the Cu^{2+}–cytidine system clearly eliminate the C_4NH_2 group as a binding site toward Cu^{2+}.[47] Although additional experimental work appears desirable, we assign ionization from protonated cytosine and cytidine to the N_3H^+ group.

The second ionization (p$K \sim 12$) from cytosine has been assigned[45] to the N_1HC_2O groupings. Lewin and Humphreys[41] suggest that the proton may ionize from either a hydroxyl or an acidic imino group. Shugar and Fox[48] favor a hydroxyl ionization. The second ionization from cytidine has been assigned to the ribose moiety.[38,49,50]

2. Uracil, Uridine, Thymine, and Thymidine

Proton ionization from uracil, thymine, and their respective ribosyl nucleosides in the strongly acid region (p$K < 0.5$) has been reported based on spectrophotometric data.[37,51] This ionization is apparently from a cationic species in these cases; however, no definitive information concerning ionization sites or pK values is available.

The proton ionization site with p$K \sim 9.6$ in uracil, uridine, thymine, and thymidine has had various assignments. From an ultraviolet spectral study of thymine, uracil, and several substituted uracils, it was concluded that ionization from neutral uracil and thymine (p$K \sim 9.6$) was from the 2-hydroxyl group, and ionization from the uracil and thymine anions (p$K \sim 13$) was from the 4-hydroxyl group.[48] However, also on the basis of ultraviolet measurements in aqueous solution, it is stated that uracil exists primarily in the diketo form, whereas in alkaline solutions it exists as an approximately 1:1 mixture of the two possible deprotonated forms.[52] Two overlapping absorption bands with λ_{max} 260 and 284 nm have been reported for proton ionization from neutral uracil with the conclusion that protons ionize simultaneously from both the N_1H and N_3H groups.[53] Microspecies formation is probable in these systems, and further experimental work is indicated to resolve the question of ionization sites. In the case of the nucleosides calorimetric results are consistent with the first proton ionizing from the N_3 position and the second proton dissociating from the sugar group.[20] Uridine has also been shown in an infrared study to exist predominately in the diketo form.[54] However, with uridine and thymidine the

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ionization is from the N_3–C_4–O group inasmuch as the N_1 position is blocked by the sugar moiety.

D. SUMMARY OF PROBABLE PROTON IONIZATION SITES

The probable sites of proton ionization from the protonated purines and pyrimidines and their nucelosides are summarized in Table II.

Table II

Most Probable Sites of Proton Ionization from Cation, Neutral, or Anion Forms of Compounds as Indicated[a]

Compound	*Cation*	*Neutral*	*Anion*
		Purine and Purine Nucleosides	
Adenine	N_1	N_9	. . .
Adenosine	N_1	Ribose: 2′-, 3′-OH	. . .
Guanine	(N_7)	(N_1)	(N_9)
Guanosine	N_7	N_1C_6O	Ribose
Hypoxanthine	N_7	N_1/C_6O	N_9/N_7
Inosine	(N_7)	N_1/C_6O	Ribose
Xanthine	(N_7)	N_1/N_3, C_6O/C_2O	N_9/N_7
Xanthosine	(N_7)	$N_1/C_6O/C_2O$	Ribose
		Pyrimidines and Pyrimidine Nucleosides	
Cytosine	N_3	N_1/C_2O	. . .
Cytidine	N_3	Ribose	. . .
Uracil	*b*	N_3C_4O/N_1C_2O	N_3C_4O/N_1C_2O
Uridine	*b*	N_3C_4O	Ribose
Thymine	*b*	N_3C_4O/N_1C_2O	N_3C_4O/N_1C_2O
Thymidine	*b*	N_3C_4O	Deoxyribose

[a] When placed in parentheses, the site is predicted by analogy to known sites. When two sites are given, the site is either uncertain or microspecies are probable (see text). Also, when possible, the negative charge will reside on O rather than N. [b] Evidence for proton ionization from a cationic species has been reported; see text.

III. *Sites of Metal Ion Coordination*

A. INTRODUCTION

The reactions of purine and pyrimidine bases, nucleosides, nucleotides, and polynucleotides with metal ions have been studied by many workers. In the past, according to their general affinity for metal ions, these ligands have been divided into two groups—those which contain phosphate moieties and those which do not. Similarly, according to their affinity for ribonucleotides, metal ions were considered to fall into three classes. Those which interact exclusively (or nearly so) with the base portion, those which interact with both base and phosphate portions, and those which interact exclusively (or nearly so) with the phosphate portion. It was thought for many years that most metal ions could rather neatly be placed in one of the above classifications. However, as pointed out by Eichhorn and Shin[55] present evidence is that the choice of a metal ion for a binding site on polynucleotides is not an all-or-nothing proposition. It has become clear that, particularly in the case of the transition metal ions, one can speak of *degrees* of binding by a given metal ion to *both* phosphate and base sites and these *degrees* vary from metal ion to metal ion. This concept is confirmed by a recent Raman study in which the binding of Mn^{2+} and Zn^{2+} to the base appears to be weaker than to the phosphate.[56] The situation is further complicated by the fact that the bases themselves offer in each case several sites for metal ion coordination, and there has been considerable difference of opinion concerning which of these sites is coordinated to a given metal ion. Furthermore, recent evidence shows the ribose group to contain sites which coordinate with metal ions either alone or in conjunction with the base and/or phosphate moieties. Much of the controversy over site assignments may be eliminated in the future by a more general application of the recent suggestion, based on proton nuclear magnetic resonance data taken in $(CH_3)_2SO$ solutions, that multiple complexation sites exist in the case of Cu^{2+}–AMP complexes.[24] Berger and Eichhorn found, in the case of the AMP isomers (2′-AMP, 2′:3′-cyclic AMP, and 3′:5′-cyclic AMP), that approximately equal amounts of Cu^{2+} are bound to sites on the pyrimidine ring near C_2 and to sites on the imidazole ring near C_8.[24] It is likely that the experimental results obtained by various investigators have often indicated the existence of one, but not other, metal–nucleic acid species thus leading to incomplete site assignments. In recent years, the sites of metal coordination have been established in many cases using techniques, *i.e.*, ^{31}P nmr, proton nmr, ^{15}N nmr, which were not available to earlier workers. In general, the earlier assignments, many of which were found to be incorrect, are not discussed, but reference to them can be traced through the cited references. Carrabine and Sundaralingam[57] point out that combined metal and proton binding disturbs the entire electronic system of the ring in the Cu^{2+}–guanine HCl system and suggest that methods which measure localized perturbations should be used with caution. However, in a proton nmr study of Cu^{2+}–adenosine and Cu^{2+}–adenosine nucleotides, Berger and Eichhorn[24] sought but were unable to find any evidence that the perturbations on either the five- or six-membered adenine ring systems were transferred to the other ring.

A concise discussion of the methods which have been used to study the structures of metal–nucleic acid complexes has appeared.[58] These methods include proton-competing reactions, cation-sensitive electrodes, ion-exchange resin, and other ligand competition reactions, spectroscopy (ultraviolet, visible, and infrared), magnetic resonance spectroscopy (electron spin resonance, ^{31}P nmr, proton nmr, ^{15}N nmr), optical rotary dispersion, thermal transition, conductometric titration, and relaxation spectrometric assay. Raman spectroscopy has also been used to study the structures of metal–nucleic acid complexes; *e.g.*, see ref 56.

The discussion which follows is organized under the headings Alkali Metal Ions, Alkaline Earth Metal Ions, First Transition Series Metal Ions, and Other Metal Ions, in that order. The probable complexation sites are indicated in each section and are summarized in section F.

B. ALKALI METAL IONS

Alkali metal ions are generally considered to be poor complexing agents although recently they have been shown to form

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rather stable complexes with many ligands, *e.g.*, cyclic polyethers,[59,60] ethane-1,1-diphosphonic acid,[61] and hexacyanoferrate ion.[62] Additional examples may be found in recent compilations.[63,64] The formation constants of the K^+-ATP and Na^+-ATP complexes were estimated by early workers[1] to be $\sim$10. Recent, direct measurements of these constants using ion selective electrodes give the values 220 and 229, respectively;[65,66a] however, there is controversy concerning the value for K^+.[66b,c] The equal degree of association of Na^+ and K^+ with ATP has been confirmed by the fact that freezing point depressions of aqueous solutions of Na_4ATP and Na_2K_2ATP are equal.[67]

Recent proton nmr work[68] indicates that electrolytes (*e.g.*, $Mg(ClO_4)_2$, $NaClO_4$, NaOAc, NaCl, $(CH_3)_4NCl$, and tetrabutylammonium chloride) can have important effects on the conformations of nucleosides and nucleotides possibly because of the effect of the electrolyte on the water structure. Dissolving DNA in distilled water is known to result in its denaturation;[69,70] however, high concentrations of alkali metal ions stabilize the double helical structure presumably by neutralization of the negative charges on the phosphates which in aqueous solution repel each other causing destabilization.[71] Also, Na^+ ions are bound more strongly by native than by denatured DNA,[72-74] and the base composition of the DNA does not affect the binding of Li^+, Na^+, K^+, or Cs^+.[75] There is general agreement that alkali metal ions bind phosphate rather than base sites. Gordon[76] cites the absence of spectral shifts in adenine, adenosine, AMP, and RNA solutions containing Li^+, Na^+, K^+, Rb^+, or Cs^+ as evidence that these metal ions bind to the phosphate moieties.

Whether the alkali metal ions act as counterions or are attached to specific sites has not been determined unambiguously. Dialysis equilibrium and conductance data suggest that sodium ions do not bind specific sites in DNA but remain close to the DNA chains as counterions.[77] This possibility receives strong support from studies of other polyelectrolytes such as polystyrenesulfonic acid where the binding of H^+ and alkali metal ions is known to be of a diffuse electrostatic type.[78] However, Donnan equilibrium data lead to the conclusion that counterion site binding may exist in DNA interactions with Li^+, Na^+, and K^+.[79] One evidence for site binding is that the Donnan equilibrium data are consistent with the binding order of $Li^+ > Na^+ > K^+$ which has also been observed for many other simple and complex phosphate compounds and which would be expected on theoretical grounds.

Specific site binding is supported by a study of the effect of ionizing radiations from a ^{60}Co source on the binding properties of Cs^+ with DNA.[80] Retention of ^{134}Cs by the DNA is decreased following irradiation presumably because of the action of the ionizing radiations in decreasing the number of phosphates available for Cs^+ binding.

The evidence indicates that alkali metal ions bind exclusively to the phosphate moieties of ribonucleotides and DNA. Additional experimental work appears necessary to fully understand the nature of the interactions.

C. ALKALINE EARTH METAL IONS

Phillips[1] summarizes the various types of evidence used to establish the binding sites of Mg^{2+} and Ca^{2+} to the adenine nucleotides and concludes that these ions bind the phosphate but not the ring portion of the nucleotides. Specifically, interaction occurs on the α-phosphate in AMP, α- and β-phosphates in ADP, and β- and γ-phosphates in ATP. In an infrared study of freeze-dried samples of Mg^{2+}-AMP, Mg^{2+}-ADP, Mg^{2+}-ATP, and Mg^{2+}-ITP, interpretation of bond shifts leads to the conclusions that Mg^{2+} binds to phosphate in all cases and that the purine nucleus is involved in complex formation in ADP and ATP, but not in AMP.[81] No information could be obtained concerning the interaction of the C_6O group of the inosine nucleotides with Mg^{2+} since the C_6O group absorbs in the region of interest. By studying Mg^{2+} interaction with adenosine and pyrophosphate or tripolyphosphate ions present together in equal molar amounts, it was shown that no interaction occurs with adenosine unless it is in the same molecule with the phosphate group. Interpretation of absorption spectra led to the conclusion that Mg^{2+} and Ca^{2+} were interacting simultaneously with ring N atoms and triphosphate O atoms in ATP and other nucleoside triphosphates;[82] however, more recent ^{15}N nmr,[83] proton mnr,[84] temperature jump,[85] and Raman[86] data have established that in aqueous solution these ions do not complex with the nitrogen atoms of ATP. The suggestion has been made,[87] however, that the small chemical shifts of the ring protons observed[84] in Mg^{2+}-ATP complexes reflect the insensitivity of the proton chemical shift to Mg^{2+} binding rather than the absence of Mg^{2+} binding. Site binding to the phosphate moieties of ribonucleotides by Ba^{2+}, Sr^{2+}, Ca^{2+}, and Mg^{2+} is indicated by the increased stability of the complex formed

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by each cation as chain length is increased in the order AMP, ADP, ATP.[88,89] The results of a potentiometric titration determination of the pK values of the base (pK = 4) and phosphate (pK = 6) groups of 3′-AMP, ADP, and ATP in the presence and absence of Mg^{2+} are given in Table III.[90] It is seen that Mg^{2+} has very little effect on the pK value of the base ionization but has a considerable effect on the pK value of the phosphate group, indicating that binding of Mg^{2+} is primarily to the phosphate group. The recent finding[90a] using proton nmr and kinetic data that certain transition metal ions coordinate to the N_7 site of ATP *via* a water bridge structure has prompted the suggestion that Mg^{2+} and/or Ca^{2+} may complex in a similar fashion. Interactions of the ATP–M–ATP type where M = Mg^{2+} or Ca^{2+} have been observed in a Raman spectra study.[90b] These complexes dissociate in the presence of excess NaCl. Possible biological implications have been noted.

Table III

pK Values of 3′-AMP, ADP, and ATP in the Presence and Absence of Mg^{2+} and Zn^{2+} (0.1 M KCl, 25°)[90]

Ligand	*Metal*	*pK (base)*	*pK (phosphate)*
3′-AMP	H^+	3.93	6.55
	Mg^{2+}	3.93	6.30
	Zn^{2+}	3.91	6.02
ADP	H^+	4.21	6.61
	Mg^{2+}	4.16	5.86
	Zn^{2+}	3.98	5.19
ATP	H^+	4.26	6.73
	Mg^{2+}	4.12	5.35
	Zn^{2+}	3.83	4.91

The change in the pH of poly(A) and poly(U) solutions upon addition of $MgCl_2$ led to the postulation[91] that the Mg^{2+} was bound to the terminal secondary phosphate group. Felsenfeld and Huang[92] conclude from conductometric titration data of Mg^{2+} and Mn^{2+} interaction with poly(A) and poly(U) that only 1 equiv of divalent cation reacts with these polynucleotides. Furthermore, their data do not support the suggestions[77] that purine bases in denatured DNA are involved in the binding of divalent cations or that cations are bound more strongly at purine than they are at pyrimidine sites. The latter conclusion is based on the observation that the binding properties of poly(A) and poly(U) are the same within experimental uncertainty.

Evidence has been presented[93] that Mg^{2+} and Ca^{2+} are integral components of tobacco mosaic virus RNA. Dialysis of a 2.5% solution of the virus nucleic acid at 4° for 24 hr against 0.1 M phosphate at pH 7 lowered the Ca^{2+} concentration (initially 210 μg/g of virus) below an experimentally detectable level and reduced the Mg^{2+} concentration (initially 1900 μg/g of virus) to about one-tenth its original value, suggesting that Mg^{2+} is more strongly bound than is Ca^{2+}. Dialysis studies[94] show Mg^{2+} to be bound preferentially over K^+ to specific sites on RNA. Only when the K^+ concentration is increased 100-fold is the amount of bound Mg^{2+} appreciably decreased. It has been demonstrated that Mg^{2+} interacts with DNA[55,70,71,74,77,95,96] although there is disagreement concerning both the site of binding and the relative affinities of native and denatured DNA for Mg^{2+}. Spectral and conductivity results agree that 0.8 equiv of Mg^{2+} per atom of DNA phosphorus is taken up by native DNA.[69] If the DNA is treated with alkali, and the salt removed by dialysis, the resulting (probably partially denatured) DNA showed an uptake of 1.0 and 0.44–0.32 equiv of Mg^{2+} from spectral and conductance data, respectively. A dialysis study[97] shows little if any difference between the affinities of denatured and native DNA for Mg^{2+}. However, Zubay[98] in a conductometric experiment concluded that Mg^{2+} is strongly bound by the NH_2 groups on adenine and guanine because denatured DNA has a greater affinity than native DNA for Mg^{2+} and there is a significant decrease in Mg^{2+} binding by denatured DNA in the presence of formaldehyde. Formaldehyde has been shown[99] to react with the adenine amino group. Absorption spectra of heat-denatured calf thymus DNA were similar in the presence and absence of Mg^{2+}, suggesting Mg^{2+} binding to the phosphate groups. However, if the DNA is denatured in the presence of Mg^{2+}, the spectra lead to the conclusion that Mg^{2+} then binds also to the bases.[100] All other evidence indicates that Mg^{2+} binds primarily to the phosphate groups of DNA. The melting temperature (T_m) of DNA is raised from 63 to 80° in the presence of Mg^{2+} indicating that the double helix is stabilized by Mg^{2+} presumably through phosphate binding.[55,95] Upon subsequent cooling the Mg^{2+} is believed to hold the single chains of DNA in close proximity, thereby permitting the hydrogen bonds broken by heating to re-form. Activity coefficient data[96] determined in the absence of competing cations lead to the conclusion that the interaction between Mg^{2+} and DNA in pure water is of a diffuse electrostatic type with very little actual site binding. This interaction would nevertheless involve the phosphate groups where the negative charges are located.

Two highly purified yeast alanine tRNA's have been found in the absence of added Mg^{2+} (where $[Mg^{2+}]$ present $<10^{-6}$ M) to exist in partially base paired (66% of the maximum) conformations.[101] Addition of Mg^{2+} ($>10^{-3}$ M) causes both tRNA's to undergo similar conformation changes involving a net increase in base pairs (33%) and a reduction of molecular volume with the Mg^{2+} coordinated to the phosphate groups.

The available evidence is that the alkaline earth metal ions interact only with the phosphate moiety in the ribonucleotides, polyribonucleotides, DNA, and RNA. No data are available in the case of Be^{2+} and, apparently, the pos-

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sible interaction of these metal ions with the ribose group has not been investigated.

D. FIRST TRANSITION SERIES METAL IONS

1. Introduction

It was early recognized[93] that the transition metal ions Fe and Cu were components of tobacco mosaic virus DNA and that they were very strongly attached to complexing sites of the nucleic acid. Wacker and Vallee[102] using chelating agents and dialysis as a function of pH were able only with difficulty to remove the metals Cr, Ni, Mn, Fe, and Cd from RNA preparations obtained from phylogenetically diverse sources. The presence of Mn in RNA isolated from human and rat tissues has been confirmed.[103] The extreme difficulty of removing these or other metals from these preparations and the observation[104] that they stabilize the ordered structure of RNA indicate that they may play a significant role in the maintenance of the configuration of the RNA molecule possibly linking purine or pyrimidine bases or both, through covalent bonds. It was further suggested[102] that metals may bear a functional relationship to protein synthesis and the transmission of genetic information. Thus, transition metal ions are known to be present in and extremely tightly bound to the naturally occurring RNA material, and it is likely that they are responsible for holding the RNA molecules in specific conformations. This last property may be an important one in the action of RNA in protein synthesis and in the transmission of genetic information.

In the following discussion the several transition metal ions are treated separately. Few interaction sites of these metal ions with the purines, pyrimidines, and their nucleosides have been determined. However, in the nucleotides, polynucleotides, DNA, and RNA interactions with base, phosphate and ribose have been reported in several instances. An interesting aspect of the coordination involving at least the bivalent metal ions is their reported[55] increasing *degree* of affinity for the base relative to phosphate site in the sequence Co^{2+}, Ni^{2+}, Mn^{2+}, Cu^{2+}. The sites of interaction for the transition metal ions are summarized in Table IV.

2. Chromium

A pulsed nmr technique has been employed[105,106] to measure the spin-lattice relaxation time of the water protons in the presence of paramagnetic ions and DNA. The observed relaxation times are a function of the concentration of metal ion and also of its environment (*i.e.*, free or bound). In the case of Cr^{3+}, interpretation of the relaxation time data leads to the conclusion that Cr^{3+} is bound to DNA at exterior sites, probably the phosphate groups. Since Cr^{3+} is concentrated in RNA rather than in proteins and it has a preference for octahedral holes (over any irregular geometry) due to the large ligand field stabilization energy arising from appropriate splitting of the 3d subshells in the field of the ligand, Williams[107] has suggested that kinetically stable RNA conformations should be formed in the presence of Cr^{3+}.

3. Manganese

Coordination of Mn^{2+} with the phosphate groups of ribonucleotides has been demonstrated in aqueous solutions for AMP, ADP, and/or ATP by ^{31}P nmr,[84,108–111] potentiometric,[88] ion exchange,[112] electron spin resonance,[113] infrared,[114] and Raman[56] studies. Interaction with all available phosphates is indicated by ^{31}P nmr spectra in the cases of AMP,[108] ADP,[84] and ATP,[84,110] but an infrared study[114] of the changes in the phosphate absorption bands in the 900–1300-cm^{-1} region in the presence of Mn^{2+} showed only the β- and γ-phosphates of ATP to be coordinated to Mn^{2+}. The presence of neither the adenine ring nor the ribose group influenced the infrared spectra. Furthermore, similar results were obtained if methyl triphosphate was substituted for ATP.

A Raman spectral study[56] of Mn^{2+}–ATP interaction shows the Mn^{2+} to bind the base moiety and to promote intramolecular base–phosphate interaction. Proton nmr data indicate that Mn^{2+} binds the adenine ring of ADP[84] and ATP.[84,111,113] Since the C_8H peak is broadened,[84] coordination apparently ocurs at the N_7 site of ADP and ATP with possible participation from the C_6NH_2 group.[87] However, Sundaralingam[115] in an examination of the conformational possibilities for metal–nucleotide interaction discounts the C_6NH_2 group as a complexing site. He points out that the amino group in adenine is highly conjugated with the ring and has considerable double bond character with a resulting lowered basicity compared to the amino groups of aniline or amino acids. Support for the binding of Mn^{2+} to the base moiety of ATP is found in a recent proton nmr and kinetic study.[90a] The experimental data were accounted for by assuming that a water molecule forms a bridge between the Mn^{2+} and the N_7 site. The remaining metal coordination sites were phosphate oxygen atoms. Optical rotary dispersion data suggest[116] an interaction of Mn^{2+} with the 2′- and 3′-OH groups of ATP.

Binding of Mn^{2+} to the phosphate moieties of DNA, poly(A), poly(I), poly(C), and poly(U) has been established by a pulsed nmr technique,[105,106,116] and binding between Mn^{2+} and the phosphate moieties of RNA has been demonstrated by a ^{31}P nmr method.[108] It is concluded from a conductometric titration study that Mn^{2+} does not bind the adenine bases in poly(A).[92] Eichhorn and Shin[55] deduce from the effect of Mn^{2+} on the melting temperature of DNA that Mn^{2+} interacts with both the phosphate and nucleic acid bases in DNA. This conclusion is based on the ability of Mn^{2+} to cause partial rewinding of the double helix at lower

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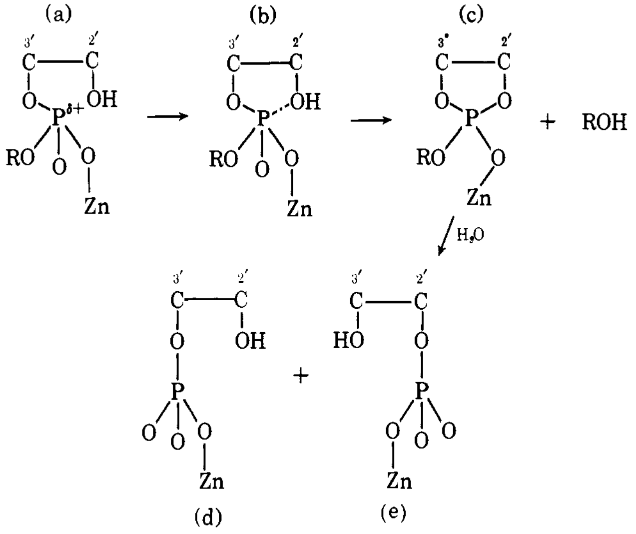
temperatures and the shift in the absorption maximum of DNA in its presence. The fact that RNA, but not DNA, is depolymerized by Mn^{2+} and several other metal ions resulted in the plausible suggestion[71,117] that degradation by metal ions proceeds through chelation of the metal between the phosphate and the 2′-hydroxyl group. However, in a later study Butzow and Eichhorn[117a] showed that this complex was not an intermediate in the degradation of polynucleotides. Working with simpler substrates, ribooligomers of defined nucleoside sequence, the kinetics of the breakage of the phosphodiester bond in the presence of Zn^{2+} were determined spectrophotometrically, and the products of the reaction were identified by thin layer chromatography. The fact that 2′:3′-cyclic phosphodiesters are found in the reaction mixture strongly suggests that the zinc degradation proceeds through a 2′:3′-cyclic phosphate form. Chelation of the Zn^{2+} with the 2′-hydroxyl group should favor the direct cleavage and, therefore, would not be expected to occur. Rather, as seen in **25** the zinc ions binding to the phosphate groups polarize the PO linkage, producing a positive dipole on the phosphorus atom which then forms a ring with a 2′-hydroxyl group. Subsequently, the phosphodiester linkage is cleaved. Presumably, other metal ions (Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, La^{3+}, Ce^{3+}, and Lu^{3+}) found to depolymerize RNA[117] also proceed through a mechanism similar to **25**.



25

Electron paramagnetic resonance data are consistent with the bonding of Mn^{2+} in RNA to the oxygen atoms in two phosphate groups.[103] Possible binding of Mn^{2+} to ribose OH groups or to the nitrogenous bases cannot be excluded by this technique, but the spectra do not support binding to the base nitrogen atoms.

4. *Iron*

The interactions of Fe^{2+} and Fe^{3+} with adenine nucleotides, poly(A), and methylated poly(A) have been studied by Mössbauer spectroscopy.[118] The Mössbauer spectral data for Fe^{3+}–AMP, -ADP, and -ATP interaction at low pH support a model in which Fe^{3+} is surrounded by an octahedral arrangement of oxygen atoms indicating binding only to the phosphate moiety of the nucleotides. When the pH is raised to 7, the resulting quadrupole splittings suggest that one or more ring nitrogen atoms have replaced oxygen donor atoms on the Fe^{3+}. The spectrum for the interaction of Fe^{3+} with poly(A) methylated at the N_1 position resembles almost exactly that for Fe^{3+}–AMP and Fe^{3+}–poly(A) showing that the N_1 position is not involved in complex formation with Fe^{3+}. Thus the N_7 and/or C_6NH_2 positions appear to be the sites of binding in the nucleotides. In this study all samples used were lyophilized powders and the temperature was 77°K.

A Mössbauer study[119] of Fe^{3+} interaction with guanine, ribose, and guanosine at liquid N_2 temperature using lyophilized samples showed the behavior of the Fe^{3+} toward ribose to be quite different from that toward the other two compounds. The Mössbauer spectra of the guanine complex is a singlet unaffected by changing pH and is characteristic of high-spin Fe(III) complexes. On formation of the Fe(III)–ribose complex there is incomplete reduction of the Fe(III); however, no reduction of Fe(III) occurs in the Fe(III)–guanosine complex.

Equilibrium constant data[120] for the reaction of Fe^{3+} with ADP, ATP, ITP, GTP, CTP, and UTP show (1) the formation constant to increase in the series ADP, ATP indicating phosphate interaction, and (2) no significant stability difference when the base is changed. The conclusion drawn from these results was that there was little or no interaction of Fe^{3+} with the bases.

Pulsed nmr data[105,106] lead to the conclusion that Fe^{2+} is bound to phosphate rather than interior base sites in DNA, but were inconclusive on whether Fe^{3+} was bound to interior base or exterior phosphate sites. However, Eichhorn[95] explains the change in optical density of Fe^{2+}–DNA solutions as the solution temperature is raised from 30 to 90° by assuming initial binding of Fe^{3+} to phosphate followed by a change to binding of the nucleotide bases after the hydrogen bonds have been broken. It would thus appear that in this case the binding sites may depend on the temperature of the system.

In a radioactive isotope study of tobacco mosaic virus RNA-Fe^{3+} interaction, Singer[121] concluded that Fe^{3+} binds to the bases of RNA since the same amount of Ca^{2+} was bound to the RNA whether the Fe^{3+} was present or not, and Ca^{2+} was assumed to bind only to the phosphate groups. Williams[107] has found that the major portion of the iron bound to RNA can be removed on dialysis and is presumably bound to the phosphate groups. However, a much smaller portion of the total iron is very difficult to remove and on the basis of spectral data is believed to be bound to the RNA bases. Spectral evidence indicates that this iron is present as low-spin Fe(II).

It would appear that formation of Fe^{2+} and Fe^{3+} complexes of these compounds is very dependent on the experimental conditions of pH, temperature, presence of other cations, etc. More work on these most interesting systems seems warranted.

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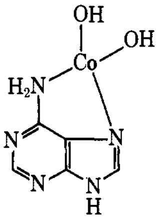
5. *Cobalt*

Adenine–cobalt complexes were first reported[122] in 1951. The complexes were studied calorimetrically by the α-nitroso-β-naphthol reaction with a parallel isotopic method, but no attempt was made to assign sites of coordination. Infrared spectral data for a solid Co^{2+}–adenine complex were interpreted to show Co^{2+} binding to the C_6NH_2 and N_7 groups with two additional OH groups bound to Co^{2+} as in **26.**[123] In strong acid solution this complex is also stable with the OH groups being converted to H_2O molecules. Brigando and Colaitis[124] in an infrared study of solid Co^{3+}–adenosine complexes report that Co^{3+} binds to the C_6NH_2 and $C_{5'}OH$ groups as indicated in **27**. They specifically eliminate binding to N_7.



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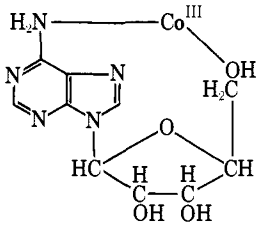

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Kan and Li[125] in an nmr study of the Co^{2+}–adenosine system in dimethyl sulfoxide observe that addition of Co^{2+} causes approximately equal downfield shifts of the C_8H and C_2H signals and a broadening of the C_6NH_2 signal. Interpretation of these data led to postulation of the N_7 and amino groups as the binding sites. However, these results and those involving Co^{2+}– and Co^{3+}–adenine interaction should be considered carefully in view of the considerable evidence that the amino group is not involved in metal[24,115] or proton[25] complexation in purines.

Cobalt(II) ion has been found[84,85,87,88,90a,108–110,112,114,116] to bind to AMP, ADP, and ATP in a manner similar to that of Mn^{2+}, and the discussion given under manganese is relevant. In a recent study employing difference spectroscopy it was concluded that Co^{2+} was bonded only to the phosphate groups of AMP, ADP, and ATP.[126] However, proton nmr data[87] show Co^{2+} to interact with all three phosphate oxygen atoms (*i.e.*, α, β, and γ) and the N_7 nitrogen of ATP with possible binding to the C_6NH_2 also. Similarly, proton nmr data show binding of Co^{2+} between the N_7 and the C_6O^- groups of ITP.[87]

Pulsed nmr data[105] lead to the conclusion that in DNA Co^{2+} is bound to phosphate rather than to interior (*i.e.*, base) sites. Eichhorn and Shin,[55] however, found in an ultraviolet spectral study of Co^{2+}–DNA interaction as a function of temperature that Co^{2+} could produce partial rewinding of the DNA double helix indicating, in addition to strong phosphate interaction, some tendency to react with the bases. They place Co^{2+} above Mg^{2+}, approximately equal to Ni^{2+} and below Mn^{2+}, Zn^{2+}, Cd^{2+}, and Cu^{2+} in its affinity for base sites. It has been concluded from ^{31}P nmr data[108] that Co^{2+} binds to phosphate in RNA. The earlier postulation of a Co^{2+} chelate involving the 2′-OH group of ribose[71,117] has been found to be incorrect.[117a]

6. *Nickel*

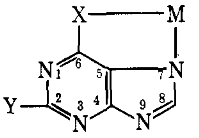
Interaction sites for Ni^{2+} have been found to be essentially the same as those for Mn^{2+} and Co^{2+}. The affinity of Ni^{2+} for base sites is reported to be similar to that of Co^{2+}.[55] Using a temperature jump procedure, Karpel, Kustin, and Wolff[126a] find a 1:1 complex formed between Ni^{2+} and adenine. The kinetic data are consistent with the formation of a chelate involving the N_7 and C_6NH_2 groups of adenine. The reader is referred to earlier sections on Mn and Co for additional literature references.

7. *Copper*

More experimental work has been reported for the binding of the ligands considered here by Cu^{2+} than by any other single metal ion. The binding studies have included Cu^{2+} interactions with bases, nucleosides, nucleotides, and nucleic acids, and they are discussed in that order.

a. Copper–Base Interactions

The purine bases have two high electron density centers which are possible sites for metal ion chelation, *viz.*, C_6NH_2/C_6O–N_7 (**28**) and N_3–N_9 (**29**). Chelation of Cu^{2+} by both sites has been suggested. Structure **28** has been proposed for solution reactions[26,32,127,128] partly on the basis of structural similarities between purines and 8-hydroxyquinoline.[32] Aqueous solution stability constant measurements for the interaction of Cu^{2+} with various substituted purines has led to the postulation[129] of structure **29** in the cases of adenine, hypoxanthine, and xanthine although there is some uncertainty in the assignment. Recent electron spin resonance[130] and X-ray crystallographic[131] studies show **29** to be the structure for solid Cu^{2+}–adenine complexes. However, infrared data using the KBr disk technique indicate that **28** is the structure in the solid Cu^{2+}–guanine complex.[132] Arguments for the inability of the amino group to bind Cu^{2+} have been presented.[115]



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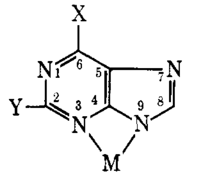

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The crystal structure of a 2:1 cytosine–copper(II) chloride complex has been shown by X-ray crystallography to consist of a copper atom binding two bases at the N_3 positions with only weak binding (if any) by the base oxygen atoms.[133]

The affinity of pyrimidine bases for Cu^{2+} appears to be much lower than that of purine bases. No evidence was found

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for complexation of Cu^{2+} with uracil in hot water[134] or with cytidine or uridine in a Raman study.[135] In a proton magnetic resonance study,[136] the addition of $CuCl_2$ to cytosine in dimethyl sulfoxide broadened the C_5H peak slightly more than the C_6H peak, leading to the conclusion that Cu(II) binds at the N_3 position. Melzer[134] treated $CuCl_2$ with methanol for 3 hr and in unbuffered aqueous solution (pH ~5.5) at 80° for 30 min obtaining two complexes having Cu to cytosine ratios of 1:2 and 1:1. Yields of 71 and 5% were obtained for the two complexes in methanol and 13 and 11% in aqueous solution. These results suggest the possibility of cytosine–Cu^{2+}–cytosine cross linkages in DNA and may explain the naure of the very stable linking of Cu^{2+} to nucleic acid found by Wacker and Vallee.[102]

Further work directed toward elucidating the sites of Cu^{2+} interaction with purines and pyrimidines, particularly in aqueous solution, is clearly needed.

b. Copper–Nucleoside Interactions

Complexes of first transition series metal ions (including Cu^{2+}) with purine and pyrimidine nucleosides in aqueous solution have been postulated.[32,137,138] No evidence is found in pH titration studies[26,32,139] for Cu^{2+}–adenosine interaction. The fact that the pH titration curve of the copper(II)–adenosine system is essentially the same as that of hydrated Cu^{2+} was later confirmed[138] for adenosine, guanosine, cytidine, and uridine. As has been pointed out,[138] the failure to observe a shift in the potentiometric titration curves could be a result of either no complex formation or complex formation proceeding without the removal of protons from the ligand molecule. Since complex formation has been demonstrated by other methods,[138,140–143] the second explanation appears to be the correct one. Proton nmr data[24] valid in $(CH_3)_2SO$ are reported for the Cu^{2+} complexes of adenosine and tubercidin (7-deazaadenosine) which has the N_7 atom replaced by C, leaving no free N on the imidazole ring to coordinate Cu^{2+}. Two potential coordinating nitrogen atoms remain on the pyrimidine ring. The major finding is that the broadening of the C_8H peak in adenosine is due to Cu^{2+} coordination at the N_7 position. The C_8H peak is not broadened by Cu^{2+} coordination to the pyrimidine moiety of tubericidin, indicating that the broadening effect is not transmitted from the six-membered ring to the five-membered ring. Tu and Friederich[144] from both conductometric (or potentiometric) titration and spectrophotometric titration data find that 1 mol of Cu^{2+} combines with 1 mol each of guanosine and inosine. In constrast, no complex formation was found up to 1×10^{-3} *M* Cu^{2+} and 1×10^{-3} *M* cytidine. Complex formation was observed at the concentration of 0.1 *M* Cu^{2+} and 0.1 *M* cytidine. Proton magnetic resonance studies of the reaction in dimethyl sulfoxide of Cu^{2+} with several deoxynucleosides lead to the conclusion that binding is to N_7 in deoxyadenosine and deoxyguanosine and N_3 in deoxycytidine.[138] The lack of proton magnetic resonance shifts in deoxythymidine lead to the conclusion that no appreciable degree of binding occurs in this case. The amino group is not involved in the binding of Cu^{2+} by adenosine in dimethyl sulfoxide.[24] No change in the aqueous Raman spectrum of either cytidine or uridine was found upon addition of $CuSO_4$;[135] however, interaction of Cu^{2+} with uridine (presumably at N_3, but possibly with some contribution from the C_4O group) has been confirmed by proton nmr spectra.[47]

Reinert and Weiss[140] report green and blue complexes with Cu^{2+} attached to the 2′ and 3′ ribose oxygen atoms of adenosine and uridine. The green complex was found in the pH region 9.5–10.5 while the blue complex was stable at pH 12. Binding of Cu^{2+} to ribose hydroxyl groups as well as the uracil base of uridine in dimethyl sulfoxide solvent has also been observed in a proton nmr study.[47] Interaction with ribose hydroxyl groups was not observed in a similar study of Cu^{2+}–adenosine interaction in dimethyl sulfoxide.[24]

c. Copper–Nucleotide Interaction

Potentiometric,[138,139] ^{31}P nmr,[84,138] and aqueous solution infrared absorption data[114] confirm the binding of Cu^{2+} to the phosphate portion of AMP, dAMP, dGMP, dCMP, ADP, and ATP. These studies are in essential agreement that Cu^{2+} binds the available phosphate groups in the mono- and dinucleotides but only the α- and β-phosphates in ATP. This latter behavior has been attributed to the square-planar stereochemical requirements of Cu^{2+}. Taqui Khan and Martell[139] find a concentration-dependent buffer region at pH values of 6.5–8 in the Cu^{2+}–ATP system which is characteristic of polynuclear complex formation. They postulate the formation of a dimer containing two Cu^{2+} and two ATP species bonded only through the α- and β-phosphates. It is interesting that reaction of Cu^{2+} with IDP and ITP apparently does not involve the phosphate groups.[145]

Based on the observed lack of reaction (from pH titration data) between Cu^{2+} and adenosine and the increased stability of Cu^{2+} complexes in the order AMP < ADP < ATP, the suggestion was made that Cu^{2+} did not react with the base moiety of ATP.[139] However, proton nmr studies have demonstrated binding of Cu^{2+} to the N_7 positions of the adenine base in dAMP,[138] the guanine bases in 2′(3′)-GMP[146] and dGMP[138] (broadening of the C_8H peaks is observed), and to the N_3 position of dCMP[138] and CMP (broadening of the C_5H peak is observed). The N_1H and NH_2 lines are relatively unaffected by Cu^{2+} in dAMP, dGMP, and dCMP, eliminating them as possible binding sites.[138] Binding to N_7 has also been demonstrated by proton nmr for ADP and ATP.[84] It is of further interest that proton nmr and electron spin resonance data show that Cu^{2+} is still involved in complexes with the GMP base at pH 2.

Reaction of Cu^{2+} with 5′-GMP,[144] 5′-IMP,[144] IDP,[145] and ITP[145] has been shown to involve the C_6O and N_7 groups. As previously noted, no interaction of Cu^{2+} with the phosphate groups was found apparently because of the square-

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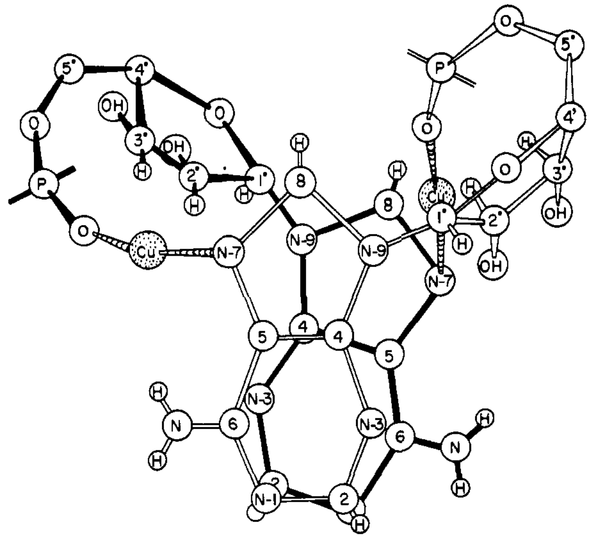
planar requirements of Cu^{2+} and its preferred binding to the C$_6$O group. Sigel[147,148] studied Cu^{2+}–ITP, –GTP, –UTP, and –TTP binding using methods based on ultraviolet difference spectra, pH titration, and H_2O_2 oxidation. His results indicate interaction at N$_7$ and perhaps C$_6$O in ITP and GTP, and N$_3$ in CTP, UTP, and TTP. The formation of a stable Cu^{2+}–TTP complex is interesting since no evidence was found[138] for interaction of Cu^{2+} with deoxythymidine or dTMP. It is likely, however, that binding with TTP may be a result of the additional phosphates present.

In a definitive proton nmr study of the binding of Cu^{2+} to adenine nucleotides in D_2O, Berger and Eichhorn[24] find that C$_8$H is broadened preferentially to C$_2$H in 3′-AMP, 5′-AMP, and poly(A); C$_8$H and C$_2$H are broadened equally in adenosine, 2′-AMP, 3′:5′-cyclic AMP, and 2′:3′-cyclic AMP; and C$_2$H is broadened preferentially to C$_8$H in tubercidin (7-deazaadenosine). They conclude that, in general, Cu^{2+} can bind to multiple sites on the adenine base, with preference for a given site influenced by molecular associations which in the different AMP isomers is governed by the position of the phosphate on the ribose. For example, the preferential broadening of the C$_8$H proton in 5′-AMP indicates that Cu^{2+} coordination to the N$_7$ position is favored by the phosphate at the 5′ position. This is in contrast to adenosine where coordination to N$_7$ and N$_1$(N$_3$) occurs in approximately equal amounts. Also, in 3′-AMP Cu^{2+} binds preferentially to N$_7$ even though the phosphate group is constrained to one side of the ribose and is unable to approach closely to the adenine. In contrast there is no site preference, N$_7$ over N$_3$ (N$_1$), in the cases of adenosine, 2′-AMP, or the 3′:5′- or 2′:3′-cyclic AMP. Their results with 3′- and 5′-AMP suggest a binuclear 2:2 Cu^{2+}/AMP complex in which the two bases are stacked with each Cu^{2+} bound to a phosphate of one AMP and N$_7$ of the other as seen in **30**. In the case of 2′-AMP a chelate involving N$_3$ and a phosphate group of the same molecule is proposed as in **31**.

From continuous variation data, it was concluded that Cu^{2+} binds poly(A), poly(C), and poly(I), but not poly(U).[149] The failure of Cu^{2+} to react with poly(U) was consistent with the earlier observations based on spectrophotometric and potentiometric data that Cu^{2+} did not react with uridine, thymidine, or 3′(2′)-UMP.[137,138,144] However, in a later proton nmr study Cu^{2+} was found to bind UMP and poly(U) near N$_3$.[47] The preferred binding site of Cu^{2+} in poly(A) is reported from proton nmr data to be N$_7$.[24] In poly(C) broadening of the C$_5$H, but not the C$_4$NH$_2$, peak indicates binding of Cu^{2+} to N$_3$.[47] Continuous variation studies[149] indicate that for $[\text{poly(A,U)}] = 1 \times 10^{-4}\ M$ if $[\text{Cu}^{2+}] \lesssim 1 \times 10^{-4}\ M$ the complexing of poly(A) with poly(U) proceeds as it would in the absence of Cu^{2+}. However, if $[\text{Cu}^{2+}] > 1 \times 10^{-4}\ M$, formation of poly(A,U) is virtually prevented. The reactions were also studied in the reverse direction where Cu^{2+} in excess of 50 mol % (sum of Cu^{2+} + polynucleotide concentrations = 1×10^{-4} M) was found to result in dissociation of the poly(A,U) complex. Cu^{2+} causes similar denaturation of poly(I,C). The explanation given for this phenomena is that Cu^{2+} when present in low concentration bonds to the phosphate stabilizing the polymer. As the mole fraction of Cu^{2+} is increased, the Cu^{2+} bonds to the bases and denatures the complex poly(A,U) or poly(I,C). The denaturation of the poly(I,C) complex can be reversed by the addition of 1 M NaCl. When heated in the presence of Cu^{2+}, poly(A) is degraded into low molecular weight oligonucleotides by cleavage of the phosphate bonds.[117] The effect of cupric ion on ordered synthetic polynucleotides is very similar (with the exception of phosphate bond cleavage) to its effect on DNA although the synthetic polynucleotides are denatured under milder conditions than those necessary for denaturation of DNA. As pointed out by Eichhorn,[138] it is important to note that binding to the bases in nucleosides, nucleotides, etc., is very different from binding to the purines and pyrimidines alone, since the position of attachment of the ribose to the bases becomes a site for metal binding in the absence of the ribose bond.



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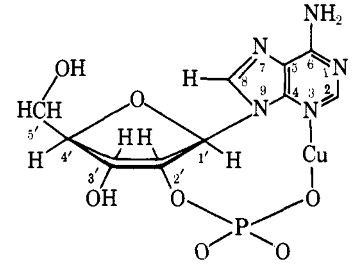

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d. Copper–Nucleic Acid Interaction

The interaction of Cu^{2+} with DNA has been subject of a number of recent studies.[55,95,105,106,150–157] Proton nmr data[105,106] show binding of Cu^{2+} with phosphate; however, at high Cu^{2+} concentrations binding to the bases appears to occur. Melting temperature (T_m) data[55,150,153] obtained under various conditions of ionic strength and Cu^{2+} concentration show that addition of small quantities of Cu^{2+} to DNA (0.5 Cu^{2+}/2DNA) causes T_m to increase, indicating increased DNA

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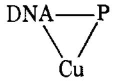
double helix stability due to binding of Cu^{2+} to the phosphate moieties, thereby reducing the electrostatic repulsion between the strands. However, as the Cu^{2+} concentration increases relative to that of the DNA, T_m decreases owing to metal ion coordination with sites on the nucleic acid bases. A sharp decrease of T_m occurs[150] between 0.5–0.8 Cu^{2+}/2DNA phosphorus and 1.5 Cu^{2+}/2DNA phosphorus. Unlike the situation with Co^{2+}, Ni^{2+}, Mn^{2+}, and Zn^{2+}, rewinding of the double helix does not occur upon cooling but does occur upon the addition of electrolyte to the cooled solution.[55,153] The interpretation of these observations is that Cu^{2+} binds more strongly to the nucleoside bases than do the aforementioned metal ions. Several studies[153–155] indicate that Cu^{2+} may be attached initially to the phosphates of native DNA, and that binding with bases occurs only upon heating although the interaction occurs readily at lower temperatures once the helix is disrupted. However, absorption spectral data comparing Cu^{2+} reactivity toward apurinic acid, apyrimidic acid, and DNA indicate some binding of Cu^{2+} to the pyrimidine moieties of DNA at 1-2°.[158] Using the techniques of gel filtration, difference spectroscopy, viscosity, and ultracentrifugation, Bryan and Frieden[151] found evidence for Cu^{2+} interaction with the bases of DNA at 26°. The viscosity data and dissociation curves obtained by them suggest that two binding sites are involved. They found that identical maximum spectral changes were produced by heated and nonheated DNA although reaction times and required Cu^{2+} concentrations were different in each case. The observation that the viscosity of Cu(II)-DNA solutions decreases before hyperchromism and sedimentation changes occur was taken to indicate the formation of an initial species, DNA-P-Cu^{2+}, which produces loosening in the coiling of the molecule prior to major changes in shape. The changes in shape are then attributed to the formation of a complex of the type **32**.



32

Bryan and Frieden[151] also calculated a binding constant that was best described by assuming that only the purine nucleotide units bind to Cu(II) in a 1:1 complex. This assumption is substantiated[128] by the observation that cupric ion catalysis of ascorbate oxidation is inhibited by DNA and its components in the order of decreasing effectiveness: purines > purine nucleotide = RNA − DNA > purine nucleoside > pyrimidine nucleotide. Evidence exists that Cu^{2+} binds to guanine sites in DNA.[150,156,159] The basis for this suggestion is the stronger destabilization of the double helix for DNA rich in guanine and cytosine[156] compared to DNA rich in adenine and thymine as well as the fact[150] that the Cu^{2+}–guanosine complex is more stable than the Cu^{2+}–adenosine complex. In addition, modification of the G·C base pairs by methylating the guanine N_7 of DNA reduces the affinity of DNA for Cu^{2+} as is evidenced by a lessening of the destabilizing effect of Cu^{2+} on the melting of DNA.[159]

Minchenkova and Ivanov[156] find that the addition of reducing agents, *i.e.*, ascorbic acid or sodium borohydride, to a DNA solution containing Cu^{2+} causes changes in the DNA absorption spectra. A new absorption band with a maximum of 280 nm is assigned to a DNA base–Cu^+ complex. The melting temperature is raised and the circular dichroism curve is quite different for DNA–Cu^+ as compared with that for DNA itself. The suggestion is made that the above effects are caused by proton transfer along the hydrogen bond from guanine to cytosine under the influence of the chelate formed between Cu^+ and the N_7, C_6O sites of guanine. Ropars and Viovy[160] in an electron paramagnetic resonance study of Cu^{2+}–DNA interaction find that Cu^{2+} binds to the phosphate groups as well as to guanine through the C_6OH and N_7 groups. Tu and Friederich[144] in a conductometric and infrared study have confirmed that Cu^{2+} binds to the N_7 and C_6O groups of the guanine base of DNA.

Little work has been reported for Cu^{2+}–RNA interaction. Cu^{2+} has been found to participate in RNA depolymerization presumably by coordination with the phosphate groups[117a] and not with the 2′-OH groups as originally postulated.[71,117]

8. Summary of Probable Transition Metal Coordination Sites

The probable complexation sites of the first-row transition metal ions are given in Table IV.

E. OTHER METAL IONS

1. Zinc

Stable complexes are formed between Zn^{2+} and the purine bases. In a proton nuclear magnetic resonance study[136] of $ZnCl_2$–purine interaction in DMSO all signals were found to be shifted downfield relative to those found in the absence of $ZnCl_2$. Since the C_8H chemical shift was greatest, it was concluded that N_7 is the preferred Zn^{2+} binding site in purine. No evidence was found for Zn^{2+}–cytidine or –uridine complexing in aqueous solution in a Raman study.[135] In the case of $ZnCl_2$–cytosine interaction in DMSO,[136] equal downfield chemical shifts were observed in the C_5H and C_6H peaks upon addition of the $ZnCl_2$, leading to the conclusion that N_3 is the preferred binding site for Zn^{2+} in cytosine. It is not particularly surprising that there have been no reports of Zn^{2+}–nucleoside interaction since Cu^{2+} has been found to form only weak complexes with nucleosides and Zn^{2+} would be expected to form still less stable complexes. One attempt[139] to detect these complexes by pH titration failed in the case of adenosine.

On the basis of successively larger formation constants in the order AMP < ADP < ATP, complexation with all available phosphates has been suggested.[88,139] The addition of $ZnCl_2$ to a solution of 0.5 *M* NaCl produces a broadening of the ^{35}Cl nmr line.[161] This broadening varies linearly with Zn^{2+} concentration and is pH independent to the point where $Zn(OH)_2$ begins to form. Ward and Happe[161] have used this nmr technique to study the Zn^{2+}–ADP interaction in the presence of Cl^- and postulate formation of Zn_2ADP, ZnADP, and $Zn(ADP)_2$ in dilute aqueous solutions. No sites were postulated although by analogy with pyrophosphate (also studied) Zn^{2+} complexation with phosphate groups was suggested. Nmr[83,84] and infrared[114,162,163] spectroscopic

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(158) Ye. T. Zakharenko and Yu. Sh. Moshovskii, *Biophysics (USSR)*, **11**, 1083 (1966).

(159) C. Zimmer and H. Venner, *Eur. J. Biochem.*, **15**, 40 (1970).

(160) C. Ropars and R. Viovy, *J. Chim. Phys., Physicochim. Biol.*, **62**, 408 (1965).

(161) R. L. Ward and J. A. Happe, *Biochem. Biophys. Res. Commun.*, **28**, 785 (1967).

(162) H. Brintzinger, *Helv. Chim. Acta*, **48**, 47 (1965).

(163) H. Brintzinger, *J. Amer. Chem. Soc.*, **87**, 1805 (1965).

Table IV

Probable Complexation Sites of First-Row Transition Metal Ions[a]

Metal ion	*Purine*	*Pyrimidine*	*Nucleoside*	*Nucleotide*	*Polynucleotide*	*DNA*	*RNA*
Cr^{3+}						Phosphate	
Mn^{2+}				Phosphate N_7/C_6NH_2	Phosphate	Phosphate Base	Phosphate
Fe^{2+}						Phosphate Base	Base
Fe^{3+}				Phosphate, base	Phosphate, Base	Phosphate	Phosphate
Co^{2+}	C_6NH_2/N_7		N_7/NH_2 (DMSO)	Phosphate N_7/C_6X		Phosphate Base	
Co^{3+}			$C_6NH_2/C_{5'}OH$ (solid)				
Ni^{2+}				Phosphate N_7/C_6X		Phosphate Base	
Cu^{2+}	C_6X/N_7 or N_3/N_9	N_3	N_7 Ribose	Phosphate Base (N_7 purines) (N_3 pyrimidines)	Phosphate Base (N_7 purines) (N_3 pyrimidines)	Phosphate Base	Phosphate (Base)?

[a] Blank spaces indicate no data available. Text should be consulted for experimental conditions.

studies indicate that in ATP Zn^{2+} is bound to the β- and γ-phosphate groups and to the adenine. A ^{15}N nmr study[83] of the Zn^{2+}–ATP complex (0.5–0.9 M in ATP) shows that Zn^{2+} causes small downfield shifts in the N_9 and amino nitrogen resonances as well as an upfield shift in the N_7 signal. These shifts are consistent with the interaction of Zn^{2+} with both the N_7 and amino groups. The reaction of Zn^{2+} with both base and phosphate moieties of ATP is also confirmed by Raman spectroscopy[56] with the additional suggestion that in the presence of Zn^{2+} there is intramolecular phosphate–base interaction. The pK data presented in Table III for proton ionization from 3′-AMP, ADP, and ATP in the presence and absence of Zn^{2+} support the binding of Zn^{2+} to both phosphate and base moieties, at least in the cases of ADP and ATP.

Zinc ion can unwind and rewind DNA reversibly when a DNA solution is heated and cooled.[55] This phenomenon is explained by assuming that Zn^{2+} holds the two chains in proximity during the unwound stage by binding to the bases (less strongly than does Cu^{2+}), and that Zn^{2+}, unlike Cu^{2+}, itself causes rewinding without the necessity of adding concentrated electrolyte as in the case of Cu^{2+}.

The depolymerization rate of RNA by Zn^{2+} is approximately ten times as rapid as with Mn^{2+}, Co^{2+}, Ni^{2+}, or Cu^{2+}. The reaction is believed to involve coordination of Zn^{2+} to the phosphate group as shown in **25**,[117a] but not to the 2′-OH group as postulated earlier.[71,117] The reasoning upon which this assignment is based is presented in section III.D.3.

In summary, Zn^{2+} binds both phosphate and base portions of nucleotides, polynucleotides, and DNA. Coordination to the 2′-OH group has also been reported. Reaction with purine bases appears to be at the N_7 site with possible contribution from C_6NH_2, while pyrimidine bases appear to interact at N_3.

2. Silver

Potentiometric and pH-Stat titrations have been used[164] to study the binding of Ag^+ by adenine and some substituted adenines. At pH ~7 insoluble compounds were obtained with 6-dimethylaminopurine, adenine, deoxyadenosine, and 9-methyladenine; a soluble polymeric species was obtained with deoxy-AMP; and barely perceptible interaction was observed with 9-methyl-6-dimethylaminopurine. Strongest binding was found with adenine and 6-dimethylaminopurine, both of which have N_9H groups. Those substances which have N_9 blocked but have amino hydrogens bind less strongly, but still very markedly, whereas binding by 9-methyl-6-dimethylaminopurine, with no N–H bonds, is still weaker and just barely perceptible under the conditions employed. It was concluded that with 6-dimethylaminopurine, a 1:1 compound is formed with the N_9 proton being displaced. With adenine, both N_9 and amino hydrogens are displaced with 1.5–2.0 Ag^+ bound per adenine and 1.5 protons displaced. In the cases of 9-methyladenine and deoxyadenosine 0.75 H^+ is released per Ag^+ bound and the reaction

$$3Ag^+ + 2HB + ClO_4^- = [(Ag_3B_2)ClO_4]_{ppt} + 2H^+$$

is suggested, where BH is the neutral purine and H is an amino hydrogen. For dAMP a soluble polymeric species is formed according to the equation

$$2Ag^+ + BHP^{2-} = (1/n)[Ag_2BP]_n^{n-} + H^+$$

where BHP^{2-} is dinegative dAMP. Although complexing is suggested with displacement of amino hydrogens (having a very high pK, *i.e.*, ~16) it is also possible that other ring nitrogens may be involved when one considers that protonation occurs on N_1, not C_6NH_2 (see section II.B.1). However, reaction with ring nitrogens would not account for the observed proton release.

From the results of a potentiometric study of Ag^+–adenosine interaction, Phillips and George[165] suggest a five-membered ring involving chelation with the N_7 and C_6NH_2 groups. Since Ag^+ forms a significantly more stable complex with adenosine than does Cu^{2+}, it is proposed[165] that Ag^+ binds primarily to the base in ATP. This conclusion has been confirmed for adenosine and extended to AMP, ADP, and ATP in a difference spectrophotometric study.[126] In a study[166] involving pH and Ag^+ titration and ultraviolet and infrared spectroscopy,

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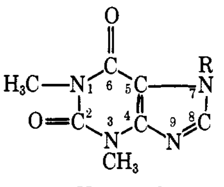
(164) K. Gillen, R. Jensen, and N. Davidson, *J. Amer. Chem. Soc.*, **86**, 2792 (1964).

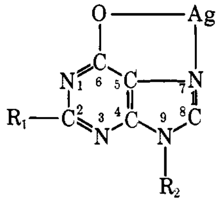
(165) R. Phillips and P. George, *Biochim. Biophys. Acta*, **162**, 73 (1968).

(166) A. T. Tu and J. A. Reinosa, *Biochemistry*, **5**, 3375 (1966).

guanosine, GMP, inosine, IMP, and theophylline were found to combine with Ag$^+$ in a 1:1 ratio whereas no reaction was observed with caffeine, uridine, and UMP. Since uridine did not complex with Ag$^+$, the imidazole portion of the purine base was assumed to be involved in the case of the purine derivatives. The possibility of complexation at N$_9$ was ruled out since this atom is connected to a ribose moiety in guanosine, GMP, inosine, and IMP, all of which reacted. The only difference between caffeine and theophylline is the group at N$_7$ (**33**). From these observations it was concluded that Ag$^+$ is chelated to the N$_7$ and C$_6$O groups in guanosine, GMP, inosine, and IMP as shown in **34**, where R$_1$ and R$_2$ are the appropriate groups for guanosine, GMP, inosine, and IMP, and that Ag$^+$ coordinates to theophylline only through N$_7$. An alternative structure **35** was also suggested[166] in which the Ag$^+$ ions lie in a colinear arragnement which is in better accord with known stereochemical requirements of Ag$^+$



33, R = H theophylline
R = CH$_3$ caffeine



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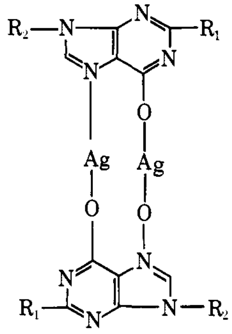
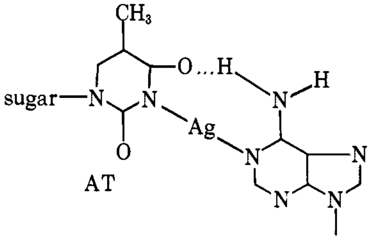


35

Complexing of Ag$^+$ with cytidine, but not uridine, has been reported in a Raman spectral study.[135]

Silver ion is reported to form at least three different (types I, II, and III) complexes with DNA[167–169] and to be bound more tightly by denatured than by native DNA.[167] In combined potentiometric and spectrophotometric studies,[167,169] the type I complex formed when the Ag(bound)/DNA base ratio was 0 to 0.2 and appeared to involve little or no proton release. Type I binding is more important for (G + C)-rich DNA than for (G + C)-poor DNA. The Ag$^+$–DNA complexes have about the same intrinsic viscosity as the uncomplexed DNA, indicating that the double helix is not denatured. Jensen and Davidson[167] suggest that in type I binding Ag$^+$ is chelated between the N$_7$ and C$_6$O groups of guanosine. However, they consider it more likely that a π complex is formed in which Ag$^+$ is sandwiched between two aromatic rings of the same strand or between the π electrons of an amino group of guanosine and a π-electron system on the next base up along the strand. A similar structure in which Ag$^+$ is sandwiched between two base pairs of DNA, one of which must be a G·C pair, has also been proposed for the type I complex.[168]

Type II binding occurs when the Ag/DNA base ratio is between 0.2 and 0.5 and is accompanied by a different spectrum, proton release, and somewhat weaker binding.[167–169] It is proposed that type II binding occurs with bases unaffected by type I binding and that it involves the conversion of an N—H···N hydrogen bond of a complementary base pair to an N–Ag–N bond as in **36** and **37**.[167] Since the sum of type I and type II binding saturates at one Ag$^+$ per base pair, it is assumed that a nucleotide involved in type I binding cannot fully participate in type II binding. Evidence has been reported[168] that the formation of the type II complex is accompanied by a change of structure and the postulate made that Ag$^+$ forms linear complexes between the bases of each strand, partially replacing bonds and producing a new helical structure which is insensitive to temperature.



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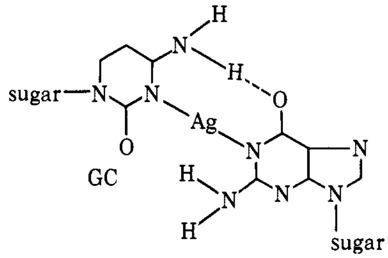

37

Type III and possibly higher complexes are formed at pH <7 and Ag$^+$(bound)/DNA ratios >0.5.[167] A precipitate is formed in this region and the type III complex has not been studied further.

The evidence to date supports the belief that Ag$^+$ binds exclusively or nearly so to the base portion of DNA. In sedimentation coefficient studies,[168] Ag$^+$ was found to react with poly(A), but not with poly(U). The nonreactivity toward poly(U) together with ultraviolet spectral and potentiometric results[168] are taken as evidence that the phosphate groups are not the binding sites in these polynucleotides.

Studies[168,170] with tobacco mosaic virus RNA show Ag$^+$ to increase the resistance of TMV–RNA to both temperature and ribonuclease. Slightly less than 1 mol of either Ag$^+$ or Hg^{2+} was found to bind independently.[121] Competition experiments with Ag$^+$ and Hg^{2+} show 0.5 mol of each to bind on specific independent sites, while an additional 0.5 mol is shared competitively with Hg^{2+} having the greater affinity. Both metals were assumed to bind bases only, and Fe^{3+}, In^{3+}, and Al^{3+} displaced Hg^{2+} and particularly Ag$^+$ from these shared sites.

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(167) R. H. Jensen and N. Davidson, *Biopolymers*, 4, 17 (1966).

(168) M. Daune, C. A. Dekker, and H. K. Schachman, *ibid.*, 4, 51 (1966).

(169) T. Yamane and N. Davidson, *Biochim. Biophys. Acta*, 55, 609 (1962).

(170) B. Singer, and H. Fraenkel-Conrat, *Biochemistry*, 1, 852 (1962).

Silver(I) ion appears to bind exclusively to the bases of the nucleotides, polynucleotides, and DNA studied. No interactions with the phosphate or ribose moieties have been reported.

3. *Cadmium*

No change is observed in the Raman spectra of aqueous solutions of either cytidine or uridine upon addition of $CdCl_2$.[135]

Melting curves for DNA in the presence of Cd^{2+} are similar to those for Cu^{2+}.[55,95] The interpretation placed on these curves is that Cd^{2+} binds more strongly than Zn^{2+}, but less strongly than Cu^{2+} to the DNA bases.[55] This interpretation is based on the following argument. The Cd^{2+}–DNA bond is sufficiently strong to remain intact on cooling without regeneration of the double helix as occurs with Zn^{2+}. Furthermore, the addition of electrolyte to a cooled DNA solution renatures DNA immediately[55] in the presence of Cd^{2+} whereas the reaction in Cu^{2+} solution takes 5 hr to go to completion.[154] This difference in renaturation times is taken[55] as evidence that the Cu^{2+}–DNA base bond is stronger than the Cd^{2+}–DNA base bond. These relative binding strengths are confirmed by equilibrium constant studies.[158]

Binding of Cd^{2+} to the phosphate moieties of ribonucleotides would probably be expected, but no data have been reported.

4. *Platinum*

Changes in absorption spectra with time are observed[171] for the K_2PtCl_4–adenine and K_2PtCl_4–hypoxanthine systems, but not for the K_2PtCl_4 systems containing guanine, thymine, or cytosine. The C_6NH_2 group of adenine was eliminated as a possible complexation site since hypoxanthine and adenine showed similar behavior. Elemental analysis indicated the formation of a 1:1 complex with adenine. Electron microscopy showed that the attachment of K_2PtCl_4 occurred nonuniformly along the thread of the DNA molecule presumably because of selectivity of the reaction with individual bases. Reduction of $PtCl_4^{2-}$ by DNA was also observed, but whether this reduction was related to complexing of adenine with $PtCl_4^{2-}$ was not clear.

5. *Lead*

An equilibrium constant study[137] of Pb^{2+}–base interactions shows Pb^{2+}–guanosine and Pb^{2+}–cytosine complexes to have formation constants approximately an order of magnitude greater than those of the corresponding Pb^{2+}–adenosine complexes. The DNA double helix is destabilized by Pb^{2+} on heating[95] in a manner similar to that seen in the case of Cd^{2+} (and also Cu^{2+} but to a lesser extent). Presumably, the Pb^{2+} coordinates with the nucleotide bases in DNA. No data are available on Pb^{2+} interaction with the phosphate moieties of the ribonucleotides or DNA.

6. *Mercury*

Eichhorn and Clark[172] conclude from an ultraviolet spectral study that at pH 9 $HgCl_2(aq)$ reacts with the amino group in cytidine since no reaction occurs between $HgCl_2$ and cytidine if the amino group is blocked by reaction with formaldehyde.[99] Simpson[173] also finds, based on an ultraviolet spectra study, that at pH 10–11 CH_3HgOH combines with the amino group of cytidine while at pH 2–6 N_3 is the site of mercuration with some contribution from the amino group. A Raman spectra study[135] shows $HgCl_2$ to bind cytidine at N_3 with release of $2Cl^-$. Although in contradiction to the conclusions of Eichhorn and Clark,[172] association at N_3 appears most likely. In an nmr study of $HgCl_2$–cytidine association in DMSO, $HgCl_2$ has been shown to bind only to the N_3 group.[174] The nmr spectra show the amino signals to be due to two protons in the amino group in the presence of $HgCl_2$ and thus confirm that no imino group is formed.

A 1:1 complex between thymidine and Hg^{2+} has been postulated[175] with complexation only at N_3. The N_3 position has also been suggested[173] as the site of complexation in uridine. In DMSO $HgCl_2$ does not react with uridine.[174]

Yamane and Davidson[175] report that the complex HgA_2^{2+} forms in excess adenosine, while in excess Hg^{2+}, HgA^+ is formed with the loss of a proton. The latter reaction is unexpected and no positive proof for the structure is given. Eichhorn and Clark[172] in an ultraviolet spectra study found $HgCl_2$ to react with adenosine in the absence, but not in the presence, of formaldehyde. Since formaldehyde is known[10,99,176] to react with the imino groups of the purine and pyrimidine nucleosides, they conclude that the $HgCl_2$ binds the amino group in adenosine. However, this conclusion is made less certain by the finding[10,177] that the acid imino groups of purine, adenine, thymine, and uracil also react with formaldehyde. Simpson[173] in an ultraviolet spectral study found that CH_3Hg^+ interacts with both the N_1 and amino groups of adenosine with the predominant reaction being at the N_1 position. In an nmr study of $HgCl_2$–adenosine interaction in dimethyl sulfoxide, Kan and Li[174] report binding to the N_7 as well as to the amino and N_1 positions.

There is agreement[172,173,175] that mercury(II) reacts with guanosine at the N_1 position displacing a proton. Simpson[173] also suggests that guanosine is complexed at the N_7 and C_2NH_2 positions by CH_3Hg^+ and at the N_7 position by $Hg(OH)_2$ and that inosine is complexed at both the N_1 and N_7 positions by CH_3HgOH.

Mercury(II) chloride combines reversibly with DNA with a large increase in molecular weight as determined by light scattering.[178] The increase in molecular weight was attributed to partial aggregation of DNA molecules. Addition of electrolyte completely reversed the complexation reaction. Examination[179] of the ultraviolet spectra of complexed and uncomplexed DNA led to the postulation that $HgCl_2$ reacts primarily with the bases although some interaction with the phosphate groups was not ruled out. It was further suggested that the $HgCl_2$ coordinates with the conjugated double bond systems in guanine, cytosine, and thymine or possibly with the NH_2 groups of adenine, guanine, and cytosine. The latter suggestion must be considered tentative in view of the findings that

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(171) B. P. Ulanov, L. F. Malysheva, and Yu. Sh. Moshkovskii, *Biophysics* (*USSR*), **12**, 371 (1967).

(172) G. L. Eichhorn and P. Clark, *J. Amer. Chem. Soc.*, **85**, 4020 (1963).

(173) R. B. Simpson, *ibid.*, **86**, 2059 (1964).

(174) L. S. Kan and N. C. Li, *ibid.*, **92**, 4823 (1970).

(175) T. Yamane and N. Davidson, *ibid.*, **83**, 2599 (1961).

(176) M. Ya. Fel'dman, *Biochemistry* (*USSR*), **25**, 432 (1960).

(177) S. Lewin and M. A. Barnes, *J. Chem. Soc. B*, 478 (1966).

(178) S. Katz, *J. Amer. Chem. Soc.*, 74, 2238 (1952).

(179) C. A. Thomas, *ibid.*, 76, 6032 (1954).

$HgCl_2$ does not react with the NH_2 group in cytidine[174] and that other bivalent metal ions (*i.e.*, Cu^{2+}) do not react with the amino groups of adenine, guanine, or cytosine nucleotides.[24,47]

Yamane and Davidson[175] report a decrease in the intrinsic viscosity and a spectral shift when Hg(II) reacts with DNA. The reaction was found to be reversible; *i.e.*, addition of substances which complex Hg(II) results in the regeneration of DNA. Also, identical spectral shifts and identical viscosity changes are produced if $Hg(ClO_4)_2$ rather than $HgCl_2$ is used as the source of Hg(II), leading to the conclusion that Hg^{2+} is the complexing form of Hg(II). In addition, no evidence was found for Hg^{2+}–phosphate interaction. Addition of Hg^{2+} results in one type of complex with a characteristic spectrum up to a ratio of one Hg(II) to two bases for the natural DNA's studied (calf thymus, *E. coli*, and *M. lysodeikticus*) irrespective of the (G + C):(A + T) ratio in the DNA. With excess Hg(II), a second, higher complex forms. Katz[180,181] later proposed a structure for the Hg^{2+}–DNA complex in which it was assumed that each Hg^{2+} is attached to purine or pyrimidine bases on two polynucleotide chains. This structure was later confirmed by a pH titration procedure.[172] Recent ultracentrifugation[182] and spectrophotometric[183] studies of CH_3HgOH–DNA interaction support the postulation that CH_3Hg^+ reacts with the imino nitrogens of thymine (N_3) and guanine (N_1) in DNA. The reaction with native DNA causes irreversible denaturation probably because CH_3Hg^+ cannot bind two complementary strands of DNA in a chelation process as Hg^{2+} can.

Marked changes in the ultraviolet absorption spectra of soluble, ribosomal, and tobacco mosaic virus RNA's upon addition of $HgCl_2$ led to the conclusion that Hg(II) interacts with RNA.[184] In a study of the enzymatic degradation of RNA in the presence of Mg^{2+}, Ag^+, and Hg^{2+} (both independently and in various combinations), Singer and Fraenkel-Conrat[170] conclude that the binding affinities and sites for these metal ions are comparatively independent of one another.

In summary, Hg(II), like Ag^+, binds to the bases of nucleosides, polynucleotides, DNA, and RNA with no evidence for Hg^{2+}–phosphate interaction.

7. *Lanthanide Metal Ions*

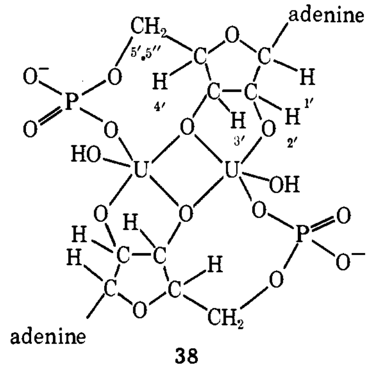
In a study of the degradation of polyribonucleotides by lanthanide ions, Eichhorn and Butzow[185] found a cleavage of the polynucleotide chains of poly(A), poly(C), poly(U), and poly(I) to occur at the 5′-phosphate linkages. In a similar study involving bivalent transition metal ions, Butzow and Eichhorn[117] report rates of degradation with Zn^{2+} to be about the same as those with La^{3+}, but about ten times faster than those with Mn^{2+}, Co^{2+}, Ni^{2+}, and Cu^{2+}. These studies[117,185] were carried out at 64° where reaction occurred in several hours compared to several days at 37°.[186]

RNA is depolymerized[71,185] by La^{3+}, Ce^{3+}, and Lu^{3+}. The fact that DNA does not depolymerize[185,186] in the presence of La^{3+} and Ce^{3+} implicates the participation of the 2′-OH group in the process probably through an intermediate of the type proposed in **25**.

No evidence has been presented that the trivalent lanthanide metal ions bind polynucleotides other than through the phosphate linkages.

8. *Uranium*

Potentiometric titrations of mixtures of $UO_2(NO_3)_2$ with H_2ATP^{2-}, $HADP^-$, AMP, adenosine, and glycerol phosphate led to the conclusion[187] that in adenosine nucleotides $UO_2{}^{2+}$ binds an O atom of the α-phosphate group, the ribose oxygen, and the N_3 atom of the adenine ring. Uranyl ion was found to interact with adenosine only when the adenosine and phosphate groups are part of the same molecule and when these groups are in a geometrical arrangement favorable for chelation with the $UO_2{}^{2+}$. Furthermore, $UO_2{}^{2+}$ does not bind to the adenine group of AMP at high pD values (7.5–11.4).[188,189] Nuclear magnetic resonance data, both ^1H and ^{31}P, indicate that in equimolar mixtures of uranyl nitrate and AMP a chelate with 1:1 stoichiometry is exclusively present above pD 10.9. In this sandwich-type chelate one uranyl group is chelated by the 2′ and 3′ ribose oxygen atoms of one 5′-AMP molecule and by a phosphate oxygen and 3′-oxygen of a second AMP molecule, and a second uranyl group is chelated by the 2′- and 3′-oxygens of the second AMP molecule and a phosphate oxygen and 3′-oxygen of the first AMP according to structure **38**.



38

U represents UO_2^{2+} ion perpendicular to the plane of the paper

Below pD 10.9 this chelate disproportionates to uncomplexed AMP, sandwich-type chelates (two forms) with 2:1 ($UO_2{}^{2+}$:AMP) stoichiometry and nonsandwich-type complexes. The extent of disproportionation increases with decrease in pH, probably because of competition between $UO_2{}^{2+}$ and H^+ for the ribose hydroxyl oxygen sites.

The absorption spectra of the $UO_2{}^{2+}$–DNA system shows that at pH 3.5 one $UO_2{}^{2+}$ is bound for every two phosphate groups.[190] This finding is consistent with the electrostatic interaction of $UO_2{}^{2+}$ with the phosphate groups of DNA. Lowering the solution pH to 2.3 or heating the DNA in the presence of formaldehyde results in a reduction of the stoichiometric ratio to one $UO_2{}^{2+}$ per three phosphate groups.

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In conclusion, under favorable conditions UO_2^{2+} appears to complex with base, phosphate, and ribose moieties of nucleotides.

9. Boron

The reaction of boric acid with D-ribose, inosine, and uridine has been shown to involve the ribose OH groups.[191]

F. SUMMARY OF PROBABLE METAL COORDINATION SITES

The metal ions which have been studied have been arranged in Table V according to their relative affinities for the phosphate, ribose, and base moieties of nucleotides and nucleic acids. This order does not necessarily parallel the thermodynamic stabilities of the complexes which are a measure of the overall reaction of the metal with the various complexation sites. Of the remaining metals studied, available evidence indicates that Pt^{IV} is primarily a base binder, and Cr^{3+} is primarily a phosphate binder; however, additional experimental data are desirable in these cases.

Table V

Summary of Metal Ion Coordination Sites with the Phosphate, Base, and Ribose Moieties of Nucleotides and Nucleic Acids

Site	*Metal ions*
Phosphate	Li^+, Na^+, K^+, Rb^+, Cs^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, trivalent lanthanides
Phosphate and ribose[a]	B(III), UO_2^{2+}
Phosphate and base[b]	$Co^{2+} = Ni^{2+}$, Mn^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Cu^{2+}
Ribose and base	Co^{3+}
Base	Ag^+, Hg^{2+}

[a] Evidence also for interaction of UO_2^{2+} with base. [b] Increasing affinity for base relative to phosphate from left to right. Order taken from ref 55 except Pb^{2+} which is from ref 95. Fe^{2+} and Fe^{3+} also fit in this group, but their positions are not known.

IV. Thermodynamic Data

The thermodynamic quantities (log K, ΔH, ΔS, and ΔC_p) for the interaction of protons and metal ions with the nucleic acids and their components together with the methods and conditions used in their determination are given in Table VI. In the cases of adenosine and the adenosine nucleotides only those values which have appeared since or were not included in the review by Phillips[1] are given.

The thermodynamic data in Table VI are arranged according to the following system. The bases are listed alphabetically and each is followed by its nucleoside; nucleoside mono-, di-, tri-, and tetraphosphate; and polynucleotide derivatives in that order. Deoxy, other sugar derivatives, and other ligands (*e.g.*, MHL) in that order follow each parent nucleoside or nucleotide ionization or metal complexation step. Mixed polynucleotides are listed alphabetically; *e.g.*, poly(A + G) is found under the main heading of adenine. The ligands are listed in order of increasing degree of protonation with metal complexation data following proton association data for each ligand. The metal ion order is that given in ref 64. Consecutive reactions are given first, followed by overall and unspecified reactions in that order. Data at specific temperatures are listed first, in order of increasing temperature, followed by data at unspecified temperatures and those valid over a temperature range. Data are listed in order of increasing ionic strength or supporting electrolyte concentration followed by data at unspecified ionic strengths. The log K and ΔH values are listed in order according to the method used in their determination. Log K: calorimetric, potentiometric, spectrophotometric, other. ΔH: calorimetric, temperature variation. Data valid in aqueous solution are given first followed by those determined in other solvents arranged alphabetically according to the solvent.

The most numerous thermodynamic data for the systems included in Table VI are equilibrium constants. Relatively few ΔH and ΔS and very few ΔC_p values have been reported. Several experimental techniques have been used to measure equilibrium constants, with the most popular being that of

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Table VI

Log K, $\Delta H°$, ΔS, and $\Delta C_p°$ Values for the Interaction of H^+ and M^{n+} with DNA, RNA, and Their Components (Listed Alphabetically)[a]

Meta	*Method*	*pH*	*Temp, °C*	*Supporting electrolyte/solvent*	*μ*	*Log K*	*ΔH°, kcal/mol*	*ΔS°, cal/(deg mol)*	*ΔCp°, cal/(deg mol)*	*Ref*
Adenine (L), N_9, $H^+ + L^- = HL$										
H^+	pH titration		20		0.006–0.012	9.96				9
H^+	pH statting		20		0.0135	9.98				177
H^+	Spectrophotometry		20		0.1	9.87				177
H^+	pH titration		25		0	9.87				192
H^+	C		25		0		−9.65	12.88		21
H^+	pH titration		25	$NaClO_4$	0.05	9.88				129
H^+	Spectrophotometric, T		25		0.1	9.72	−9.5	12.4		53
H^+	pH titration		25	0.15 *M* NaCl		9.75				5
H^+	Hydrogen electrode		25			9.80				4
H^+	pH titration		25			9.7				26
H^+	pH titration		25	50 vol % dioxane	0.01	10.65				8
H^+	pH titration		25	50 vol % dioxane		10.7				26
H^+	pH titration		30		0.006–0.012	9.67				9
H^+	pH statting		30		0.0135	9.75				177
H^+	Spectrophotometry		30		0.1	9.70				177
H^+	pH titration		38	0.15 *M* NaCl		9.52				5
H^+	pH titration		40		0.006–0.012	9.49				9
H^+	pH statting		40		0.0135	9.53				177
H^+	Spectrophotometry		40		0.1	9.45				177
H^+	pH statting		50		0.0135	9.37				177
H^+	Spectrophotometry		50		0.1	9.21				177
H^+	pH statting		20–50		0.0135		−9.1	14.8[b]		177
H^+	Spectrophotometry		20–50		0.1		−9.6	12.8[b]		177
H^+	pH titration, T		20–30				−11.0	5 (25°)		9
H^+	pH titration, T		30–40				−8.2	14 (35°)		9
Adenine (L), $M^{n+} + L^- = ML^{+n-1}$										
Co^{2+}	pH titration		25			4.2				26
Ni^{2+}	pH titration		20			4.37				127
Ni^{2+}	pH titration		25			4.8				26
Ni^{2+}	Temperature jump		25	KNO_3	0.1	5.3				126a
Ni^{2+}	pH titration		25	50 vol % dioxane	0.01	6.18				8
Cu^{2+}	pH titration		25			7.1				26
Cu^{2+}	pH titration		25	50 vol % dioxane		9.0				26
Cu^{2+}	pH titration		25	50 vol % dioxane	0.01	8.94				8
Zn^{2+}	pH titration		25	50 vol % dioxane	0.01	6.42				8
Adenine (L), $ML^{+n-1} + L^- = ML_2^{+n-2}$										
Cu^{2+}	pH titration		25			6.4				26
Cu^{2+}	pH titration		25	50 vol % dioxane		8.0				26
Adenine (L), $M^{n+} + 2L^- = ML_2^{+n-2}$										
Cu^{2+}	pH titration		20			14.22				127
Adenine (L), N_1, $H^+ + HL = H_2L^+$										
H^+	pH titration		10			4.33				26
H^+	pH titration		20		0.006–0.012	4.22				9
H^+	pH titration		20			4.22				32
H^+	pH titration		20			4.22				193
H^+	pH titration		25		0	4.20				192
H^+	C		25		0		−4.9	2.7		15
H^+	C		25		0		−4.81	3.17		21
H^+	pH titration		25	$NaClO_4$	0.05	4.22				129
H^+	Spectrophotometric, T		25		0.1	4.12	−4.2	4.7		53
H^+	C		25	NaCl	0.1		−4.0			194
H^+	pH titration		25	0.15 *M* NaCl		4.12				5
H^+	pH titration		25			4.18				26

Table VI (Continued)

Metal	*Method*	*pH*	*Temp, °C*	*Supporting electrolyte/solvent*	*μ*	*Log K*	*ΔH°, kcal/mol*	*ΔS°, cal/(deg mol)*	*ΔC$_p$°, cal/(deg mol)*	*Ref*
H^+	Potentiometric		25			4.1				195
H^+	Hydrogen electrode		25			4.15				4
H^+	pH titration		25	50 vol % dioxane	0.01	3.54				8
H^+	pH titration		25	50 vol % dioxane		3.43				26
H^+	pH titration		30		0.006–0.012	4.12				9
H^+	pH titration		38	0.15 *M* NaCl		4.07				5
H^+	pH titration		40		0.006–0.012	4.06				9
H^+	pH titration		40			4.02				26
H^+	T		10–40		0.005		−4.2	5 (25°)		26
H^+	T		20–30				−3.8	5 (25°)		9
H^+	T		30–40				−2.7	10 (35°)		9
Adenine (L), N_7(?), $H^+ + H_2L^+ = H_3L^{2+}$										
H^+	pH titration		20			<1				193
Adenosine^c (L), 2′-OH, 3′-OH, $H^+ + L^- = HL$										
H^+	C		25		0	12.35	−9.7	24.0		19
H^+	Optical rotation, pH titration		25			12.5				27
Adenosine^c (L), N_1, $H^+ + HL = H_2L^+$										
H^+	pH titration		20	1 *M* $NaNO_3$		3.703				137
H^+	pH titration		20			3.52				32
H^+	C		25		0		−3.1	5.7		15
H^+	pH titration, C		25		0	3.50	−3.91	2.92		21
H^+	pH titration		25	$NaClO_4$	0.05	3.57				140
H^+	Spectrophotometric, T		25		0.1	3.55	−3.80	3.4		53
H^+	Potentiometric		25			3.6				195
H^+	Spectrophotometry		. .^{b}			3.5				173
Adenosine^c (L), $M^{n+} + HL = MHL^{n+}$										
Cu^{2+}	pH titration	3.5	20	1 *M* $NaNO_3$		0.70				137
Ag^+	Potentiometric, Ag^+		25		0	2.02				165
Ag^+	Potentiometric, Ag^+, T		10–40		0		−5.49	−9.1 (25°)		165
Hg^{2+}	Spectrophotometry	2–4	. .^{b}			~3.0				173
$HgCl_2$	Proton nmr		36	Dimethyl sulfoxide		0.86				174
Pb^{2+}	pH titration	3.5	20	1 *M* $NaNO_3$		−0.52				137
Adenosine^c (L), $MHL^{n+} + HL = M(HL)_2^{n+}$										
Ag^+	Potentiometric, Ag^+		25		0	1.84				165
Ag^+	Potentiometric, Ag^+, T		10–40		0		−3.66	−3.8 (25°)		165
Deoxyadenosine^c (L), N_1, $H^+ + L = HL^+$										
H^+	pH titration		25	$NaClO_4$	0.05	3.77				140
H^+	C		25	NaCl	0.1		−3.87			194
9-β-D-Xylofuranosyladenine (L), 2′-OH, 3′-OH, $H^+ + L^- = HL$										
H^+	C		25		0	12.34	−8.4	28.3		196
2′-AMP (L), Phosphate, $H^+ + L^{2-} = HL^-$										
H^+	pH titration		0.4	KNO_3	0.1	6.12				197
H^+	pH titration		12	KNO_3	0.1	6.07				197
H^+	pH titration		24.5			6.17				40
H^+	pH titration		25	KNO_3	0.1	6.01				197
H^+	pH titration		25	0.15 *M* NaCl		6.15				5
H^+	pH titration		38	0.15 *M* NaCl		6.05				5
H^+	pH titration		40	KNO_3	0.1	5.95				197
H^+	pH titration, T		0.4–40	KNO_3	0.1		−1.6	22.2 (25°)		197
2′-AMP (L), $M^{n+} + L^{2-} = ML^{+n-2}$										
Mg^{2+}	pH titration		0.4	KNO_3	0.1	1.71				197
Mg^{2+}	pH titration		12	KNO_3	0.1	1.82				197

Table VI (Continued)

Metal	*Method*	*pH*	*Temp, °C*	*Supporting electrolyte/solvent*	*μ*	*Log K*	*ΔH°, kcal/mol*	*ΔS°, cal/(deg mol)*	*ΔC$_p$°, cal/(deg mol)*	*Ref*
Mg^{2+}	pH titration		25	KNO_3	0.1	1.93				197
Mg^{2+}	pH titration		40	KNO_3	0.1	2.05				197
Mg^{2+}	pH titration, T		0.4–40	KNO_3	0.1		3.5	20.5 (25°)		197
Ca^{2+}	pH titration		0.4	KNO_3	0.1	1.87				197
Ca^{2+}	pH titration		12	KNO_3	0.1	1.85				197
Ca^{2+}	pH titration		25	KNO_3	0.1	1.83				197
Ca^{2+}	pH titration		40	KNO_3	0.1	1.81				197
Ca^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−0.6	6.5 (25°)		197
Sr^{2+}	pH titration		0.4	KNO_3	0.1	1.85				197
Sr^{2+}	pH titration		12	KNO_3	0.1	1.79				197
Sr^{2+}	pH titration		25	KNO_3	0.1	1.74				197
Sr^{2+}	pH titration		40	KNO_3	0.1	1.71				197
Sr^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−1.0	4.5 (25°)		197
Ba^{2+}	pH titration		0.4	KNO_3	0.1	1.82				197
Ba^{2+}	pH titration		12	KNO_3	0.1	1.77				197
Ba^{2+}	pH titration		25	KNO_3	0.1	1.71				197
Ba^{2+}	pH titration		40	KNO_3	0.1	1.64				197
Ba^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−2.0	1.2 (25°)		197
Mn^{2+}	pH titration		0.4	KNO_3	0.1	2.43				197
Mn^{2+}	pH titration		12	KNO_3	0.1	2.41				197
Mn^{2+}	pH titration		25	KNO_3	0.1	2.38				197
Mn^{2+}	pH titration		40	KNO_3	0.1	2.35				197
Mn^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−1.0	7.5 (25°)		197
Co^{2+}	pH titration		0.4	KNO_3	0.1	2.15				197
Co^{2+}	pH titration		12	KNO_3	0.1	2.19				197
Co^{2+}	pH titration		25	KNO_3	0.1	2.24				197
Co^{2+}	pH titration		40	KNO_3	0.1	2.28				197
Co^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−0.7	8.5 (25°)		197
Ni^{2+}	pH titration		0.4	KNO_3	0.1	2.86				197
Ni^{2+}	pH titration		12	KNO_3	0.1	2.84				197
Ni^{2+}	pH titration		25	KNO_3	0.1	2.81				197
Ni^{2+}	pH titration		40	KNO_3	0.1	2.78				197
Ni^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−1.0	9.5 (25°)		197
Cu^{2+}	pH titration		0.4	KNO_3	0.1	3.28				197
Cu^{2+}	pH titration		12	KNO_3	0.1	3.23				197
Cu^{2+}	pH titration		25	KNO_3	0.1	3.16				197
Cu^{2+}	pH titration		40	KNO_3	0.1	3.10				197
Cu^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−1.9	8.0 (25°)		197
Zn^{2+}	pH titration		0.4	KNO_3	0.1	2.72				197
Zn^{2+}	pH titration		12	KNO_3	0.1	2.68				197
Zn^{2+}	pH titration		25	KNO_3	0.1	2.64				197
Zn^{2+}	pH titration		40	KNO_3	0.1	2.60				197
Zn^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−1.2	8.0 (25°)		197
2′-AMP (L), N_1, $H^+ + HL^- = H_2L$										
H^+	pH titration		0.4	KNO_3	0.1	4.03				197
H^+	pH titration		12	KNO_3	0.1	3.88				197
H^+	pH titration		24.5			3.81				40
H^+	pH titration		25	KNO_3	0.1	3.71				197
H^+	pH titration		25	0.15 *M* NaCl		3.80				5
H^+	pH titration		38	0.15 *M* NaCl		3.60				5
H^+	pH titration		40	KNO_3	0.1	3.54				197
H^+	pH titration, T		0.4–40	KNO_3	0.1		−4.7	1.2 (25°)		197
3′-AMP (L), Phosphate, $H^+ + L^{2-} = HL^-$										
H^+	pH titration		0.4	KNO_3	0.1	5.93				197
H^+	pH titration		12	KNO_3	0.1	5.88				197
H^+	pH titration		24.5			5.92				40
H^+	pH titration		25	KNO_3	0.1	5.80				88
H^+	pH titration		25	KNO_3	0.1	5.83				197
H^+	pH titration		25	KCl	0.1	6.55				90
H^+	pH titration		25	0.15 *M* NaCl		5.88				5
H^+	pH titration		38	0.15 *M* NaCl		5.82				5
H^+	pH titration		40	KNO_3	0.1	5.78				197
H^+	pH titration		0.4–40	KNO_3	0.1		−1.5	22.3 (25°)		197

Table VI (Continued)

Metal	Method	pH	Temp, °C	Supporting electrolyte/solvent	μ	Log K	ΔH°, kcal/mol	ΔS°, cal/(deg mol)	ΔC$_p$°, cal/(deg mol)	Ref
				3′-AMP (L), $M^{n+} + L^{2-} = ML^{+n-2}$						
Mg^{2+}	pH titration		0.4	KNO_3	0.1	1.68				197
Mg^{2+}	pH titration		12	KNO_3	0.1	1.78				197
Mg^{2+}	pH titration		25	KNO_3	0.1	1.89				88, 197
Mg^{2+}	pH titration		25	KCl	0.1	1.73				90
Mg^{2+}	pH titration		40	KNO_3	0.1	2.01				197
Mg^{2+}	pH titration, T		0.4–40	KNO_3	0.1		3.5	20.5 (25°)		197
Ca^{2+}	pH titration		0.4	KNO_3	0.1	1.86				197
Ca^{2+}	pH titration		12	KNO_3	0.1	1.84				197
Ca^{2+}	pH titration		25	KNO_3	0.1	1.80				88, 197
Ca^{2+}	pH titration		40	KNO_3	0.1	1.78				197
Ca^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−0.6	6.5 (25°)		197
Sr^{2+}	pH titration		0.4	KNO_3	0.1	1.81				197
Sr^{2+}	pH titration		12	KNO_3	0.1	1.75				197
Sr^{2+}	pH titration		25	KNO_3	0.1	1.71				88, 197
Sr^{2+}	Ion exchange	7.2–7.3	25		0.16	1.4				198
Sr^{2+}	pH titration		40	KNO_3	0.1	1.68				197
Sr^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−0.9	4.5 (25°)		197
Ba^{2+}	pH titration		0.4	KNO_3	0.1	1.81				197
Ba^{2+}	pH titration		12	KNO_3	0.1	1.75				197
Ba^{2+}	pH titration		25	KNO_3	0.1	1.69				88, 197
Ba^{2+}	pH titration		40	KNO_3	0.1	1.62				197
Ba^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−1.9	1.2 (25°)		197
Mn^{2+}	pH titration		0.4	KNO_3	0.1	2.34				197
Mn^{2+}	pH titration		12	KNO_3	0.1	2.31				197
Mn^{2+}	pH titration		25	KNO_3	0.1	2.28				88, 197
Mn^{2+}	pH titration		25	$(CH_3)_4NBr$	0.1	1.98				199
Mn^{2+}	Ion exchange		25	$KClO_4$	0.1	1.86				199
Mn^{2+}	pH titration		40	KNO_3	0.1	2.25				197
Mn^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−0.9	7.6 (25°)		197
Co^{2+}	pH titration		0.4	KNO_3	0.1	2.11				197
Co^{2+}	pH titration		12	KNO_3	0.1	2.15				197
Co^{2+}	pH titration		25	KNO_3	0.1	2.20				197
Co^{2+}	pH titration		25	KNO_3	0.1	2.10				199
Co^{2+}	pH titration		25	$(CH_3)_4NBr$	0.1	2.19				199
Co^{2+}	Ion exchange		25	$KClO_4$	0.1	2.08				199
Co^{2+}	pH titration		25	KNO_3	0.1	2.24				88
Co^{2+}	pH titration		40	KNO_3	0.1	2.24				197
Co^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−0.6	8.5 (25°)		197
Ni^{2+}	pH titration		0.4	KNO_3	0.1	2.85				197
Ni^{2+}	pH titration		12	KNO_3	0.1	2.82				197
Ni^{2+}	pH titration		25	KNO_3	0.1	2.79				88, 197
Ni^{2+}	pH titration		40	KNO_3	0.1	2.75				197
Ni^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−1.0	9.6 (25°)		197
Cu^{2+}	pH titration		0.4	KNO_3	0.1	3.06				197
Cu^{2+}	pH titration		12	KNO_3	0.1	3.00				197
Cu^{2+}	pH titration		25	KNO_3	0.1	2.96				88, 197
Cu^{2+}	pH titration		40	KNO_3	0.1	2.90				197
Cu^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−1.7	8.0 (25°)		197
Zn^{2+}	pH titration		0.4	KNO_3	0.1	2.65				197
Zn^{2+}	pH titration		12	KNO_3	0.1	2.62				197
Zn^{2+}	pH titration		25	KNO_3	0.1	2.60				88, 197
Zn^{2+}	Ion exchange		25	$KClO_4$	0.1	2.48				199
Zn^{2+}	pH titration		25	KCl	0.1	2.69				90
Zn^{2+}	pH titration		40	KNO_3	0.1	2.56				197
Zn^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−1.1	8.2 (25°)		197
				3′-AMP (L), N_1, $H^+ + HL^- = H_2L$						
H^+	pH titration		0.4	KNO_3	0.1	3.95				197
H^+	pH titration		12	KNO_3	0.1	3.80				197
H^+	pH titration		24.5			3.74				40
H^+	pH titration		25	KNO_3	0.1	3.63				88
H^+	pH titration		25	KNO_3	0.1	3.65				197
H^+	pH titration		25	KCl	0.1	3.93				90
H^+	pH titration		25	0.15 *M* NaCl		3.65				5

Table VI (Continued)

Metal	Method	pH	Temp, °C	Supporting electrolyte/solvent	μ	Log K	$\Delta H°$, kcal/mol	$\Delta S°$, cal/(deg mol)	$\Delta C_p°$, cal/(deg mol)	Ref
H^+	pH titration		38	0.15 *M* NaCl		3.50				5
H^+	pH titration		40	KNO_3	0.1	3.49				197
H^+	pH titration, T		0.4–40	KNO_3	0.1		−4.6	1.3 (25°)		197
				5′-AMP (L), Ribose OH, $H^+ + L^{3-} = HL^{2-}$						
H^+	C		25		0	13.06	−10.9	23.3		19
				5′-AMP^c (L), Phosphate, $H^+ + HL^{2-} = H_2L^-$						
H^+	pH titration		0.4	KNO_3	0.1	6.38				197
H^+	pH titration		12	KNO_3	0.1	6.31				197
H^+	pH titration, T		25		0	6.67	0.85	33.4		200
H^+	pH titration		25	$NaClO_4$	0.1	6.14				201
H^+	pH titration		25	KNO_3	0.1	6.23				197
H^+	pH titration		25	$NaClO_4$/ 10% dioxane	0.1	6.43				202
H^+	pH titration		40	KNO_3	0.1	6.16				197
H^+	pH titration		0.4–40	KNO_3	0.1		−1.9	22.0 (25°)		197
				5′-AMP^c (L), $M^{n+} + HL^{2-} = MHL^{+n-2}$						
Mg^{2+}	pH titration		0.4	KNO_3	0.1	1.75				197
Mg^{2+}	pH titration		12	KNO_3	0.1	1.85				197
Mg^{2+}	pH titration		25	KNO_3	0.1	1.97				197
Mg^{2+}	pH titration		40	KNO_3	0.1	2.09				197
Mg^{2+}	pH titration, T		0.4–40	KNO_3	0.1		3.4	20.4 (25°)		197
Ca^{2+}	pH titration		0.4	KNO_3	0.1	1.88				197
Ca^{2+}	pH titration		12	KNO_3	0.1	1.87				197
Ca^{2+}	pH titration		25	KNO_3	0.1	1.85				197
Ca^{2+}	pH titration		40	KNO_3	0.1	1.83				197
Ca^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−0.6	6.4 (25°)		197
Sr^{2+}	pH titration		0.4	KNO_3	0.1	1.88				197
Sr^{2+}	pH titration		12	KNO_3	0.1	1.83				197
Sr^{2+}	pH titration		25	KNO_3	0.1	1.79				197
Sr^{2+}	pH titration		40	KNO_3	0.1	1.74				197
Sr^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−1.4	4.4 (25°)		197
Ba^{2+}	pH titration		0.4	KNO_8	0.1	1.85				197
Ba^{2+}	pH titration		12	KNO_3	0.1	1.80				197
Ba^{2+}	pH titration		25	KNO_3	0.1	1.73				197
Ba^{2+}	pH titration		40	KNO_3	0.1	1.66				197
Ba^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−2.0	1.2 (25°)		197
Mn^{2+}	pH titration		0.4	KNO_3	0.1	2.46				197
Mn^{2+}	pH titration		12	KNO_3	0.1	2.43				197
Mn^{2+}	pH titration		25	KNO_3	0.1	2.40				197
Mn^{2+}	pH titration		25	KNO_3	0.1	2.35				199
Mn^{2+}	pH titration		40	KNO_3	0.1	2.37				197
Mn^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−1.0	7.6 (25°)		197
Co^{2+}	pH titration		0.4	KNO_3	0.1	2.44				197
Co^{2+}	pH titration		12	KNO_3	0.1	2.49				197
Co^{2+}	pH titration		25	KNO_3	0.1	2.53				197
Co^{2+}	pH titration		25	KNO_3	0.1	2.57				199
Co^{2+}	pH titration		25	$NaClO_4$/10% dioxane	0.1	2.34				202
Co^{2+}	pH titration		40	KNO_3	0.1	2.57				197
Co^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−1.1	8.4 (25°)		197
Ni^{2+}	pH titration		0.4	KNO_3	0.1	2.90				197
Ni^{2+}	pH titration		12	KNO_3	0.1	2.87				197
Ni^{2+}	pH titration		25	KNO_3	0.1	2.84				197
Ni^{2+}	pH titration		25	KNO_3	0.1	2.67				199
Ni^{2+}	pH titration		25	$NaClO_4$/10% dioxane	0.1	2.88				202
Ni^{2+}	pH titration		40	KNO_3	0.1	2.84				197
Ni^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−1.0	9.6 (25°)		197
Cu^{2+}	pH titration		0.4	KNO_3	0.1	3.30				197
Cu^{2+}	pH titration		12	KNO_3	0.1	3.24				197
Cu^{2+}	pH titration		25	KNO_3	0.1	3.18				197
Cu^{2+}	pH titration		25	$NaClO_4$/10% dioxane	0.1	3.22				202

Table VI (Continued)

Metal	*Method*	*pH*	*Temp, °C*	*Supporting electrolyte/solvent*	*μ*	*Log K*	*ΔH°, kcal/mol*	*ΔS°, cal/(deg mol)*	*ΔC$_p$°, cal/(deg mol)*	*Ref*
Cu^{2+}	pH titration		40	KNO_3	0.1	3.12				197
Cu^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−2.0	8.0 (25°)		197
Zn^{2+}	pH titration		0.4	KNO_3	0.1	2.80				197
Zn^{2+}	pH titration		12	KNO_3	0.1	2.76				197
Zn^{2+}	pH titration		25	KNO_3	0.1	2.72				197
Zn^{2+}	pH titration		25	$NaClO_4$/10% dioxane	0.1	2.40				202
Zn^{2+}	pH titration		40	KNO_3	0.1	2.68				197
Zn^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−1.2	8.2 (25°)		197
5′-AMP^c (L), N_1, $H^+ + H_2L^- = H_3L$										
H^+	pH titration		0.4	KNO_3	0.1	4.15				197
H^+	pH titration		12	KNO_3	0.1	3.98				197
H^+	Spectrophotometric titration		19–22	0.025 *M* NaH_2PO_4–Na_2HPO_4		3.70				203
H^+	Spectrophotometric titration		19–22	0.025 *M* NaH_2PO_4–Na_2HPO_4 + 8 *M* urea		4.20				203
H^+	Spectrophotometric titration		19–22	0.025 *M* NaH_2PO_4–Na_2HPO_4 + 48% sucrose		3.75				203
H^+	pH titration		25	KNO_3	0.1	3.80				197
H^+	pH titration		40	KNO_3	0.1	3.62				197
H^+	pH titration, T		0.4–40	KNO_3	0.1		−4.9	1.0 (25°)		197
5′-AMP (L), $M^{n+} + H_2L^- = MH_2L^{+n-1}$										
Mg^{2+}	pH titration		25	$NaClO_4$	0.1	1.63				201
Ca^{2+}	pH titration		25	$NaClO_4$	0.1	1.39				201
Ba^{2+}	pH titration		25	$NaClO_4$	0.1	1.14				201
Mn^{2+}	pH titration		25	$NaClO_4$	0.1	2.14				201
Co^{2+}	pH titration		25	$NaClO_4$	0.1	2.19				201
Ni^{2+}	pH titration		25	$NaClO_4$	0.1	2.62				201
Cu^{2+}	pH titration		25	$NaClO_4$	0.1	3.04				201
Zn^{2+}	pH titration		25	$NaClO_4$	0.1	2.23				201
AMP (L)^d										
Co^{2+}	Difference spectrophotometry	6.0	25			3.9				126
Ni^{2+}	Difference spectrophotometry	6.0	25			4.0				126
Ag^+	Difference spectrophotometry	6.0	25			4.2				126
5′-dAMP (L), Phosphate, $H^+ + L^{2-} = HL^-$										
H^+	pH titration, T		25		0	6.65	1.04	33.8		200
5′-dAMP (L), N_1, $H^+ + HL^- = H_2L$										
H^+	C		25	NaCl	0.1		−2.6			194
5′-AMP–Mg^{2+} (L), $H^+ + L = HL^+$										
H^+	pH titration		25	$NaClO_4$	0.1	5.89				201
5′-AMP–Ca^{2+} (L), $H^+ + L = HL^+$										
H^+	pH titration		25	$NaClO_4$	0.1	5.99				201
5′-AMP–Ba^{2+} (L), $H^+ + L = HL^+$										
H^+	pH titration		25	$NaClO_4$	0.1	6.03				201
5′-AMP–Mn^{2+} (L), $H^+ + L = HL^+$										
H^+	pH titration		25	$NaClO_4$	0.1	5.58				201
5′-AMP–Co^{2+} (L), $H^+ + L = HL^+$										
H^+	pH titration		25	$NaClO_4$	0.1	5.52				201
5′-AMP–Ni^{2+} (L), $H^+ + L = HL^+$										
H^+	pH titration		25	$NaClO_4$	0.1	5.47				201
5′-AMP–Cu^{2+} (L), $H^+ + L = HL^+$										
H^+	pH titration		25	$NaClO_4$	0.1	5.34				201

Table VI (Continued)

Metal	Method	pH	Temp, °C	Supporting electrolyte/solvent	μ	Log K	ΔH°, kcal/mol	ΔS°, cal/(deg mol)	ΔC_p°, cal/(deg mol)	Ref
5′-AMP–Zn^{2+} (L), $H^+ + L = HL^+$										
H^+	pH titration		25	$NaClO_4$	0.1	5.53				201
ADP[c] (L), Phosphate, $H^+ + L^{3-} = HL^{2-}$										
H^+	pH titration		0.4	KNO_3	0.1	6.51				197
H^+	pH titration		12	KNO_3	0.1	6.48				197
H^+	pH titration		25		0	7.00				192
H^+	pH titration		25		0	7.20	1.37	37.4		200
H^+	C		25		0		1.3	36		15
H^+	pH titration		25	KNO_3	0.1	6.44				197
H^+	pH titration		40	KNO_3	0.1	6.41				197
H^+	pH titration, T		0.4–40	KNO_3	0.1		−1.2	25.4 (25°)		197
ADP[c] (L), $M^{n+} + L^{3-} = ML^{+n-3}$										
Mg^{2+}	pH titration		0.4	KNO_3	0.1	2.94				197
Mg^{2+}	pH titration		12	KNO_3	0.1	3.05				197
Mg^{2+}	pH titration		25	KNO_3	0.1	3.17				197
Mg^{2+}	pH titration		40	KNO_3	0.1	3.30				197
Mg^{2+}	pH titration, T		0.4–40	KNO_3	0.1		3.6	26.6 (25°)		197
Ca^{2+}	pH titration		0.4	KNO_3	0.1	2.91				197
Ca^{2+}	pH titration		12	KNO_3	0.1	2.88				197
Ca^{2+}	Ion exchange	8.2	23	0.1 *M* NaCl		2.82				112
Ca^{2+}	pH titration		25	KNO_3	0.1	2.86				197
Ca^{2+}	pH titration		25	KNO_3	0.1	2.80				197
Ca^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−1.2	9.1 (25°)		197
Sr^{2+}	pH titration		0.4	KNO_3	0.1	2.70				197
Sr^{2+}	pH titration		12	KNO_3	0.1	2.63				197
Sr^{2+}	pH titration		25	KNO_3	0.1	2.54				197
Sr^{2+}	pH titration		40	KNO_3	0.1	2.43				197
Sr^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−2.68	5.5 (25°)		197
Ba^{2+}	pH titration		0.4	KNO_3	0.1	2.53				197
Ba^{2+}	pH titration		12	KNO_3	0.1	2.45				197
Ba^{2+}	pH titration		25	KNO_3	0.1	2.36				197
Ba^{2+}	pH titration		40	KNO_3	0.1	2.25				197
Ba^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−2.9	1.1 (25°)		197
Mn^{2+}	pH titration		0.4	KNO_3	0.1	4.47[e]				197
Mn^{2+}	pH titration		12	KNO_3	0.1	4.24				197
Mn^{2+}	Nmr	8.0	25	0.05 *M* *N*-ethylmorpholine HCl		4.40				204
Mn^{2+}	pH titration		25	KNO_3	0.1	4.16				197
Mn^{2+}	pH titration		40	KNO_3	0.1	4.06				197
Mn^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−2.4	11.0 (25°)		197
Co^{2+}	pH titration		0.4	KNO_3	0.1	4.63[f]				197
Co^{2+}	pH titration		12	KNO_3	0.1	4.27				197
Co^{2+}	pH titration		25	KNO_3	0.1	4.20				197
Co^{2+}	pH titration		40	KNO_3	0.1	4.12				197
Co^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−2.0	12.5 (25°)		197
Ni^{2+}	pH titration		0.4	KNO_3	0.1	4.62				197
Ni^{2+}	pH titration		12	KNO_3	0.1	4.57				197
Ni^{2+}	pH titration		25	KNO_3	0.1	4.50				197
Ni^{2+}	pH titration		40	KNO_3	0.1	4.42				197
Ni^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−1.9	14.1 (25°)		197
Cu^{2+}	pH titration		0.4	KNO_3	0.1	6.16				197
Cu^{2+}	pH titration		12	KNO_3	0.1	6.04				197
Cu^{2+}	pH titration		25	KNO_3	0.1	5.90				197
Cu^{2+}	pH titration		40	KNO_3	0.1	5.75				197
Cu^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−4.1	13.0 (25°)		197
Zn^{2+}	pH titration		0.4	KNO_3	0.1	4.40				197
Zn^{2+}	pH titration		12	KNO_3	0.1	4.35				197
Zn^{2+}	pH titration		25	KNO_3	0.1	4.28				197
Zn^{2+}	pH titration		40	KNO_3	0.1	4.20				197
Zn^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−2.0	12.5 (25°)		197
ADP[c] (L), N_1, $H^+ + HL^{2-} = H_2L^-$										
H^+	pH titration		0.4	KNO_3	0.1	4.20				197
H^+	pH titration		12	KNO_3	0.1	4.09				197

Table VI (Continued)

Metal	*Method*	*pH*	*Temp, °C*	*Supporting electrolyte/solvent*	*μ*	*Log K*	*ΔH°, kcal/mol*	*ΔS°, cal/(deg mol)*	*ΔCp°, cal/(deg mol)*	*Ref*
H^+	pH titration		25		0	4.20				192
H^+	pH titration		25	KNO_3	0.1	3.93				197
H^+	C		25		0		−4.1	5.4		15
H^+	pH titration		40	KNO_3	0.1	3.73				197
H^+	pH titration, T		0.4–40	KNO_3	0.1		−4.8	1.9 (25°)		197
				ADP^c (L), $M^{n+} + HL^{2-} = MHL^{+n-2}$						
Mg^{2+}	pH titration		0.4	KNO_3	0.1	1.39				197
Mg^{2+}	pH titration		12	KNO_3	0.1	1.51				197
Mg^{2+}	pH titration		25	KNO_3	0.1	1.64				197
Mg^{2+}	pH titration		40	KNO_3	0.1	1.78				197
Mg^{2+}	pH titration, T		0.4–40	KNO_3	0.1		3.9	20.5 (25°)		197
Ca^{2+}	pH titration		0.4	KNO_3	0.1	1.61				197
Ca^{2+}	pH titration		12	KNO_3	0.1	1.60				197
Ca^{2+}	pH titration		25	KNO_3	0.1	1.58				197
Ca^{2+}	pH titration		40	KNO_3	0.1	1.54				197
Ca^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−0.6	5.2 (25°)		197
Sr^{2+}	pH titration		0.4	KNO_3	0.1	1.60				197
Sr^{2+}	pH titration		12	KNO_3	0.1	1.57				197
Sr^{2+}	pH titration		25	KNO_3	0.1	1.53				197
Sr^{2+}	pH titration		40	KNO_3	0.1	1.48				197
Sr^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−1.2	3.0 (25°)		197
Ba^{2+}	pH titration		0.4	KNO_3	0.1	1.55				197
Ba^{2+}	pH titration		12	KNO_3	0.1	1.50				197
Ba^{2+}	pH titration		25	KNO_3	0.1	1.44				197
Ba^{2+}	pH titration		40	KNO_3	0.1	1.37				197
Ba^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−1.8	0.6 (25°)		197
Mn^{2+}	pH titration		0.4	KNO_3	0.1	2.00				197
Mn^{2+}	pH titration		12	KNO_3	0.1	1.95				197
Mn^{2+}	pH titration		25	KNO_3	0.1	1.89				197
Mn^{2+}	pH titration		40	KNO_3	0.1	1.81				197
Mn^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−1.9	2.2 (25°)		197
Co^{2+}	pH titration		0.4	KNO_3	0.1	2.12				197
Co^{2+}	pH titration		12	KNO_3	0.1	2.07				197
Co^{2+}	pH titration		25	KNO_3	0.1	2.01				197
Co^{2+}	pH titration		40	KNO_3	0.1	1.93				197
Co^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−1.9	2.8 (25°)		197
Ni^{2+}	pH titration		0.4	KNO_3	0.1	2.43				197
Ni^{2+}	pH titration		12	KNO_3	0.1	2.37				197
Ni^{2+}	pH titration		25	KNO_3	0.1	2.30				197
Ni^{2+}	pH titration		40	KNO_3	0.1	2.22				197
Ni^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−2.1	3.3 (25°)		197
Cu^{2+}	pH titration		0.4	KNO_3	0.1	2.80				197
Cu^{2+}	pH titration		12	KNO_3	0.1	2.72				197
Cu^{2+}	pH titration		25	KNO_3	0.1	2.63				197
Cu^{2+}	pH titration		40	KNO_3	0.1	2.52				197
Cu^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−2.7	3.0 (25°)		197
Zn^{2+}	pH titration		0.4	KNO_3	0.1	2.15				197
Zn^{2+}	pH titration		12	KNO_3	0.1	2.11				197
Zn^{2+}	pH titration		25	KNO_3	0.1	2.04				197
Zn^{2+}	pH titration		40	KNO_2	0.1	1.96				197
Zn^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−1.9	3.0 (25°)		197
				ADP^d						
Fe^{3+}	Spectrophotometry	2	25			5.66				120
Co^{2+}	Difference spectrophotometry	6.0	25			4.3				126
Ni^{2+}	Difference spectrophotometry	6.0	25			4.3				126
Ag^+	Difference spectrophotometry	6.0	25			4.2				126
				ADP (L), $H^+ + M(OH)L^{2-} = ML^-$						
Cu^{2+}	pH titration		25	KNO_3	0.1	7.08				88
Zn^{2+}	pH titration		25	KNO_3	0.1	8.51				88

Table VI (Continued)

Metal	*Method*	*pH*	*Temp, °C*	*Supporting electrolyte/solvent*	*μ*	*Log K*	*ΔH°, kcal/mol*	*ΔS°, cal/(deg mol)*	*ΔC$_p$°, cal/(deg mol)*	*Ref*
ADP (L), $2H^+ + [M(OH)L]_2^{4-} = 2ML^-$										
Cu^{2+}	pH titration		25	KNO_3	0.1	10.73				88
Zn^{2+}	pH titration		25	KNO_3	0.1	13.68				88
ATP^c (L), Phosphate, $H^+ + L^{4-} = HL^{3-}$										
H^+	pH titration		0.4	KNO_3	0.1	6.56				205
H^+	pH titration		12	KNO_3	0.1	6.54				205
H^+	pH titration		20	$NaClO_4$	0.1	6.47				143
H^+	pH titration, T		25		0	7.68	1.68	40.7		200
H^+	pH titration		25	KNO_3	0.1	6.53				205
H^+	pH titration		30	$(CH_3)_4NBr$	0.1	6.81				206
H^+	pH titration		40	KNO_3	0.1	6.52				205
H^+	pH titration, T		0.4–40	KNO_3	0.1		−0.5	27.8 (25°)		205
ATP^c (L), $M^{n+} + L^{4-} = ML^{+n-4}$										
Li^+	pH titration		25			1.74				207
Na^+	pH titration		25			1.23				207
Na^+	Ion selective electrode		25			2.36				66a
K^+	pH titration		25			0.95				207
K^+	Ion selective electrode		25			2.34				65, 66a
Rb^+	pH titration		25			0.90				207
Cs^+	pH titration		25			0.85				207
Mg^{2+}	pH titration		0.4	KNO_3	0.1	3.97				205
Mg^{2+}	pH titration		12	KNO_3	0.1	4.10				205
Mg^{2+}	Ion exchange	8.2	23	0.1 *M* NaCl		4.04				112
Mg^{2+}	pH titration		25	KNO_3	0.1	4.22				205
Mg^{2+}	Raman spectra		25			3.9				90b
Mg^{2+}	pH titration		40	KNO_3	0.1	4.28				205
Mg^{2+}	pH titration, T		0.4–40	KNO_3	0.1		2.6	27.5 (25°)		205
Ca^{2+}	pH titration		0.4	KNO_3	0.1	4.10				205
Ca^{2+}	pH titration		12	KNO_3	0.1	3.99				205
Ca^{2+}	pH titration		25	KNO_3	0.1	3.97				205
Ca^{2+}	Raman spectra		25			4.5				90b
Ca^{2+}	pH titration		40	KNO_3	0.1	3.94				205
Ca^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−0.9	12 (25°)		205
Sr^{2+}	pH titration		0.4	KNO_3	0.1	3.80				205
Sr^{2+}	pH titration		12	KNO_3	0.1	3.66				205
Sr^{2+}	pH titration		25	KNO_3	0.1	3.54				205
Sr^{2+}	pH titration		40	KNO_3	0.1	3.45				205
Sr^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−3.0	6 (25°)		205
Ba^{2+}	pH titration		0.4	KNO_3	0.1	3.58				205
Ba^{2+}	pH titration		12	KNO_3	0.1	3.42				205
Ba^{2+}	pH titration		25	KNO_3	0.1	3.29				205
Ba^{2+}	pH titration		40	KNO_3	0.1	3.12				205
Ba^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−3.9	2 (25°)		205
Mn^{2+}	pH titration		0.4	KNO_3	0.1	4.97				205
Mn^{2+}	pH titration		12	KNO_3	0.1	4.82				205
Mn^{2+}	pH titration		22	KCl	0.1	4.78				209
Mn^{2+}	Nmr	7.5	25	0.05 *M N*-ethylmorpholine HCl		5				204
Mn^{2+}	pH titration		25	KNO_3	0.1	4.78				205
Mn^{2+}	pH titration		30	$(CH_3)_4NBr$	0.1	5.19				206
Mn^{2+}	pH titration		40	KNO_3	0.1	4.63				205
Mn^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−3.0	12 (25°)		205
Co^{2+}	pH titration		0.4	KNO_3	0.1	4.80				205
Co^{2+}	pH titration		12	KNO_3	0.1	4.69				205
Co^{2+}	pH titration		25	$NaClO_4$	0.1	4.86				202
Co^{2+}	pH titration		25	KNO_3	0.1	4.66				205
Co^{2+}	pH titration		30	$(CH_3)_4NBr$	0.1	5.21				206
Co^{2+}	pH titration		40	KNO_3	0.1	4.55				205
Co^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−2.2	14 (25°)		205
Ni^{2+}	pH titration		0.4	KNO_3	0.1	5.18				205
Ni^{2+}	pH titration		12	KNO_3	0.1	5.05				205
Ni^{2+}	pH titration		25	$NaClO_4$	0.1	4.85				202

Table VI (Continued)

Metal	*Method*	*pH*	*Temp, °C*	*Supporting electrolyte/solvent*	*μ*	*Log K*	*ΔH°, kcal/mol*	*ΔS°, cal/(deg mol)*	*ΔC$_p$°, cal/(deg mol)*	*Ref*
Ni^{2+}	pH titration		25	KNO_3	0.1	5.02				205
Ni^{2+}	pH titration		30	$(CH_3)_3NBr$	0.1	5.32				206
Ni^{2+}	pH titration		40	KNO_3	0.1	4.90				205
Ni^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−2.5	15 (25°)		205
Cu^{2+}	pH titration		0.4	KNO_3	0.1	6.42				205
Cu^{2+}	pH titration		12	KNO_3	0.1	6.20				205
Cu^{2+}	pH titration		20	$NaClO_4$	0.1	6.30				143
Cu^{2+}	pH titration		25	$NaClO_4$	0.1	6.38				202
Cu^{2+}	pH titration		25	KNO_3	0.1	6.13				205
Cu^{2+}	pH titration		40	$(CH_3)_4NBr$	0.1	6.83				206
Cu^{2+}	pH titration		40	KNO_3	0.1	5.97				205
Cu^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−4.3	14 (25°)		205
Zn^{2+}	pH titration		0.4	KNO_3	0.1	5.00				205
Zn^{2+}	pH titration		12	KNO_3	0.1	4.88				205
Zn^{2+}	pH titration		25	$NaClO_4$	0.1	5.21				202
Zn^{2+}	pH titration		25	KNO_3	0.1	4.85				205
Zn^{2+}	pH titration		25	KCl	0.1	4.76				90
Zn^{2+}	pH titration		30	$(CH_3)_4NBr$	0.1	5.52				206
Zn^{2+}	pH titration		40	KNO_3	0.1	4.71				205
Zn^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−2.7	13 (25°)		205
				ATP^c (L), N_1, $H^+ + HL^{3-} = H_2L^{2-}$						
H^+	pH titration		0.4	KNO_3	0.1	4.29				205
H^+	pH titration		12	KNO_3	0.1	4.14				205
H^+	pH titration		20	$NaClO_4$	0.1	4.10				143
H^+	pH titration		25	KNO_3	0.1	4.06				205
H^+	pH titration		30	$(CH_3)_4NBr$	0.1	3.83				206
H^+	pH titration		40	KNO_3	0.1	3.87				205
H^+	pH titration, T		0.4–40	KNO_3	0.1		−4.1	4.5 (25°)		205
				ATP^c (L), $M^{n+} + HL^{3-} = MHL^{+n-3}$						
Li^+	pH titration		25			0.78				207
Na^+	pH titration		25			0.60				207
K^+	pH titration		25			~0.30				207
Rb^+	pH titration		25			−0.30				207
Cs^+	pH titration		25			−0.30				207
Mg^{2+}	pH titration		0.4	KNO_3	0.1	1.95				205
Mg^{2+}	pH titration		12	KNO_3	0.1	2.16				205
Mg^{2+}	pH titration		25	KNO_3	0.1	2.24				205
Mg^{2+}	pH titration		40	KNO_3	0.1	2.29				205
Mg^{2+}	pH titration, T		0.4–40	KNO_3	0.1		3.4	21.5 (25°)		205
Ca^{2+}	pH titration		0.4	KNO_3	0.1	2.34				205
Ca^{2+}	pH titration		12	KNO_3	0.1	2.21				205
Ca^{2+}	pH titration		25	KNO_3	0.1	2.13				205
Ca^{2+}	pH titration		40	KNO_3	0.1	2.13				205
Ca^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−0.3	9 (25°)		205
Sr^{2+}	pH titration		0.4	KNO_3	0.1	2.17				205
Sr^{2+}	pH titration		12	KNO_3	0.1	2.11				205
Sr^{2+}	pH titration		25	KNO_3	0.1	2.05				205
Sr^{2+}	pH titration		40	KNO_3	0.1	2.00				205
Sr^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−1.6	4 (25°)		205
Ba^{2+}	pH titration		0.4	KNO_3	0.1	2.02				205
Ba^{2+}	pH titration		12	KNO_3	0.1	1.92				205
Ba^{2+}	pH titration		25	KNO_3	0.1	1.85				205
Ba^{2+}	pH titration		40	KNO_3	0.1	1.75				205
Ba^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−2.1	2 (25°)		205
Mn^{2+}	pH titration		0.4	KNO_3	0.1	2.55				205
Mn^{2+}	pH titration		12	KNO_3	0.1	2.48				205
Mn^{2+}	pH titration		25	KNO_3	0.1	2.39				205
Mn^{2+}	pH titration		30	$(CH_3)_4NBr$	0.1	2.62				206
Mn^{2+}	pH titration		40	KNO_3	0.1	2.30				205
Mn^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−2.3	3 (25°)		205
Co^{2+}	pH titration		0.4	KNO_3	0.1	2.45				205
Co^{2+}	pH titration		12	KNO_3	0.1	2.39				205
Co^{2+}	pH titration		25	KNO_3	0.1	2.32				205
Co^{2+}	pH titration		30	$(CH_3)_4NBr$	0.1	2.65				206

Table VI (Continued)

Metal	*Method*	*pH*	*Temp, °C*	*Supporting electrolyte/solvent*	*μ*	*Log K*	*$\Delta H°$, kcal/mol*	*$\Delta S°$, cal/(deg mol)*	*$\Delta C_p°$, cal/(deg mol)*	*Ref*
Co^{2+}	pH titration		40	KNO_3	0.1	2.24				205
Co^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−2.1	4 (25°)		205
Ni^{2+}	pH titration		0.4	KNO_3	0.1	2.88				205
Ni^{2+}	pH titration		12	KNO_3	0.1	2.80				205
Ni^{2+}	pH titration		25	KNO_3	0.1	2.72				205
Ni^{2+}	pH titration		30	$(CH_3)_4NBr$	0.1	2.98				206
Ni^{2+}	pH titration		40	KNO_3	0.1	2.59				205
Ni^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−2.4	4 (25°)		205
Cu^{2+}	pH titration		0.4	KNO_3	0.1	3.32				205
Cu^{2+}	pH titration		12	KNO_3	0.1	3.20				205
Cu^{2+}	pH titration		25	KNO_3	0.1	3.12				205
Cu^{2+}	pH titration		30	$(CH_3)_4NBr$	0.1	3.97				206
Cu^{2+}	pH titration		40	KNO_3	0.1	3.01				205
Cu^{2+}	pH titration, T		0.4-40	KNO_3	0.1		−3.0	4 (25°)		205
Zn^{2+}	pH titration		0.4	KNO_3	0.1	2.81				205
Zn^{2+}	pH titration		12	KNO_3	0.1	2.73				205
Zn^{2+}	pH titration		25	KNO_3	0.1	2.67				205
Zn^{2+}	pH titration		30	$(CH_3)_4NBr$	0.1	2.91				206
Zn^{2+}	pH titration		40	KNO_3	0.1	2.58				205
Zn^{2+}	pH titration, T		0.4–40	KNO_3	0.1		−2.4	4 (25°)		205
ATP^c (L), $M^{n+} + ML^{+n-4} = M_2L^{+2n-4}$										
Li^+	pH titration		25			0.53				207
Na^+	pH titration		25			0.93				207
K^+	pH titration		25			−0.22				207
Rb^+	pH titration		25			− ∞				207
Cs^+	pH titration		25			− ∞				207
ATP^d										
Fe^{3+}	Spectrophotometry	2.0	25	$(H^+ + Na^+)ClO_4$	0.1	6.59				120
Co^{2+}	Difference spectrophotometry	6.0	25			4.3				126
Ni^{2+}	Difference spectrophotometry	6.0	25			4.3				126
Ag^+	Difference spectrophotometry	6.0	25			4.3				126
(Adenosine Tetraphosphate) AQP (L), Phosphate, $H^+ + L^{5-} = HL^{4-}$										
H^+	pH titration		25	n-$(C_3H_7)_4NBr$	0.2	7.27				210
AQP (L), $M^{n+} + L^{5-}$ (?) $= ML^{+n-5}$ (?)										
Li^+	pH titration		25	n-$(C_3H_7)_4NBr$	0.2	1.90				210
Na^+	pH titration		25	n-$(C_3H_7)_4NBr$	0.2	1.43				210
K^+	pH titration		25	n-$(C_3H_7)_4NBr$	0.2	1.29				210
Poly(A), N_1, $H^+ + L = HL^+$ (Adenine Residues)										
H^+	C	7		0.1 *M* KCl/0.01 *M* sodium cacodylate			−2.4		80	211
Cytosine (L), N_1/C_2O, $H^+ + L^- = HL$										
H^+	C		10		0	12.62	−12.05	15.2		20
H^+	C		25		0	12.15	−11.5	17.0		49
H^+	pH titration		25			12.16				212
H^+	Spectrophotometric, T		25		0.1	11.82	−11.0	17.1		53
H^+	Spectrophotometry		30		0.2	12.30				41
H^+	C		40		0	11.68	−11.07	18.1		20
H^+	Spectrophotometry		40		0.2	11.97				41
H^+	Spectrophotometric, T		30–40		0.2		−7.24	7.0^b		41
H^+	C		10–40		0				33	20
Cytosine (L), N_3, $H^+ + HL = H_2L^+$										
H^+	pH titration, C		10		0	4.79	−5.25	3.4		20
H^+	Potentiometric		20		0	4.91				41
H^+	Potentiometric		20		0.20	4.69				41
H^+	Spectrophotometry		20		0.20	4.67				41
H^+	pH titration, C		25		0	4.58	−5.14	3.7		49
H^+	pH titration		25	$NaClO_4$	0.05	4.58				129

Table VI (Continued)

Metal	*Method*	*pH*	*Temp, °C*	*Supporting electrolyte/solvent*	*μ*	*Log K*	*$\Delta H°$, kcal/mol*	*$\Delta S°$, cal/(deg mol)*	*$\Delta C_p°$, cal/(deg mol)*	*Ref*
H$^+$	C		25	NaCl	0.1		−4.47			194
H$^+$	Spectrophotometric, T		25		0.1	4.5	−5.0	3.7		53
H$^+$	pH titration		25			4.60				212
H$^+$	Potentiometric		30		0	4.82				41
H$^+$	Potentiometric		30		0.2	4.60				41
H$^+$	Spectrophotometry		30		0.2	4.64				41
H$^+$	C		40		0	4.42	−4.98	4.3		20
H$^+$	Potentiometric		40		0	4.72				41
H$^+$	Potentiometric		40		0.2	4.50				41
H$^+$	Spectrophotometry		40		0.2	4.54				41
H$^+$	Potentiometric		50		0	4.62				41
H$^+$	Potentiometric		50		0.2	4.40				41
H$^+$	Spectrophotometry		50		0.2	4.45				41
H$^+$	Spectrophotometry		. .^{b}			4.45				48
H$^+$	Spectrophotometry		. .^{b}			4.61				37
H$^+$	T (potentiometric)		20–50		0		−3.85	7.10^b		41
H$^+$	T (potentiometric)		20–50		0.2		−4.22	7.06^b		41
H$^+$	T (potentiometric)		20–50		0.2		−4.07	7.16^b		41
H$^+$	C		10–40		0				9	20
Cytidine (L), Ribose, $H^+ + L^- = HL$										
H$^+$	C		10		0	13.1	−10.6	22.3		20
H$^+$	C		25		0	12.5	−10.3	22.9		20
H$^+$	pH titration		25			12.3				3
H$^+$	C		40		0	12.0	−10.2	22.5		20
Cytidine (L), N_3, $H^+ + HL = H_2L^+$										
H$^+$	pH titration, C		10		0	4.29	−5.31	0.9		20
H$^+$	Potentiometric		20		0	4.54				41
H$^+$	Potentiometric		20		0.2	4.32				41
H$^+$	Spectrophotometry		20		0.2	4.24				41
H$^+$	pH titration		20	1 *M* $NaNO_3$		4.229				46
H$^+$	pH titration, C		25		0	4.08	−5.11	1.5		20, 49
H$^+$	Spectrophotometric, T		25		0.1	4.22	−4.4	5.0		53
H$^+$	pH titration		25			4.22				3
H$^+$	Potentiometric		30		0	4.44				41
H$^+$	Potentiometric		30		0.2	4.22				41
H$^+$	Spectrophotometry		30		0.2	4.16				41
H$^+$	Potentiometric		40		0	4.37				41
H$^+$	pH titration, C		40		0	3.92	−4.83	2.5		20
H$^+$	Potentiometric		40		0.2	4.15				41
H$^+$	Spectrophotometry		40		0.2	4.10				41
H$^+$	Potentiometric		50		0	4.29				41
H$^+$	Potentiometric		50		0.2	4.06				41
H$^+$	Spectrophotometry		50		0.2	4.00				41
H$^+$	Spectrophotometry		. .^{b}		0.05	4.11				50, 213
H$^+$	Spectrophotometry	2–4	. .^{b}			4.2				173
H$^+$	T (potentiometric)		20–50		0		−3.70	8.66^b		41
H$^+$	T (potentiometric)		20–50		0.2		−3.75	8.1^b		41
H$^+$	T (spectrophotometric)		20–50		0.2		−3.71	7.11^b		41
H$^+$	C		10–40		0				16	20
Cytidine (L), $M^{n+} + HL = MHL^{n+}$										
Cu^{2+}	pH titration	4.00	20	1 *M* $NaNO_3$		1.59				137
Hg^{2+}	Spectrophotometry	2–4	. .^{b}			4.6				173
$HgCl_2$	Proton nmr		36	Dimethyl sulfoxide		1.53				174
Pb^{2+}	pH titration	4.00	20	1 *M* $NaNO_3$		0.97				137
Cytidine (L), $HgCl_2 + HL = HgHL^{2+} + 2Cl^-$										
$HgCl_2$	Raman spectra	5–6	35			−0.30				135
Deoxycytidine (L), N_3, $H^+ + L = HL^+$										
H$^+$	Spectrophotometry		. .^{b}		0.05	4.25				50, 213
H$^+$	C	25		NaCl	0.1		−4.30			194

Table VI (Continued)

Metal	*Method*	*pH*	*Temp, °C*	*Supporting electrolyte/solvent*	*μ*	*Log K*	*ΔH°, kcal/mol*	*ΔS°, cal/(deg mol)*	*ΔCp°, cal/(deg mol)*	*Ref*
2′-CMP (L), Phosphate, $H^+ + L^{2-} = HL^-$										
H^+	pH titration		24.5			6.19				40
H^+	pH titration		..^{b}			6.2				214
2′-CMP (L), N_3, $H^+ + HL^- = H_2L$										
H^+	pH titration		24.5			4.44				40
H^+	pH titration		..^{b}			4.3				214
H^+	Spectrophotometry		..^{b}		0.05	4.30				50
2′-CMP (L), Phosphate, $H^+ + H_2L = H_3L^+$										
H^+	pH titration		..^{b}			0.7				214
3′-CMP (L), Phosphate, $H^+ + L^{2-} = HL^-$										
H^+	pH titration		24.5			6.04				40
H^+	pH titration		..^{b}			6.0				214
3′-CMP (L), $M^{n+} + L^{2-} = ML^{+n-2}$										
Sr^{2+}	Ion exchange	7.2–7.3	25		0.16	1.6				198
3′-CMP (L), N_3, $H^+ + HL^- = H_2L$										
H^+	pH titration		24.5			4.31				40
H^+	pH titration		..^{b}			4.3				214
H^+	Spectrophotometry		..^{b}		0.05	4.16				50
5′-CMP (L), Ribose, $H^+ + L^{3-} = HL^{2-}$										
H^+	pH titration		25			13.2				3
5′-CMP (L), Phosphate, $H^+ + HL^{2-} = H_2L^-$										
H^+	pH titration		25		0	6.62	1.35	34.8		200
H^+	pH titration		25	KCl	0.1	6.35				90
H^+	pH titration		25			5.97				3
5′-CMP (L), $M^{n+} + HL^{2-} = MHL^{+n-2}$										
Zn^{2+}	pH titration		25	KCl	0.1	2.54				90
5′-CMP (L), N_3, $H^+ + H_2L^- = H_3L$										
H^+	Spectrophotometric titration		19–22			4.28				203
H^+	Spectrophotometric titration		19–22	0.025 *M* NaH_2PO_4-Na_2HPO_4		4.30				203
H^+	Spectrophotometric titration		19–22	1 *M* NaCl		4.21				203
H^+	pH titration		25	KCl	0.1	4.35				90
H^+	pH titration		25			4.24				3
H^+	Spectrophotometry, pH titration, T		25	0.1 *M* Na_3PO_4			−5.2			215
H^+	Spectrophotometric titration		19–22	0.025 *M* NaH_2PO_4–Na_2HPO_4 + 48% sucrose		4.24				203
5′-CMP (L), Phosphate, $H^+ + H_3L = H_4L^+$										
H^+	pH titration		25			0.80				3
H^+	pH titration		..^{b}			0.7				214
5′-dCMP (L), N_3, $H^+ + L^- = HL$										
H^+	Spectrophotometry		..^{b}		0.05	4.44				50
H^+	C		25	NaCl	0.1		−4.28			194
CDP (L), Phosphate, $H^+ + L^{3-} = HL^{2-}$										
H^+	pH titration, T		25		0	7.18	1.34	37.4		200
CTP (L), Phosphate, $H^+ + L^{4-} = HL^{3-}$										
H^+	pH titration, T		25		0	7.65	1.75	40.8		200
CTP (L), N_3, $M^{n+} + L^{4-} = ML^{+n-4}$										
Mg^{2+}	Ion exchange	8.2	23	0.1 *M* NaCl		4.01				112
Ca^{2+}	Ion exchange	8.2	23	0.1 *M* NaCl		3.81				112

Table VI (Continued)

Metal	*Method*	*pH*	*Temp, °C*	*Supporting electrolyte/solvent*	*μ*	*Log K*	*ΔH°, kcal/mol*	*ΔS°, cal/(deg mol)*	*ΔC$_p$°, cal/(deg mol)*	*Ref*
Mn^{2+}	Ion exchange	8.2	23	0.1 *M* NaCl		4.78				112
Co^{2+}	Ion exchange	8.2	23	0.1 *M* NaCl		4.48				112
				CTP[d]						
Fe^{3+}	Spectrophotometry	2	25			6.18				120
				Poly(C,U) (CMP:UMP ratio 1:8), Cytosine, N_3, $H^+ + L^? = HL^{+?-}$						
H^+	Spectrophotometric pH titration		25	0.1 *M* Na_3PO_4		4.5				215
				Guanine (L), N_9, $H^+ + L^{2-} = HL^-$						
H^+	Spectrophotometry		20			12.62				31
H^+	Hydrogen electrode		25			12.3				4
				Guanine (L), N_1, $H^+ + HL^- = H_2L$						
H^+	Spectrophotometry		20			9.32				31
H^+	Spectrophotometric, T		25		0.1	9.42	−10.1	9.1		53
H^+	Hydrogen electrode		25			9.2				4
				Guanine (L), N_7, $H^+ + H_2L = H_3L^+$						
H^+	Spectrophotometry		20			2.95				31
H^+	Hydrogen electrode		25			3.3				4
				Guanosine (L), Ribose, $H^+ + L^{2-} = HL^-$						
H^+	C		10		0	12.83	−11.04	19.7		21
H^+	C		25		0	12.33	−10.85	20.0		21
H^+	C		40		0	11.60	−10.86	18.4		21
H^+	C		10–40		0				6	21
				Guanosine (L), N_1, $H^+ + HL^- = H_2L$						
H^+	pH titration		20			9.31				32
H^+	pH titration	10.00	20	1 *M* $NaNO_3$		9.24				137
H^+	C		25		0	9.25	−7.65	16.7		21
H^+	Spectrophotometric, T		25		0.1	9.24	−8.7[g]	13.0		53
H^+	pH titration		25			9.16				3
H^+	Spectrophotometry	9–12	..[b]			9.2				173
				Guanosine (L), $M^{n+} + HL^- = MHL^{+n-1}$						
Mn^{2+}	pH titration		20			3.0				32
Fe^{2+}	pH titration		20			4.3				32
Co^{2+}	pH titration		20			3.2				32
Ni^{2+}	pH titration		20			3.8				32
Cu^{2+}	pH titration		20			6				32
Cu^{2+}	pH titration	5.00	20	1 *M* $NaNO_3$		4.34				137
Zn^{2+}	pH titration		20			4.6				32
Cd^{2+}	pH titration		20			4.0				32
Hg^{2+}	Spectrophotometry	9–12	..[b]			∼8.1				173
$HgCl_2$	Proton nmr		36	Dimethyl sulfoxide		0.77				174
Pb^{2+}	pH titration	5.00	20	1 *M* $NaNO_3$		3.48				137
				Guanosine (L), N_7, $H^+ + H_2L = H_3L^+$						
H^+	Spectrophotometric pH titration		15.3	NaCl	0.1	2.231				216
H^+	Spectrophotometric pH titration		15.3	NaCl, D_2O	0.1	2.655				216
H^+	pH titration	2.00	20	1 *M* $NaNO_3$		2.20				137
H^+	C		25		0	1.9	−3.2	−2.1		21
H^+	Spectrophotometric pH titration, T		25	NaCl	0.1	2.174	−2.22	2.5		216
H^+	Spectrophotometric, T		25		0.1	1.6	−1.0	4.0		53
H^+	pH titration		25			1.6				3
H^+	C		25	NaCl	0.1		−1			194
H^+	Spectrophotometric pH titration, T		25	NaCl, D_2O	0.1	2.593	−2.08	4.9		216
H^+	Spectrophotometric pH titration		35.2	NaCl	0.1	2.122				216

Table VI (Continued)

Metal	*Method*	*pH*	*Temp, °C*	*Supporting electrolyte/solvent*	*μ*	*Log K*	*ΔH°, kcal/mol*	*ΔS°, cal/(deg mol)*	*ΔCp°, cal/(deg mol)*	*Ref*
H^+	Spectrophotometric pH titration		35.2	NaCl, D_2O	0.1	2.554				216
H^+	Spectrophotometric	2–4	..[b]			~2.4				173
H^+	Spectrophotometry		..[b]			2.15				217
Guanosine (L), $M^{n+} + H_2L = MH_2L^{n+}$										
Cu^{2+}	pH titration	2.00	20	1 *M* $NaNO_3$		2.15				137
Pb^{2+}	pH titration	2.00	20	1 *M* $NaNO_3$		0.48				137
Th^{4+}	pH titration	2.00	20	1 *M* $NaNO_3$		0.94				137
UO_2^{2+}	pH titration	2.00	20	1 *M* $NaNO_3$		0.72				137
Hg^{2+}	Spectrophotometry	2–4	..[b]			4.5				173
Deoxyguanosine (L), N_1, $H^+ + L^- = HL$										
H^+	pH titration		25	$NaClO_4$	0.05	9.26				140
Deoxyguanosine (L) $Cu^{2+} + L^- = CuL^+$										
Cu^{2+}	pH titration		25	NaCl	0.05	5.58				140
Deoxyguanosine (L), N_7, $H^+ + HL = H_2L^+$										
H^+	pH titration		25	$NaClO_4$	0.05	2.27				140
H^+	C		25	NaCl	0.1		−1.91			194
H^+	Spectrophotometric		..[b]			2.80				217
5′-GMP (L), N_1, $H^+ + L^{3-} = HL^{2-}$										
H^+	Spectrophotometric titration		19–22			9.56				203
H^+	Spectrophotometric titration		19–22	0.025 *M* Na_3PO_4–Na_2HPO_4		9.53				203
H^+	Spectrophotometric titration		19–22	0.15 *M* KCl		9.47				203
H^+	Spectrophotometric titration		19–22	0.025 *M* Na_3PO_4–Na_2HPO_4 + 48% sucrose		9.24				203
H^+	Spectrophotometric titration		19–22	0.025 *M* Na_3PO_4–Na_2HPO_4 + 8 *M* urea		9.94				203
H^+	pH titration		25			9.36				3
5′-GMP (L), Phosphate, $H^+ + HL^{2-} = H_2L^-$										
H^+	pH titration, T		25		0	6.66	1.45	35.3		200
H^+	pH titration		25			5.92				3
5′-GMP (L), N_7(?) $H^+ + H_2L^- = H_3L$										
H^+	pH titration		25			2.3				3
5′-GMP (L), Phosphate (?), $H^+ + H_3L = H_4L^+$										
H^+	pH titration		25			0.7				3
5′-dGMP (L), Phosphate/N_7 (?), $H^+ + L^{3-} = HL^{2-}$										
H^+	C		25	NaCl	0.1		−0.14			194
GDP (L), Phosphate, $H^+ + L^{4-} = HL^{3-}$										
H^+	pH titration, T		25		0	7.19	1.48	37.7		200
GTP (L), N_1, $H^+ + L^{5-} = HL^{4-}$										
H^+	Spectrophotometry		25		0	10.1				148
H^+	Spectrophotometry		25	$NaClO_4$	0.1	9.5				148
GTP (L), $M^{n+} + L^{5-} = ML^{+n-5}$										
Mg^{2+}	Ion exchange	8.2	23	0.1 *M* NaCl		4.02				112
Ca^{2+}	Ion exchange	8.2	23	0.1 *M* NaCl		3.58				112
Mn^{2+}	Ion exchange	8.2	23	0.1 *M* NaCl		4.73				112
Co^{2+}	Ion exchange	8.2	23	0.1 *M* NaCl		4.63				112
GTP[d]										
Fe^{3+}	Spectrophotometry	2	25			6.40				120
GTP (L), Phosphate, $H^+ + HL^{4-} = H_2L^{3-}$										
H^+	pH titration, T		25		0	7.65	1.75	40.8		200

Table VI (Continued)

Metal	*Method*	*pH*	*Temp, °C*	*Supporting electrolyte/solvent*	*μ*	*Log K*	*ΔH°, kcal/mol*	*ΔS°, cal/(deg mol)*	*ΔC$_p$°, cal/(deg mol)*	*Ref*
GTP–Cu^{2+} (L), $H^+ + L^{?-} = HL^{+?-}$ (ionic charge of GTP-Cu^{2+} not given)										
H^+	Spectrophotometry		25		0	8.1				148
H^+	Spectrophotometry		25	$NaClO_4$	0.1	7.5				148
Hypoxanthine (L), N_9/N_7, $H^+ + L^{2-} = HL^-$										
H^+	C		10		0	12.64	−9.81	23.2		21
H^+	pH titration		20			12.10				193
H^+	pH titration, C		25		0	12.0	−10.0	21.5		218
H^+	C		25		0	12.07	−9.53	23.3		21
H^+	C		40		0	11.81	−9.00	25.3		21
H^+	C		10–40		0				27	21
Hypoxanthine (L), $M^{n+} + L^{2-} = ML^{+n-2}$										
Fe^{2+}	pH titration		20			3.9				32
Co^{2+}	pH titration		20			3.8				32
Ni^{2+}	pH titration		20			4.7				32
Cu^{2+}	pH titration		20			6.2				32
Hypoxanthine (L), N_1C_6O, $H^+ + HL^- = H_2L$										
H^+	pH titration		20			8.94				32, 193
H^+	pH titration, C		25		0	8.8	−8.0	13.4		218
H^+	pH titration, C		25		0	8.91	−7.88	14.4		21
H^+	pH titration		25	$NaClO_4$	0.05	8.88				129
H^+	Spectrophotometry, T		25		0.1	8.8	−7.20	16.1		53
H^+	Potentiometric		25			8.7				195
H^+	Spectrophotometry		..[b]			8.79				219
Hypoxanthine (L), N_7, $H^+ + H_2L = H_3L^+$										
H^+	pH titration		20			1.98				32, 193
H^+	pH titration, C		25		0	1.9	−2.5	−0.3		218
H^+	C		25		0	1.79	−2.95	−1.7		21
Inosine (L), Ribose, $H^+ + L^{2-} = HL^-$										
H^+	C		10		0	12.99	−10.4	22.8		21
H^+	C		25		0	12.36	−10.65	20.9		21
H^+	Optical rotation, pH titration		25			12.33				27
H^+	C		40		0	11.84	−10.60	20.3		21
H^+	C		10–40		0				−7	21
Inosine (L), N_1/C_6O, $H^+ + HL^- = H_2L$										
H^+	pH titration		20			8.82				32
H^+	C		25		0	8.96	−6.50	19.2		21
H^+	Spectrophotometric, T		25		0.1	8.9	−7.2	16.4		53
H^+	Optical rotation, pH titration		25			8.75				27
H^+	pH titration		25			8.72				3
H^+	Potentiometric		25			8.7				195
H^+	Spectrophotometry	7–9	..[b]			8.8				173
Inosine (L), $M^{n+} + HL^- = MHL^{+n-1}$										
Fe^{2+}	pH titration		20			3				32
Co^{2+}	pH titration		20			2.6				32
Ni^{2+}	pH titration		20			3.3				32
Cu^{2+}	pH titration		20			5				32
Hg^{2+}	Spectrophotometry	7–9	..[b]			8.2				173
Inosine (L), N_7, $H^+ + H_2L = H_3L^+$										
H^+	pH titration		20			1.5				32
H^+	Spectrophotometry	2–4				~1.2				173
Inosine (L), N_7, $CH_3Hg^+ + H_2L = CH_3HgH_2L^+$										
CH_3Hg^+	Spectrophotometry	2–4	..[b]			3.7				173
Inosine (L), $H_2BO_3^- + 2H_2L = H_2BO_3(H_2L)_2^-$										
B(III)	pH titiation		25	0.1 *M* KCl		3.42				191

Table VI (Continued)

Metal	*Method*	*pH*	*Temp, °C*	*Supporting electrolyte/solvent*	*μ*	*Log K*	*ΔH°, kcal/mol*	*ΔS°, cal/(deg mol)*	*ΔC$_p$°, cal/(deg mol)*	*Re*
5′-IMP (L), Ribose, $H^+ + L^{4-} = HL^{3-}$										
H^+	Optical rotation, pH titration		25			12.2				27
5′-IMP (L), N_1/C_6O, $H^+ + HL^{3-} = H_2L^{2-}$										
H^+	Optical rotation, pH titration		25			8.88				27
5′-IMP (L), Phosphate, $H^+ + H_2L^{2-} = H_3L^-$										
H^+	pH titration, T		25		0	6.66	1.43	35.3		200
H^+	Optical rotation, pH titration		25			6.04				27
5′-IMP (L), Phosphate (?), $H^+ + H_3L^- = H_4L$										
H^+	Optical rotation, pH titration		25			1.54				27
IDP (L), Phosphate, $H^+ + L^{3-} = HL^{2-}$										
H^+	pH titration		25		0	7.18	1.34	37.4		200
ITP (L), N_1/C_6O, $H^+ + L^{5-} = HL^{4-}$										
H^+	Spectrophotometry		25		0	9.5				148
H^+	Spectrophotometry		25	$NaClO_4$	0.1	9.0				148
ITP (L), Phosphate, $H^+ + HL^{4-} = H_2L^{3-}$										
H^+	pH titration, T		25		0	7.68	1.61	40.5		200
ITP (L), $M^{n+} + HL^{4-}$ (?) = ML^{+n-4} (?)										
Mg^{2+}	Ion exchange	8.2	23	0.1 *M* NaCl		4.04				112
Mg^{2+}	Fluorescence	8.1–8.2	25	0.1 *M* triethanolamine	~0.1	4.90–5.00				220
Mg^{2+}	Fluorescence	8.2	25	0.1 *M* tris(hydroxy)methylaminomethane	~0.1	4.78				220
Ca^{2+}	Ion exchange	8.2	23	0.1 *M* NaCl		3.76				112
Mn^{2+}	Ion exchange	8.2	23	0.1 *M* NaCl		4.57				112
Co^{2+}	Ion exchange	8.2	23	0.1 *M* NaCl		4.74				112
ITP^d										
Fe^{3+}	Spectrophotometry	2	25			6.78				120
ITP–Cu^{2+} (L), $H^+ + L^{?-} = HL^{+?-}$ (Base) (ionic charge of ITP–Cu^{2+} not given)										
H^+	Spectrophotometry		25		0	7.7				148
H^+	Spectrophotometry		25	$NaClO_4$	0.1	7.2				148
Thymine (L), N_3C_4O/N_1C_2O, $H^+ + L^{2-} = HL^-$										
H^+	Spectrophotometry		..^{b}			>13				48
Thymine (L), N_3C_4O/N_1C_2O, $H^+ + HL^- = H_2L$										
H^+	pH titration, C		10		0	10.18	−8.50	16.6		20
H^+	pH statting		20		0	9.90				177
H^+	pH statting		20		0.0051	9.89				177
H^+	pH statting		20		0.05	9.87				177
H^+	pH statting		20		0.10	9.84				177
H^+	pH statting		20		0.15	9.81				177
H^+	pH statting		20		0.20	9.78				177
H^+	C		25		0		−8.15	18.0		20
H^+	pH titration		25		0	9.90				49
H^+	pH titration		25			9.94				212
H^+	pH statting		30		0	9.68				177
H^+	pH statting		30		0.0051	9.70				177
H^+	pH statting		30		0.05	9.64				177
H^+	pH statting		30		0.10	9.62				177
H^+	Spectrophotometry		30		0.1	9.68				177
H^+	pH statting		30		0.15	9.58				177
H^+	pH statting		30		0.20	9.54				177
H^+	C		40		0	9.52	−7.79	18.7		20
H^+	pH statting		40		0	9.51				177
H^+	pH statting		40		0.0051	9.55				177

Table VI (Coutinued)

Metal	*Method*	*pH*	*Temp, °C*	*Supporting electrolyte/solvent*	*μ*	*Log K*	*ΔH°, kcal/mol*	*ΔS°, cal/(deg mol)*	*ΔC_p°, cal/(deg mol)*	*Ref*
H^+	pH statting		40		0.05	9.48				177
H^+	pH statting		40		0.10	9.44				177
H^+	pH statting		40		0.15	9.40				177
H^+	pH statting		40		0.20	9.37				177
H^+	pH statting		50		0	9.34				177
H^+	pH statting		50		0.0051	9.34				177
H^+	pH statting		50		0.05	9.29				177
H^+	pH statting		50		0.10	9.26				177
H^+	pH statting		50		0.15	9.22				177
H^+	pH statting		50		0.20	9.17				177
H^+	Spectrophotometry		..[b]			9.9				48
H^+	pH statting, T		20–50		0		−10.5	17		177
H^+	pH statting, T		20–40		0.0051		−8.0	18		177
H^+	C		10–40		0				24	20
Thymidine (L), N_3C_4O, $H^+ + L^- = HL$										
H^+	pH titration, C		10		0	10.14	−7.87	18.6		20
H^+	pH titration	10.00	20	1 *M* $NaNO_3$		9.65				137
H^+	pH titration		25	0.1 *M* NaCl		9.55				183
H^+	pH titration, C		25		0	9.79	−7.32	20.2		20
H^+	pH titration, C		40		0	9.57	−6.97	21.7		20
H^+	C		10–40		0				30	20
H^+	Spectrophotometry		..[b]			9.8				213
Thymidine (L), $M^{n+} + L^- = ML^{+n-1}$										
Cu^{2+}	pH titration	5.00	20	1 *M* $NaNO_3$		4.68				137
Pb^{2+}	pH titration	5.00	20	1 *M* $NaNO_3$		3.70				137
1-β-D-Arabinofuranosylthymine (L), N_3C_4O, $H^+ + L^- = HL$										
H^+	Spectrophotometry		..[b]			9.8				221
1-β-D-Lyxofuranosylthymine (L), N_3C_4O, $H^+ + L^- = HL$										
H^+	pH titration		..[b]			9.89				221
H^+	Spectrophotometry		..[b]			9.92				221
1-β-D-Ribofuranosylthymine (L) N_3C_4O, $H^+ + L^- = HL$										
H^+	Spectrophotometry		..[b]			9.68				221
1-β-D-Xylofuranosylthymine (L), N_3C_4O, $H^+ + L^- = HL$										
H^+	pH titration		..[b]			9.68				221
H^+	Spectrophotometry		..[b]			9.75				221
TTP (L), N_3C_4O, $H^+ + L^{5-} = HL^{4-}$										
H^+	Spectrophotometry		25		0	10.7				148
H^+	Spectrophotometry		25	$NaClO_4$	0.1	10.1				148
TTP–Cu^{2+} (L), $H^+ + L^{?-} = HL^{+?-}$ (ionic charge of TTP–Cu^{2+} not given)										
H^+	Spectrophotometry		25		0	8.5				148
H^+	Spectrophotometry		25	$NaClO_4$	0.1	7.9				148
Uracil (L), N_3C_4O/N_1C_2O, $H^+ + L^{2-} = HL^-$										
H^+	pH titration		25			13.56				3, 212
H^+	Spectrophotometry		..[b]			>13				48
Uracil (L), N_3C_4O/N_1C_2O, $H^+ + HL^- = H_2L$										
H^+	pH titration, C		10		0	9.74	−8.30	15.2		20
H^+	Spectrophotometry, pH titration		20	NaCl	0.01	9.47				10
H^+	pH titration		25		0	9.46				49
H^+	Spectrophotometry		25		0	9.51				52
H^+	C		25		0		−7.85	16.9		20
H^+	pH titration		25	$NaClO_4$	0.05	9.43				129
H^+	pH titration		25			9.28				3, 212
H^+	pH titration		25			9.45				212
H^+	Spectrophotometry, pH titration		30		0.01	9.28				10
H^+	pH titration, C		40		0	9.14	−7.49	17.9		20
H^+	Spectrophotometry, pH titration		40		0.01	9.15				10

Table VI (Continued)

Metal	*Method*	*pH*	*Temp, °C*	*Supporting electrolyte/solvent*	*μ*	*Log K*	*ΔH°, kcal/mol*	*ΔS°, cal/(deg mol)*	*ΔC_p°, cal/(deg mol)*	*Ref*
H^+	Spectrophotometry, pH titration		50		0.01	8.98				10
H^+	Spectrophotometry		. . [b]			9.5				48
H^+	Spectrophotometry, pH titration, T		20–50	NaCl	0.01		−7.2	24 (35°)		10
H^+	C		10–40		0				27	20
				Uridine (L), Ribose, $H^+ + L^{2-} = HL^-$						
H^+	C		10		0	13.03	−11.6	18.8		20
H^+	pH titration, C		25		0	12.59	−10.59	21.0		49
H^+	pH titration		25			12.52				3, 212
				Uridine (L), N_3C_4O, $H^+ + HL^- = H_2L$						
H^+	pH titration, C		10		0	9.61	−7.67	16.9		20
H^+	pH titration	10.00	20	1 *M* $NaNO_3$		9.20				137
H^+	pH titration		25		0	9.30				49
H^+	pH titration		25	$NaClO_4$	0.05	9.27				140
H^+	Spectro-photometric, T		25		0.1	9.51	−8.0	16.8		53
H^+	pH titration		25			9.17				3, 212
H^+	C		25		0		−7.24	18.3		20
H^+	pH titration, C		40		0	9.07	−6.8	19.8		20
H^+	Spectrophotometry		. . [b]			9.25				213
H^+	Spectrophotometry	6–12	. . [b]			9.2				173
H^+	C		10–40		0				29	20
				Uridine (L), $M^{n+} + HL^- = MHL^{+n-1}$						
Cu^{2+}	pH titration	5.00	20	1 *M* $NaNO_3$		4.18				137
Pb^{2+}	pH titration	5.00	20	1 *M* $NaNO_3$		3.38				137
Hg^{2+}	Spectrophotometry	6–12	. . [b]			9.0				173
				Uridine (L), $H_2BO_3^- + 2H_2L = H_2BO_3(H_2L)^-$						
B(III)	pH titration		25	0.1 *M* KCl		3.68				191
				Deoxyuridine (L), N_3C_4O, $H^+ + L^- = HL$						
H^+	Spectrophotometry		. . [b]			9.3				213
				5′-UMP (L), Ribose, $H^+ + L^{4-} = HL^{3-}$						
H^+	pH titration		25			13.9				3, 212
				5′-UMP (L), N_3C_4O, $H^+ + HL^{3-} = H_2L^{2-}$						
H^+	Spectrophotometric titration		19–22	0.025 *M* Na_3PO_4–Na_2HPO_4		9.44				203
H^+	Spectrophotometric titration		19–22	0.025 *M* Na_3PO_4–Na_2HPO_4 + 8 *M* urea		9.96				203
H^+	pH titration		20		0.015	9.71				222
H^+	pH titration		20		0.1	9.43				222
H^+	pH titration		20		0.2	9.34				222
H^+	pH titration		20		0.3	9.24				222
H^+	pH titration		25			9.43				3, 212
H^+	pH titration		30		0.015	9.55				222
H^+	pH titration		30		0.1	9.28				222
H^+	pH titration		30		0.2	9.18				222
H^+	pH titration		30		0.3	9.07				222
H^+	pH titration		40		0.015	9.38				222
H^+	pH titration		40		0.1	9.11				222
H^+	pH titration		40		0.2	9.01				222
H^+	pH titration		40		0.3	8.91				222
H^+	pH titration		50		0.015	9.25				222
H^+	pH titration		50		0.1	8.95				222
H^+	pH titration		50		0.2	8.85				222
H^+	pH titration		50		0.3	8.76				222
H^+	pH titration, T		20–50		0.015		−6.6	21.3[b]		222
H^+	pH titration, T		20–50		0.1		−6.6	19.1[b]		222
H^+	pH titration, T		20–50		0.2		−6.6	19.0[b]		222
H^+	pH titration, T		20–50		0.3		−6.6	18.8[b]		222

Table VI (Continued)

Metal	*Method*	*pH*	*Temp, °C*	*Supporting electrolyte/solvent*	*μ*	*Log K*	*ΔH°, kcal/mol*	*ΔS°, cal/(deg mol)*	*ΔC$_p$°, cal/(deg mol)*	*Ref*
5′-UMP (L), $M^{n+} + HL^{3-} = MHL^{+n-3}$										
Mg^{2+}	Ion exchange	8.2	23	0.1 *M* NaCl		2.25				112
Mg^{2+}	Proton nmr		30	D_2O		1.78				68
5′-UMP (L), Phosphate, $H^+ + H_2L^{2-} = H_3L^-$										
H^+	pH titration, T		25		0	6.63	1.12	34.0		200
H^+	pH titration		25			5.88				3, 212
5′-UMP (L), Phosphate, $H^+ + H_3L^- = H_4L$										
H^+	pH titration		25			1.02				3, 212
UDP (L), Phosphate, $H^+ + L^{3-} = HL^{2-}$										
H^+	pH titration, T		25		0	7.16	1.08	36.4		200
UDP (L), $M^{n+} + L^{3-} = ML^{+n-3}$										
Mg^{2+}	Ion exchange	8.2	23	0.1 *M* NaCl		3.17				112
UTP (L), N_3C_4O, $H^+ + L^{5-} = HL^{4-}$										
H^+	Spectrophotometry		25		0	10.2				148
H^+	Spectrophotometry		25	$NaClO_4$	0.1	9.6				148
UTP (L), Phosphate, $H^+ + HL^{4-} = H_2L^{3-}$										
H^+	pH titration, T		25		0	7.58	2.02	41.4		200
UTP (L), $M^{n+} + HL^{4-} = ML^{+n-4}$										
Mg^{2+}	Ion exchange	8.2	23	0.1 *M* NaCl		4.02				112
Ca^{2+}	Ion exchange	8.2	23	0.1 *M* NaCl		3.71				112
Mn^{2+}	Ion exchange	8.2	23	0.1 *M* NaCl		4.78				112
Co^{2+}	Ion exchange	8.2	23	0.1 *M* NaCl		4.55				112
UTP–Cu^{2+} (L), $H^+ + L^{?-} = HL^{+?-}$ (ionic charge of UTP–Cu^{2+} not given)										
H^+	Spectrophotometry		25		0	8.4				148
H^+	Spectrophotometry		25	$NaClO_4$	0.1	7.8				148
UTP^d										
Fe^{3+}	Spectrophotometry	2	25			6.53				120
Poly(U) (L), $H^+ + L^{?-} = HL^{+?-}$										
H^+	pH titration		10		0.1	10.32				222
H^+	pH titration		20		0.004	10.81				222
H^+	pH titration		20		0.1	10.26				222
H^+	pH titration		30		0.1	9.96				222
H^+	pH titration		40		0.1	9.85				222
Poly(C,U) (see entry following CTP)										
Xanthine (L), N_9, N_7, $H^+ + L^{2-} = HL^-$										
H^+	C		10		0	12.36	−10.18	20.6		21
H^+	pH titration		20			11.12				193
H^+	C		25		0	11.84	−9.61	22.0		21
H^+	C		40		0	11.51	−9.61	23.4		21
H^+	Spectrophotometry		. .^{b}		0.05	11.63				34
H^+	C		10–40		0				34	21
Xanthine (L), N_1/N_3, C_6O/C_2O, $H^+ + HL^- = H_2L$										
H^+	H_2 electrode		18			7.7				33
H^+	H_2 electrode		18	90% vol % ethanol		9.3				33
H^+	pH titration		20			7.44				193
H^+	Spectrophotometry		20			7.70				35
H^+	C		25		0	7.53	−6.33	13.2		21
H^+	pH titration		25	$NaClO_4$	0.05	7.52				129
H^+	Spectrophotometry		. .^{b}		0.05	7.53				34
Xanthosine (L), Ribose, $H^+ + L^{2-} = HL^-$										
H^+	C		10		0	12.85	−11.02	19.9		21
H^+	C		25		0	12.00	−10.86	18.9		21
H^+	C		40		0	11.76	−10.75	19.5		21
H^+	Spectrophotometry		. .^{b}		0.05	~13				34
H^+	C		10–40		0				9	21

Table VI (Continued)

Metal	*Method*	*pH*	*Temp, °C*	*Supporting electrolyte/solvent*	*μ*	*Log K*	*ΔH°, kcal/mol*	*ΔS°, cal/(deg mol)*	*ΔCp°, cal/(deg mol)*	*Ref*
Xanthosine (L), N_1/N_3, C_6O/C_2O, $H^+ + HL^- = H_2L$										
H^+	pH titration		20			5.67				32
H^+	H_2 electrode		20			6.0				33
H^+	H_2 electrode		20	90 vol % ethanol		6.6				33
H^+	C		25		0	5.67	−3.74	13.4		21
H^+	Spectrophotometry		..^{b}		0.05	5.50				34
Xanthosine, $M^{n+} + HL^- = MHL^{+n-1}$										
Fe^{2+}	pH titration		20			<2				32
Co^{2+}	pH titration		20			2.8				32
Ni^{2+}	pH titration		20			3.0				32
Cu^{2+}	pH titration		20			3.4				32
Zn^{2+}	pH titration		20			2.4				32
Xanthosine (L), N_7, $H^+ + H_2L = H_3L^+$										
H^+	pH titration		20			<2.5				32
Ribose (L), $H^+ + L^- = HL$										
H^+	C		25		0	12.22	−8.1	28.7		19
Ribose (L), $H_2BO_3^- + 2HL = H_2BO_3(HL)_2^-$										
B(III)	pH titration		25	0.1 *M* KCl		3.01				191
2-Deoxyribose (L), $H^+ + L^- = HL$										
H^+	C		25		0	12.67	−7.7	32.1		19
Ribose 5-Phosphate (L), Ribose, $H^+ + L^{3-} = HL^{2-}$										
H^+	C		25		0	13.05	−6.1	39.4		19
Ribose 5-Phosphate (L), Phosphate, $H^+ + HL^{2-} = H_2L^-$										
H^+	pH titration		25		0	6.70				192
H^+	C		25		0		2.7	40		15
DNA–Adenine Residues, N_1										
H^+	pH titration		25	0.05 *M* NaCl		3.65				223
H^+	pH titration		..^{b}		0	4.25				224
DNA–Cytosine Residues, N_3										
H^+	pH titration		25	0.05 *M* NaCl		4.95–5.20				223
H^+	pH titration		..^{b}		0	5.25				224
DNA-dCMP (L), N_3										
H^+	Spectrophotometry		..^{b}	Calf thymus DNA, Na salt		6.0–6.3				225
H^+	Spectrophotometry		..^{b}	Calf thymus DNA, Na salt; 0.01 *M* NaCl		5.1–5.2				225
H^+	Spectrophotometry		..^{b}	Calf thymus DNA, Na salt; 0.1 *M* NaCl		3.8–4.0				225
DNA–Guanine Residues, N_7 (?)										
H^+	pH titration		..^{b}		0	3.45				224
DNA-dGMP (L), N_1										
H^+	Spectrophotometry		..^{b}	Calf thymus DNA, Na salt		11.6				225
DNA-dGMP (L), N_7 (?)										
H^+	Spectrophotometry		..^{b}	Calf thymus DNA, Na salt		4.0				225
H^+	Spectrophotometry		..^{b}	Calf thymus DNA, Na salt; 0.01 *M* NaCl		3.6–3.7				225
H^+	Spectrophotometry		..^{b}	Calf thymus DNA, Na salt; 0.1 *M* NaCl		2.7				225
DNA–Thymine Residues, N_3C_4O										
H^+	pH titration		..^{b}	0.05 *M* NaCl		10.1				223

Table VI (Continued)

Metal	*Method*	*pH*	*Temp, °C*	*Supporting electrolyte/solvent*	*μ*	*Log K*	*ΔH°, kcal/mol*	*ΔS°, cal/(deg mol)*	*ΔC$_p$°, cal/(deg mol)*	*Re*
				DNA–TMP (L), N_3C_4O						
H^+	Spectrophotometry		..^{b}	Calf thymus DNA, Na salt		11.6				225
				$M^{n+} + DNA^{?-} = MDNA^{n+?-}$						
Li^+	Donnan equilibrium		25			0.32–0.61				79
Na^+	Donnan equilibrium		25			−0.15–0.20				79
K^+	Donnan equilibrium		25			−0.11–0				79
Mn^{2+}	Nmr		20			4.30				226
Cu^{2+}	Paramagnetic resonance		22	$NaClO_4$	0.01	4.10				160
Cu^{2+}	Kinetics		25	Bonding to phosphate		4.11				157
Cu^{2+}	Gel filtration	6.1	26			4.30				151
Cu^{2+}	Spectrophotometry		..^{b}	Bonding to amino and enol		7.18–7.25				157
Cu^{2+}	Polarography		..^{b}	Calf thymus DNA, NaCl	0.14	3.25				158
Cu^{2+}	Polarography	5.4	..^{b}	Native calf thymus DNA		1.60				155
Cu^{2+}	Polarography	4.9	..^{b}	Denatured calf thymus DNA		1.85				155
Ag^+	Potentiometric, Ag|Ag^+	5.60	22–24	Calf thymus 1, $NaClO_4$	0.1	6.32				170
Ag^+	Potentiometric, Ag|Ag^+	5.60	22–24	Calf thymus 2, $NaClO_4$	0.1	6.34				170
Ag^+	Potentiometric, Ag|Ag^+	5.60	22–24	Calf thymus 2 (denatured) $NaClO_4$	0.1	6.62				170
Ag^+	Potentiometric, Ag|Ag^+	5.60	22–24	*E. coli*, $NaClO_4$	0.1	6.34				170
Ag^+	Potentiometric, Ag|Ag^+	5.60	22–24	*M. lysodeikticus*, $NaClO_4$	0.1	6.49				170
				$MDNA + DNA = M(DNA)_2$						
Ag^+	Potentiometric, Ag|Ag^+	5.60	22–24	Calf thymus 1, $NaClO_4$	0.1	4.61				170
Ag^+	Potentiometric, Ag|Ag^+	5.60	22–24	Calf thymus 2, $NaClO_4$	0.1	4.87				170
Ag^+	Potentiometric, Ag|Ag^+	5.60	22–24	Calf thymus 2 (denatured $NaClO_4$)	0.1	5.11				170
Ag^+	Potentiometric, Ag|Ag^+	5.60	22–24	*E. coli* $NaClO_4$	0.1	4.76				170
Cd^{2+}	Polarography	5.8	..^{b}	Native calf thymus DNA		1.40				155
Cd^{2+}	Polarography		..^{b}	Calf thymus DNA, NaCl	0.14	2.40				158
Cd^{2+}	Polarography		..^{b}	Denatured calf thymus DNA		1.83				155
Ag^+	Potentiometric, Ag|Ag^+	5.60	22–24	*M. lysodeikticus*, $NaClO_4$	0.1	5.38				170
				DNA (Salmon Sperm)^d						
Mg^{2+}	Spectrophotometry	9.0–10.2	..^{b}	Calf thymus DNA, 0.002 *M* NaCl		5.30				226a
Co^{2+}	Difference spectrophotometry	6.0	25			3.9				126
Ag^+	Difference spectrophotometry	6.0	25			4.4				126
UO_2^{2+}	Spectrophotometry	3.5	..^{b}	0.15 *M* NaCl, 0.015 *M* Na citrate salmon sperm DNA		6.90				190

Table VI (Continued)

Metal	*Method*	*pH*	*Temp, °C*	*Supporting electrolyte/solvent*	μ	*Log K*	$\Delta H°$, *kcal/mol*	$\Delta S°$, *cal/(deg mol)*	$\Delta C_p°$, *cal/(deg mol)*	*Ref*
				RNA–Cytosine (L)						
H^+	Spectrophotometry, pH titration		25	0.1 M Na_3PO_4		4.7				215
				RNA (Nuclear)[d]						
Co^{2+}	Difference spectrophotometry	6.0	25			4.6				126

[a] The temperature listed is that at which the log K and/or ΔH values are valid. The $\Delta S°$ values are valid at the stated temperatures unless otherwise indicated. When a temperature interval is given, the indicated log K, $\Delta H°$, or $\Delta C_p°$ value is valid over the range. In each case the ligand is indicated by the symbol L, and L^{n-} is taken to be the ionized species for removal of all protons for which data are available. Other ionizable protons may be present, but this fact is not indicated unless data are given. Therefore, for each ligand the species to which L^{n-} refers must be known before the protonated species, H_mL^{+m-n}, can be identified. Proton binding sites are indicated in each case. For assignment of the M^{n+} binding sites, see Tables IV and V and the text. [b] Temperature not specified. [c] Additional data compiled by Phillips.[1] [d] Reaction not specified. Reaction stoichiometry 1:1 in ref 120 and ref 126; 1:2 UO_2^{2+}:P in ref 190. [e] Log K value reported in ref 197, 2.47, appears to be a typographical error. [f] Log K value reported in ref 197, 2.63, appears to be a typographical error. [g] Calculated from the $\Delta G°$ and $\Delta S°$ values reported in ref 53. The $\Delta H°$ value reported in ref 53, −3.6 kcal/mol, appears to be in error.

making pH titrations using glass and calomel electrodes. However, the important question has been raised concerning whether the metal ion binds to the same base site from which the proton was ionized in certain of these systems, *viz.*, M^{n+}–nucleoside[138,142] and M^{n+}–nucleotide.[142] This question is of obvious importance in studies which attempt to relate hydrogen ion concentrations to metal stability constants—and deserves further attention. The equilibrium constant determinations have been made under a variety of temperature, ionic strength, and solvent conditions, making comparisons difficult. The variations in ionic strength are large ranging from $\mu = 0$ to >1. There are probably two primary reasons for reporting equilibrium constants at ionic strengths other than 0. First, the computational procedure is simplified since activity coefficient corrections are usually not made, and second it is sometimes desired to make the measurement under conditions of μ more nearly approximating those found in living systems. In some cases the difference between equilibrium constants obtained at low (<0.05) and zero ionic strength is considered negligible and no correction is made. Some investigators have defined a standard state as some specific medium (*e.g.*, 0.100 M (KNO_3)).[205] In these cases one must realize that the thermodynamic values are valid only at the specific experimental conditions and are not necessarily comparable with data obtained under different conditions. It is also well to realize that often the substance used as the added electrolyte interacts to a significant extent with the ligand or metal being studied so that one has competing reactions taking place which can affect the values obtained. For example, log K values obtained in one study[148] at $\mu = 0$ and 0.1 ($NaClO_4$) differed by as much as 0.6 log K unit using the same techniques for determining the pK values of ITP, GTP, UTP, and TTP. Since Na^+ and K^+ which are extensively used as supporting electrolytes have been shown to interact significantly with nucleotides,[66,67,207,208,210,227] particular care should be exercised in these cases.

A further complication is encountered in the determination of $\Delta H°$ values by calorimetric procedures where use of added electrolyte can result in errors which are often undetected. The hydration sphere of the reacting metal ion is changed in the presence of added electrolyte to contain some (usually unknown) number of coordinated anions. Heat effects involved in the replacement of these anions by the coordinating ligand could be significantly different from those involved in replacing water molecules. Thus, in most cases neither the log K nor $\Delta H°$ value is known for the reaction of the added electrolyte, leaving the $\Delta H°$ values for formation of the metal complex in error by some unknown amount.

The $\Delta H°$ values in Table VI have often been calculated from the variation of the equilibrium constant with temperature. This method of calculating $\Delta H°$ values involves a differentiation process and results in a certain loss in accuracy. This loss in accuracy of the calculated $\Delta H°$ values can be minimized if the experimental work is very carefully done at many temperatures in the temperature range studied. Therefore, one should be careful not to attach undue significance to the very small standard deviations often reported for $\Delta H°$ values calculated from temperature-dependent work. The errors propagated through differentiation in calculating $\Delta H°$ values from equilibrium constant data as a function of temperature have been discussed.[228] In general, the direct methods of calorimetry are preferable to temperature-dependent methods for the determination of $\Delta H°$ values.

Enthalpy change, $\Delta S°$, and $\Delta C_p°$ values when known in addition to equilibrium constants provide additional information regarding sites of binding and the interactions of the metal ion or proton with the ligand and the effect of the solvent on these interactions. The magnitudes of the $\Delta H°$ values are indicative of (a) the types of binding sites (*i.e.*, ether, carboxyl, amino, sulfhydryl, etc.; *e.g.*, see discussion of proton ionization from adenine and cytosine) and (b) the number of binding sites. The magnitudes of the $\Delta S°$ values are indicative of solvent–solute interactions and supply information about relative degrees of hydration of the metal ion, ligand, and complex, the loss of degrees of freedom of the ligand when complexed with the metal ion, and the charge types involved in the reaction. In addition, comparison of the $\Delta H°$ and $\Delta S°$ values for systems gives rise to analogies among these systems and helps to point out similarities and differences among such systems.

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Determination of the $\Delta H°$ values as a function of temperature allows $\Delta C_p°$ values to be calculated. These data are available only for proton ionization from the purines, pyrimidines, and nucleosides. The $\Delta C_p°$ values reflect changes in the solvent due to conformational changes of the ligands upon proton or metal complexation. These $\Delta C_p°$ data could be very informative, and it is desirable that more work be done in determining values particularly by calorimetric procedures. The need for such data has been recognized.[229]

One of the most useful pieces of information concerning metal binding would be to have reliable log K values, particularly for the nucleosides, nucleotides, and polynucleotides, so that trends in the binding could be directly observed. Unfortunately, no systematic study has been made for any systems more complicated than the nucleotides, and available data are limited primarily to the adenine nucleotides.

Acknowledgment. Appreciation is expressed to the U. S. Public Health Service for financial support of this work through NIH Grant RG-09430-09 and Career Development Awards to J. J. C. (No. 1-K3-GM-24, 361-03) and R. M. I. (No. 1-K3-GM-35, 250-03).

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