(26) J. Kraut and J. J. Reed, *Acta Crystallogr.*, **15**, 747 (1962).
(27) I. L. Karle and K. Britts, *Acta Crystallogr.*, **20**, 118 (1966).
(28) J. Pletcher, M. Sax, S. Sengupta, J. Chu, and C. S. Yoo, *Acta Crystallogr., Sect. B*, **28**, 2928 (1972).
(29) C. H. Carlisle and D. S. Cook, *Acta Crystallogr., Sect. B*, **25**, 1359 (1969).
(30) A. F. Cameron, K. P. Forrest, and G. Ferguson, *J. Chem. Soc. A*, 1286 (1971).
(31) T. V. Long, II, A. W. Herlinger, E. F. Epstein, and I. Bernal, *Inorg. Chem.*, **9**, 459 (1970).
(32) J. T. Veal and D. J. Hodgson, *Inorg. Chem.*, 11, 597 (1972).
(33) W. C. Hamilton and J. A. Ibers, "Hydrogen Bonding in Solids", W. A. Benjamin, New York, N.Y., 1968.
(34) I. D. Brown, Department of Physics, McMaster University, Hamilton, Ontario, private communication, 1974.
(35) L. Pauling, "The Nature of the Chemical Bond", 3rd ed, Cornell University Press, Ithaca, N.Y., 1960, p 260.
(36) F. Jordan, *J. Am. Chem. Soc.*, **96**, 3623 (1974).

Nucleic Base–Metal Ion Interactions. Acidity of the N(1) or N(3) Proton in Binary and Ternary Complexes of Mn^{2+}, Ni^{2+}, and Zn^{2+} with the 5′-Triphosphates of Inosine, Guanosine, Uridine, and Thymidine

Helmut Sigel

Contribution from the Institute of Inorganic Chemistry, University of Basel, CH-4056 Basel, Switzerland.
Received August 20, 1974

Abstract: By uv difference spectra and potentiometric titrations the acidity constants, K^{H}_{NTP}, of the nucleic base residues, i.e., the proton at N(1) in ITP^{4-} and GTP^{4-}, or at N(3) in UTP^{4-} and TTP^{4-}, and of their complexes with Mn^{2+}, Ni^{2+}, and Zn^{2+} ($K^{H}_{M(NTP)}$) have been determined; the data for the Cu^{2+} and Mg^{2+} complexes were taken from earlier work. The influence of the metal ions was characterized by $\Delta pK_A = pK^{H}_{NTP} - pK^{H}_{M(NTP)}$, which is in the order of 0.2 for Mg^{2+}, 0.3 for Mn^{2+}, 0.9 for Ni^{2+}, 1.9 for Cu^{2+}, and 1.2 for Zn^{2+}. As the difference in pK_A for the proton of N(1) or N(3) for the nucleotides and the corresponding nucleosides is about 0.4 log units, the following is concluded. (i) There is a considerable interaction between the metal ion and the nucleic base residue in the nucleotide complexes of Ni^{2+}, Cu^{2+}, and Zn^{2+}. (ii) On the structure of the complexes with Mg^{2+} and Mn^{2+} nothing can be said, as the small shift in acidity may simply be due to the partial neutralization of the negatively charged phosphate chain by the metal ion. In the mixed-ligand 2,2′-bipyridyl nucleotide complexes with Ni^{2+}, Cu^{2+}, and Zn^{2+} $\Delta pK_{A(Bipy)}$ is always about 0.6, thus indicating similar structures. Based on known structures, a folded form is suggested which allows an intramolecular charge-transfer interaction between the purine or pyrimidine and pyridyl moieties. The tendency of the binary complexes to form hydroxo species, $M(L)(OH)^{n-}$, increases for a given nucleotide, with $Mn^{2+} < Ni^{2+} < Zn^{2+} < Cu^{2+}$, while it decreases for a given metal ion, with L = $CTP^{4-} \sim ATP^{4-} > (UTP\text{-}1H)^{5-} \sim (TTP\text{-}1H)^{5-} > (ITP\text{-}1H)^{5-} \sim (GTP\text{-}1H)^{5-}$, hence, indicating in the latter series an increasing degree of saturation of the coordination sphere of the metal ion.

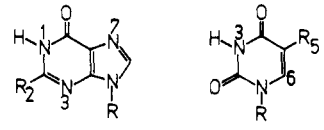
Virtually all enzymes requiring nucleoside phosphates as substrates need in addition a divalent metal ion.[1] To understand the role of these metal ions, it is necessary to learn more about the structures of their binary[2-5] and ternary (mixed-ligand)[6-8] nucleotide complexes. So far the 3d metal ion–adenine nucleotide complexes are best studied; several of the corresponding ATP complexes exist in the ring bound form,[9-11] i.e., the metal ion coordinates both to the phosphate chain *and* to N(7) of the adenine moiety. Considerably less is known on the interaction between metal ions and the base moieties of other nucleotides.

Even though the stability of divalent earth alkali and 3d metal ion nucleotide complexes is governed by the coordination tendency of the phosphate chains,[2,3,12-14] the nucleic bases determine at least in part the specificity because in most enzyme reactions the nucleotides are not freely interchangeable. Hence, there is a clear relationship between structure and reactivity. This is also evident from the Cu^{2+} promoted dephosphorylation of nucleoside 5′-di- and -triphosphates, where the rate depends on the extent of the metal ion–nucleic base interaction,[15,16] which in turn is dependent on the nucleic base.

Before the understanding of such structure–reactivity relationships will improve, it is necessary to study metal ion–nucleic base interactions more in detail. It is the aim of this study to shed some light on the structures of the complexes formed by Mn^{2+}, Ni^{2+}, and Zn^{2+} with ITP, GTP, UTP, and TTP. The IUPAC numbering system is indicated on Chart I; for convenience N(1) of the purine and N(3) of the pyrimidine nucleotides are both labeled N(A).

In contrast to ATP and CTP, the nucleotides of Chart I

Chart I



R = ribosyl 5′-triphosphate

ITP: R_2 = –H UTP: R_5 = –H

GTP: R_2 = $-NH_2$ TTP: R_5 = $-CH_3$

Abbr: N(A) = N(1) of ITP or GTP, and N(3) of UTP or TTP

have a proton at N(A). In case the base moieties of the nucleotides participate in complex formation one expects that this proton ionizes at a lower pH than in the free ligands.[17] Due to the recent work of Clarke and Taube[18] on guanosine–ruthenium complexes, it is now possible to reach conclusive results also for "labile" metal ions, like Ni^{2+} and Zn^{2+}. Certainly, if the base moieties are prevented from a

direct coordination by the formation of ternary complexes, e.g., with 2,2'-bipyridyl, the acidification of the proton at N(A) should be considerably smaller; this is found indeed.

Experimental Section

Materials. All materials were reagent grade and used without further purification. The sodium salts of the nucleoside 5'-triphosphates ("Type I" or "Sigma grade") were purchased from Sigma Chemical Co., St. Louis, Mo. The perchlorates of Mn^{II}, Ni^{II}, Zn^{II} (all purum) and Na^{I} (purissimum), and 2,2'-bipyridyl (purissimum) were from Fluka AG, Buchs, Switzerland. The exact metal ion concentration of the perchlorate stock solutions was determined with EDTA.

Apparatus. The potentiometric titrations were carried out with a Metrohm Potentiograph E 336 and Metrohm EA 121 UX glass electrodes. The absorption spectra were recorded with a Beckman spectrophotometer DB, connected with a W+W Electronic Hi-speed Recorder 202. The pH measurements were performed with a Metrohm potentiometer E 353 B with microglass electrodes EA 147.

Potentiometric Measurements. Stock solutions of NTP^{4-} (cf. ref 19) were always freshly prepared by rapidly titrating with NaOH the dissolved di- or trisodium salts to the equivalence point. Also, reaction solutions containing metal ions were immediately titrated after mixing to prevent dephosphorylation.[15,16]

The acidity constant for the deprotonation of N(A) was determined from automatic titrations of aqueous solutions containing 2×10^{-4} *M* $HClO_4$ and $NaClO_4$ ($I = 0.1$) in the presence and absence of NTP^{4-} (8×10^{-4} *M*) under N_2 with 0.1 *M* NaOH (25°). The difference of such a pair of titrations was evaluated and the constants were calculated from the pH range which corresponded to a degree of neutralization between 0.1 and 0.9. Of TTP only limited amounts were available; therefore, the titrations were carried out in solutions containing 3.2×10^{-4} *M* TTP^{4-} by using 4×10^{-2} *M* NaOH.

The acidity constants for the deprotonation of N(A) in the binary complexes were determined in solutions that contained in addition the metal ion in a ratio of 1:1 with regard to NTP^{4-}. The binary complexes are formed under these conditions to a high degree. The same is true for the ternary complexes containing 2,2'-bipyridyl, where the acidity constants were determined from solutions containing NTP^{4-}, metal ion, and Bipy in a ratio of 1:1:1.

For the binary systems the neutralization degree exceeded a value of 1, indicating the formation of hydroxo complexes. The corresponding constants were calculated by taking into account (where necessary) the overlapping buffer regions.[20]

ATP^{4-} or CTP^{4-} and M^{2+} (10^{-3} *M*) in 1:1 solutions were also titrated and the constants for the hydroxo complexes calculated. Constant values were only obtained within the range of the degree of neutralization 0.1–~0.6 (cf. also ref 15). These values for the ATP complexes agree reasonably with those determined by Brintzinger.[21] Certainly, these constants were obtained only from titrations at a single concentration and, therefore, do not exclude the possible formation of dimers, e.g., $[Zn(ATP)(OH)]_2^{6-}$,[3b] but they completely satisfy the titration curves within the given limits.

Spectrophotometric Measurements. The stock solutions of NTP^{4-} were prepared as described. The deprotonation of N(A) in the nucleotides and in their binary complexes results in a spectral change (cf. ref 17) that may be used to determine the acidity constants.

To obtain a clear-cut situation, uv difference spectra (cf. ref 17) had to be recorded ($I = 0.1$, $NaClO_4$; 25°). They were taken by placing in the reference beam one quartz cuvette with 4×10^{-4} *M* NTP^{4-} (pH 6.2-6.8) in aqueous 0.1 *M* $NaClO_4$ and a second one with the metal ion perchlorate (4×10^{-4} *M*) also in 0.1 *M* $NaClO_4$; the sample beam contained one quartz cuvette with the mixed system in 0.1 *M* $NaClO_4$ (with values of pH between 5 and 10) and one with the solvent (aqueous 0.1 *M* $NaClO_4$). As the absorption of the reference solutions was high, the spectrophotometer was used on "manual" with the slit at 2,2 mm. With ITP and GTP 2-mm cuvettes were used and with UTP and TTP 1-cm cuvettes. The desired pH in the mixed NTP–metal ion system was adjusted under N_2 by dotting with a glass stick and 2 *N* NaOH (or 2 *N* $HClO_4$); the change in volume was negligibly small.

The acidity constants were determined according to the procedure given by Benesi and Hildebrand.[22] Plots of $[H^+]$ vs. $1/\Delta E$ resulted in straight lines[17] that were calculated by the least-squares method (cf. Figure 1). In the case of ITP the spectra were evaluated at approximately 210, 238, and 260 nm, with GTP at 214, 248, and 280 nm, with UTP at 228 and 276 nm, and with TTP at 231 and 280 nm.

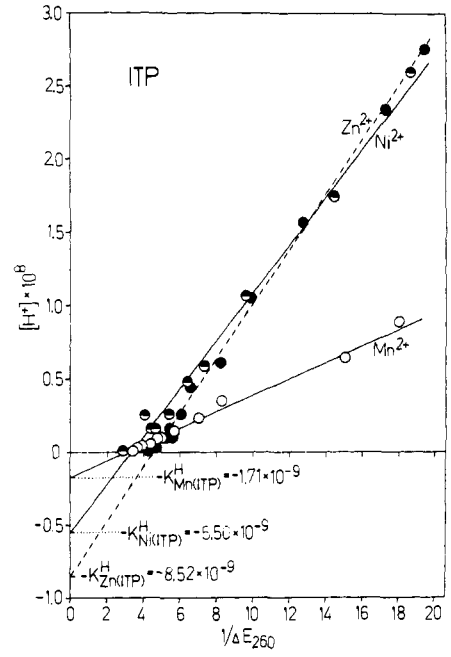


Figure 1. Determination of $K^H_{M(ITP)}$ for the deprotonation at N(1) in binary complexes ($I = 0.1$, $NaClO_4$; 25°; $[ITP^{4-}] = [M^{2+}] = 4 \times 10^{-4}$ *M*).

Results

The nucleotides, ITP^{4-}, GTP^{4-}, UTP^{4-}, and TTP^{4-}, have a proton at N(A). The ionization of this proton should be facilitated in metal ion–nucleotide complexes if the metal ion coordinates not only to the stability determining[2,3,12-14] phosphate chain but also to the nucleic base residue. The easiest approach to determine such an influence is to measure the corresponding acidity constants of the free nucleotides (eq 1) and of their complexes (eq 2). The acidifying influence of a metal ion may then be characterized by eq 3.[19] Similarly, for the mixed-ligand complexes containing 2,2'-bipyridyl, the analogous definitions hold as expressed by eq 4 and 5.

$$NTP^{4-} \rightleftharpoons (NTP\text{-}1H)^{5-} + H^+ \quad (1)$$

$$K^H_{NTP} = [(NTP\text{-}1H)^{5-}][H^+]/[NTP^{4-}]$$

$$M(NTP)^{2-} \rightleftharpoons M(NTP\text{-}1H)^{3-} + H^+ \quad (2)$$

$$K^H_{M(NTP)} = [M(NTP\text{-}1H)^{3-}][H^+]/[M(NTP)^{2-}]$$

$$\Delta pK_A = pK^H_{NTP} - pK^H_{M(NTP)} \quad (3)$$

$$M(Bipy)(NTP)^{2-} \rightleftharpoons M(Bipy)(NTP\text{-}1H)^{3-} + H^+ \quad (4)$$

$$K^H_{M(Bipy)(NTP)} = [M(Bipy)(NTP\text{-}1H)^{3-}][H^+]/M(Bipy)(NTP)^{2-}]$$

$$\Delta pK_{A(Bipy)} = pK^H_{NTP} - pK^H_{M(Bipy)(NTP)} \quad (5)$$

The results obtained from potentiometric titrations are given in Table I. The values of ΔpK_A indicate that the proton at N(A) is increasingly acidic; $Mn^{2+} < Ni^{2+} < Zn^{2+}$. A comparison between the data of the binary and corresponding ternary complexes shows in all cases $\Delta pK_A > \Delta pK_{A(Bipy)}$.[23]

For the binary metal ion–nucleotide systems the degree

Table I. Acidity Constants, Determined from Potentiometric Titrations, for the Deprotonation of N(A) in Nucleoside 5'-Triphosphates,[a,b] in Their Binary Mn^{2+}, Ni^{2+}, or Zn^{2+} Complexes, and in Their Corresponding Ternary Complexes[23] Formed with 2,2'-Bipyridyl (I = 0.1, $NaClO_4$; 25°)[b]

M	NTP	$pK^H_{M(NTP)}$	ΔpK_A	$pK^H_{M(Bipy)(NTP)}$	$\Delta pK_{A(Bipy)}$
Mn^{2+}	ITP	8.93 ± 0.02	0.33		
	GTP	9.36 ± 0.01	0.43		
	UTP	9.45 ± 0.02	0.25		
	TTP	9.67 ± 0.03	0.22		
Ni^{2+}	ITP	8.39 ± 0.03	0.87	8.77 ± 0.05	0.59
	GTP	8.64 ± 0.03	1.15	9.16 ± 0.04	0.63
	UTP	9.10 ± 0.03	0.60	9.24 ± 0.03	0.46
	TTP	9.08 ± 0.09	0.81	9.42 ± 0.05	0.47
Zn^{2+}	ITP	8.31 ± 0.04	0.95	8.87 ± 0.02	0.39
	GTP	8.39 ± 0.05	1.40	9.20 ± 0.03	0.59
	UTP	8.71 ± 0.04	0.99	9.13 ± 0.02	0.57
	TTP	8.35 ± 0.05	1.54	9.06 ± 0.05	0.83

[a] pK^H_{NTP} = 9.26 ± 0.01 for ITP, 9.79 ± 0.02 for GTP, 9.70 ± 0.01 for UTP, and 9.89 ± 0.03 for TTP. [b] The results are the average of the constants calculated from three independent titrations. The single exceptions are the data for the TTP systems; these were titrated only once due to scarcity of this nucleotide. The range of error is three times the standard deviation.

Table II. Tendency for the Formation of Hydroxo Complexes (I = 0.1, $NaClO_4$; 25°)[a]

Ligand	$pK^H_{ML(H_2O)_x}$			
	Mn^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}
CTP^{4-}	10.87 ± 0.13	9.58 ± 0.15	7.6[b]	8.79 ± 0.05
ATP^{4-}	10.7	9.41 ± 0.08	7.9,[b] 8.17[c]	8.87 ± 0.04
$(UTP\text{-}1H)^{5-}$	11.1	9.7	8.4[b]	9.24 ± 0.11
$(TTP\text{-}1H)^{5-}$	11.2	9.9	8.2[b]	9.2
$(ITP\text{-}1H)^{5-}$	11.24 ± 0.15	10.6	9.2[b]	9.4
$(GTP\text{-}1H)^{5-}$	11.3	10.57 ± 0.05	9.3[b]	9.48 ± 0.07

[a] The range of error is three times the standard deviation; where no number is given the corresponding range is approximately ±0.2 log units (cf. footnote *b* of Table I). [b] From ref 17. [c] From ref 15.

Table III. Acidity Constants, Determined from Spectrophotometric Measurements, for the Deprotonation of N(A) in Nucleoside 5'-Triphosphates and in Their Binary Mn^{2+}, Ni^{2+}, or Zn^{2+} Complexes (I = 0.1, $NaClO_4$; 25°)[a]

NTP	pK^H_{NTP}	$pK^H_{Mn(NTP)}$	$\Delta pK_{A/Mn}$	$pK^H_{Ni(NTP)}$	$\Delta pK_{A/Ni}$	$pK^H_{Zn(NTP)}$	$\Delta pK_{A/Zn}$
ITP	9.0[b]	8.8	0.2	8.2	0.8	8.2	0.8
GTP	9.5[b]	9.3	0.2	8.6	0.9	8.3	1.2
UTP	9.6[b]	9.3	0.3	9.1	0.5	8.8	0.8
TTP	10.1[b]	9.6	0.5	9.3	0.8	8.7	1.4

[a] The results are the average constants obtained from at least two (usually three) series of spectral measurements. The range of error corresponding to three times the standard deviation is approximately ±0.2 log units, with the exception of Zn(GTP) and the TTP systems; in these latter cases only a single determination could be carried out and hence the error may be rather large. [b] From ref 17.

of neutralization exceeded unity in the potentiometric titrations (cf. Experimental Section). This was attributed to the formation of hydroxo complexes according to eq 6. For

$$M(NTP\text{-}1H)(H_2O)_x^{3-} \rightleftharpoons M(NTP\text{-}1H)(OH)^{4-} + H^+ \quad (6)$$

$$K^H_{M(NTP\text{-}1H)(H_2O)} = [M(NTP\text{-}1H)(OH)^{4-}][H^+]/[M(NTP\text{-}1H)^{3-}]$$

comparison the formation of hydroxo complexes containing CTP^{4-} or ATP^{4-} was also studied. The results are summarized in Table II.

The ionization of the proton at N(A) is coupled with a significant spectral change in all these nucleotides,[17] as well as in their binary metal ion complexes. Hence, it was possible to determine by an independent method, i.e., by recording uv difference spectra, the acidity constants for the binary complexes according to eq 2. The results are given in Table III, and their comparison with the data of Tables I and II demonstrates that the (so far) tentative assignments for the acidity constants obtained from the potentiometric titrations are correct; i.e., the first acidity constant (Table I) is always due to the deprotonation of N(A) in the binary nucleotide complexes while the second one (Table II) must then refer to the formation of hydroxo complexes. The values of ΔpK_A determined by potentiometry and spectrophotometry agree satisfactorily.

Discussion

As we have seen, the ionization of the proton at N(A) is facilitated in all the binary metal ion-nucleotide complexes. The characteristic quantities, i.e., the values of ΔpK_A, are summarized in Table IV, together with the data of Cu^{2+} and those obtained by Sari and Belaich[24] for the Mg^{2+} complexes. The proton at N(A) in the binary nucleotide complexes is increasingly acidic within the series $Mg^{2+} < Mn^{2+} < Ni^{2+} < Zn^{2+} < Cu^{2+}$.

On the Structure of the Ni^{2+}, Cu^{2+}, and Zn^{2+} Nucleotides. For a further evaluation of the results it is helpful to compare the data of these nucleotide complexes with those of nucleoside ones. In the latter complexes no phosphate groups are present and therefore the only binding sites are those of the base. From the data given by Fiskin and Beer[25] for the guanosine-Cu^{2+} system one may calculate[17] the shift of the acidity constant for the proton of N(1) under the influence of Cu^{2+} coordinated to N(7) and obtains: $\Delta pK_A = pK^H_{Guo} - pK^H_{M(Guo)} = 9.24 - 7.05 = 2.2$. Obviously, the increased acidity of the proton at N(1) is only stronger by a factor of ~2 (0.3 log units), compared with the corresponding Cu^{2+} nucleotides (Table IV). Hence, in these latter complexes a Cu^{2+}-nucleic base interaction definitely exists. The somewhat smaller increase of the acidity

Table IV. Values of $\Delta pK_A = pK^H_{NTP} - pK^H_{M(NTP)}$ for Several M^{2+} Nucleotide Systems

NTP	Mg^{2+}	Mn^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}
ITP	0.2[a]	0.33	0.87	1.7[b]	0.95
GTP	0.2[a]	0.43	1.15	1.9[b]	1.40
UTP	0.2[a]	0.25	0.60	1.7[b]	0.99
TTP		0.22	0.81	2.1[b]	1.54

[a] Values from Sari and Belaich.[24] [b] From ref 17.

in the nucleotide complexes is certainly due to the additional coordination of Cu^{2+} to the fourfold negative phosphate chain.

Of further substantial help are the data of Clarke and Taube[18] on pentaammineruthenium-guanosine complexes, where Ru(II) or Ru(III) is firmly coordinated to N(7): $\Delta pK_{A/Ru(II)} = 0.8$ and $\Delta pK_{A/Ru(III)} = 2.2$. These results allow to conclude that values of ΔpK_A as small as ~0.8 still evidence a considerable metal ion-base interaction.

For further reasonings on the structure of these complexes it should be noted that Sternlicht et al.[26] proposed in 1968 that under the conditions $[M^{2+}]/[ATP^{4-}] \ll 1$ the predominant species is $M(ATP)_2^{6-}$, where the metal ion simultaneously binds to the phosphate moiety of one nucleotide and to N(7) of the second. However, this complex has *not* been observed for Mn^{2+},[27a] Ni^{2+},[27b] or Mg^{2+} (cf. also ref 5);[27c] additionally, it was concluded that $Mn(ATP)^{2-}$ is the predominant species even at high concentrations of ATP.[11] Furthermore, Sternlicht et al.[26] themselves conclude that at $[ATP^{4-}] = 10^{-3}$ *M* the 1:1 complex predominates and that the coordination of a second ligand over the adenine moiety is negligible. As in *this* study $[M^{2+}] = [NTP^{4-}] \leq 8 \times 10^{-4}$ *M* it is expected that the concentration of polymeric species is negligible and that predominantly 1:1 complexes will exist. Moreover, as in $Mn(ATP)^{2-}$ the metal ion-base interaction is *intra*molecular,[11] this is even more expected in dilute solutions of other nucleotide-metal ion systems with a greater tendency to interact.[28] This conclusion is in accord with studies on the dephosphorylation of NTP in the presence of M^{2+}.[15,16] Hence, by taking into account these arguments and the mentioned results obtained for the guanosine complexes it is concluded that the complexes $M(NTP\text{-}1H)^{3-}$ exist with Ni^{2+}, Cu^{2+}, and Zn^{2+} predominantly in the form of a macrochelate, i.e., the metal ion is bound both to the phosphate chain and to the nucleic base residue [although not necessarily over N(A)].

However, this does not necessarily mean that the complexes $M(NTP)^{2-}$, where M = Ni^{2+}, Cu^{2+}, and Zn^{2+}, exist also predominantly in the ring bound form. It means only that such a ring-bound form *must* exist at least in equilibrium with a simple phosphate-bound one, because only the ring-bound form induces a shift of the acidity of the proton at N(A). Certainly, as soon as N(A) is deprotonated this atom and the neighboring oxygen(s) (cf. Chart I) show an increased coordination tendency, which will shift the equilibria more to the ring-bound form. For example, as shown by ^1H NMR spectroscopy, $Cu(ATP)^{2-}$ and $Cu(GTP)^{2-}$ exist mainly as a macrochelate involving binding to N(7),[29a] while in $Cu(ITP)^{2-}$ such a macrochelate exists only to ~10%;[29b] this different binding behavior is also reflected in the reactivity of these complexes.[16] Furthermore, in $Cu(GTP\text{-}1H)^{3-}$ the metal ion is bound to N(7) and O(6), while in Cu(ITP-1H) to the predominating part only the N(1),O(6) site is involved. Similarly, Kotowycz et al.[11] have recently shown that in $Mn(ATP)^{2-}$ approximately 20% of the manganese ions are ring coordinated.

Acidification by Mg^{2+} and Mn^{2+}. In case of the Mn^{2+} and Mg^{2+} nucleotides it is more difficult to reach a firm conclusion as the values of ΔpK_A (cf. Table IV) are so small. Here it appears wise to assess at first the influence of the triphosphate chain on the acidity of the proton at N(A). Indeed, the data assembled in Table V demonstrate unequivocally that the presence of the negatively charged triphosphate chain leads to a decrease in acidity. Hence, if the fourfold negative charge of the triphosphate chain is partially neutralized by coordination of a metal ion, we expect an increase in acidity of the proton at N(A), which may account for a decrease of the acidity constant by up to 0.4 log units. As the values of ΔpK_A observed for the Mg^{2+} and Mn^{2+} nucleotides are only about 0.2 and 0.3 log units, respectively, we *must* conclude that the complete shift in acidity may be due to a simple charge neutralization. Hence, the recent conclusion[24] that the ΔpK_A of about 0.2 log units indicates a Mg^{2+}-nucleic base moiety interaction must be rejected. Based on the observed shifts in acidity *no* conclusions can be drawn neither for the Mg^{2+} nor for the Mn^{2+} nucleotides.

Table V. Comparison of the Acidity Constants ($pK_{A,N(A)}$) for the Deprotonation of N(A) in Nucleosides and Nucleoside 5'-Triphosphates with Several Values of $\Delta pK_{A(Bipy)}$ (Cf. Eq 5)

	$pK_{A,N(A)}$			$\Delta pK_{A(Bipy)}$		
NTP	NTP	Nucleoside	Δ[a]	Ni^{2+}	Cu^{2+}	Zn^{2+}
ITP	9.26, 9.0[b]	8.7,[c] 8.82[d]	0.4	0.59	0.5[b]	0.39
GTP	9.79, 9.5[b]	9.24,[e] 9.31[d]	0.4	0.63	0.6[b]	0.59
UTP	9.70, 9.6[b]	9.20[e]	0.5	0.46	0.5[b]	0.57
TTP	9.89, 10.1[b]	9.65,[e,f] 9.55[g]	0.4	0.47	0.7[b]	0.83

[a] Average difference between the values of $pK_{A,N(A)}$ for the nucleotides and nucleosides. [b] From ref 17 (cf. Table III). [c] From ref 29b. [d] A. Albert, *Biochem. J.*, 54, 646 (1953). [e] Values from Fiskin and Beer.[25] [f] Deoxyribonucleoside. [g] D. W. Gruenwedel and N. Davidson, *J. Mol. Biol.*, 21, 129 (1966).

Of course, the above conclusion does not preclude any Mg^{2+}- or Mn^{2+}-base interactions. In $Mn(ATP)^{2-}$ the ring-bound form exists to approximately 20%,[11] and the acidifying qualities of Mg^{2+} and Mn^{2+} are known to be quite generally rather low, compared to those of Ni^{2+}, Cu^{2+}, and Zn^{2+}; in accord herewith, their tendency to form hydroxo complexes is small (cf., e.g., Table II). This means, large values for ΔpK_A could neither be expected for Mg^{2+} nor for Mn^{2+} nucleotides. But in analogy to $Mn(ATP)^{2-}$, one may expect that some interaction occurs also in $M(NTP\text{-}1H)^{5-}$, where the base moiety carries a negative charge.

Mixed-Ligand Complexes. Some time ago ^1H NMR measurements for $Cu(Bipy)(ATP)^{2-}$ showed that a base moiety may be prevented from a direct interaction with the metal ion by the formation of a mixed-ligand complex containing 2,2'-bipyridyl.[29c] More recently, it was found that 2,2'-bipyridyl and the purine moiety form charge-transfer adducts; this holds also for metal ion containing ternary systems; $Cu(Bipy)(ATP)^{2-}$, $Cu(Bipy)(ITP)^{2-}$,[8,29b] and $Cu(Bipy)(GTP)^{2-}$ (cf. ref 29a) exist in a folded form that allows a charge-transfer interaction between the pyridyl and purine moieties.

The results on the acidity of the proton at N(A) in the mixed-ligand systems (Table V) show values for $\Delta pK_{A(Bipy)}$ that are significantly smaller than the corresponding values of ΔpK_A (Table IV). This demonstrates that the base moieties are prevented from a direct coordination at the metal ion. The values of $\Delta pK_{A(Bipy)}$ are practically independent on the metal ion and on the nucleotide, indicating that all these ternary complexes have approximately the same structure. As not only purines but also pyrimidines form

Table VI. Acidity Constants of Ligands and Equilibrium Constants for Some Binary M^{2+} NTP Systems (I = 0.1; 25°)

Equilibrium	Log K GTP	ATP	ITP	UTP	Ref
$H_2NTP^{2-} \rightleftharpoons HNTP^{3-} + H^+$	−2.8[a]	−4.06[b]	−2.1[c]		
$HNTP^{3-} \rightleftharpoons NTP^{4-} + H^+$	−6.42	−6.42	−6.42	−6.42	*d*, 29c
$NTP^{4-} \rightleftharpoons (NTP\text{-}1H)^{5-} + H^+$	−9.79		−9.26	−9.70	*e*
$Zn^{2+} + HNTP^{3-} \rightleftharpoons Zn(HNTP)^-$		2.67[b]	0.7[f]		
$Zn(HNTP)^- \rightleftharpoons Zn(NTP)^{2-} + H^+$		−3.88	−1.91		*g*
$Zn^{2+} + NTP^{4-} \rightleftharpoons Zn(NTP)^{2-}$		5.21	5.21	5.21	*d*, 29c
$Zn(NTP)(H_2O)^{2-} \rightleftharpoons Zn(NTP)(OH)^{3-} + H^+$		−8.87			*e*
$Zn(NTP)^{2-} \rightleftharpoons Zn(NTP\text{-}1H)^{3-} + H^+$			−8.31	−8.71	*e*
$Zn(NTP\text{-}1H)(H_2O)^{3-} \rightleftharpoons Zn(NTP\text{-}1H)(OH)^{4-} + H^+$			−9.4	−9.24	*e*
		Mn^{2+}	Ni^{2+}	Zn^{2+}	
$M^{2+} + HGTP^{3-} \rightleftharpoons M(HGTP)^-$		1.1	1.4	1.4	*f*
$M(HGTP)^- \rightleftharpoons M(GTP)^{2-} + H^+$		−2.74	−2.97	−2.61	*g*
$M^{2+} + GTP^{4-} \rightleftharpoons M(GTP)^{2-}$		4.78[b]	4.85	5.21	*d*, 29c
$M(GTP)^{2-} \rightleftharpoons M(GTP\text{-}1H)^{3-} + H^+$		−9.36	−8.64	−8.39	*e*
$M(GTP\text{-}1H)(H_2O)^{3-} \rightleftharpoons M(GTP\text{-}1H)(OH)^{4-} + H^+$		−11.3	−10.57	−9.48	*e*

[a] $I \sim 0.3$; 37°.[29a] [b] M. M. Taqui Khan and A. E. Martell, *J. Am. Chem. Soc.*, **88**, 668 (1966). [c] $I \sim 0.2$; 37°.[29b] [d] Value of the corresponding ATP system;[29c] this is justified because the acidity [cf. R. Phillips, P. Eisenberg, P. George, and R. J. Rutman, *J. Biol. Chem.*, 240, 4393 (1965)] and stability constants concerning the phosphates are rather independent of the base moiety.[2,3,12-14] [e] This work. [f] These values are estimated: e.g., $pK^H_{H_2ITP} - pK^H_{H_2ATP} = 2.1 - 4.06 = -2.0$; hence, $\log K^{Zn}_{Zn(HITP)} = \log K^{Zn}_{Zn(HATP)} - 2.0 = 2.67 - 2.0 = 0.7$. [g] Calculated from other data listed in this table.

charge-transfer adducts,[30] one must assume that all these complexes exist in a folded form and may be considered as metal ion bridged charge-transfer complexes. The distance between the aromatic planes in the mentioned examples[29b] has been estimated to be 3.5 ± 0.4 Å and the distance between the metal ion and H(2) as approximately 6.5 Å. Hence, the distance between the proton at N(1) and the metal ion will be in the order of 6 Å. This agrees with

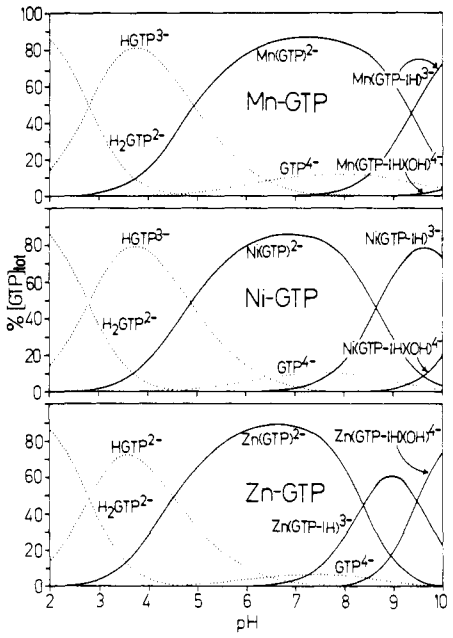


Figure 2. Influence of pH on the concentrations of several species present in an aqueous solution (I = 0.1; 25°) of M^{2+} and NTP, given as the percentage of the total NTP or M^{2+} present; computed with the constants of Table VI for concentrations of 10^{-3} M for each reactant. Complexes with a partially protonated phosphate chain were not taken into account in the calculations because the corresponding constants are not known. However, if such species occur they would exist only below pH 3. Upper part: Mn^{2+} and GTP [concentrations of $Mn(HGTP)^-$ and $(GTP\text{-}1H)^{5-}$ are ≤0.9 and ≤5.2%, respectively]. Middle part: Ni^{2+} and GTP [concentrations of $Ni(HGTP)^-$ and $(GTP\text{-}1H)^{5-}$ are ≤1.8 and ≤2.1%]. Lower part: Zn^{2+} and GTP [concentrations of $Zn(HGTP)^-$ and $(GTP\text{-}1H)^{5-}$ are ≤1.6 and ≤0.6%].

$\Delta pK_{A(Bipy)} \sim 0.6$ (and $\Delta pK_{A/Mg,Mn} \sim 0.2$–0.3) and the difference between the acidity constants of the nucleotides and nucleosides of ~0.4 log units (Table V), i.e., the observation of a small but significant increase in acidity caused by the metal ion in these ternary complexes. Similarly, in Cu^{2+} adenosine 5′-monophosphate $N(1)$-oxide, in which the metal ion is bound to the deprotonated *o*-amino *N*-oxide group, the pK_A value of the phosphate group is about 0.6 log units lower than the one of the free ligand.[31]

Hydroxo Complexes. The tendency toward the formation of the hydroxo complexes, $M(NTP\text{-}1H)(OH)^{4-}$, depends strongly on the kind of metal ion: $Mn^{2+} < Ni^{2+} < Zn^{2+} < Cu^{2+}$ (cf. Table II). However, the formation of the hydroxo complexes depends also on the kind of nucleotide involved. For a given metal ion the tendency decreases within the series, $CTP^{4-} \sim ATP^{4-} > (UTP\text{-}1H)^{5-} \sim (TTP\text{-}1H)^{5-} > (ITP\text{-}1H)^{5-} \sim (GTP\text{-}1H)^{5-}$. This must mean that within the latter series the extent of the metal ion–base moiety interaction increases, because the tendency for hydrolysis of a given metal ion depends on the degree of saturation of its coordination sphere.

One further point should be considered. A comparison of the constants due to $M(ATP)(OH)^{3-}$ and $M(CTP)(OH)^{3-}$ of Table II with those of Tables I and III for the species $M(NTP\text{-}1H)^{3-}$ reveals that in some cases the formation of these complexes occurs in similar ranges of pH. Hence, it appears possible that small amounts of $M(NTP)(OH)^{3-}$, where NTP = ITP^{4-}, GTP^{4-}, UTP^{4-}, or TTP^{4-}, are formed along with the isocharged species $M(NTP\text{-}1H)^{3-}$ and that with increasing pH the former species changes completely into $M(NTP\text{-}1H)^{3-}$. Small amounts of $M(NTP)(OH)^{3-}$ cannot be expected to be detected either by potentiometric or by uv spectrophotometric measurements, but from dephosphorylation experiments[16] some evidence was obtained for the existence of $Cu(GTP)(OH)^{3-}$ in small concentrations.

General Conclusions

Though the detailed structure of the species $M(NTP)^{2-}$ and $M(NTP\text{-}1H)^{3-}$ remains in part unknown, the constants determined in this work may be used, together with data from the literature (cf. Table VI), to calculate the pH distribution of the several complexes. The results of Figure 2

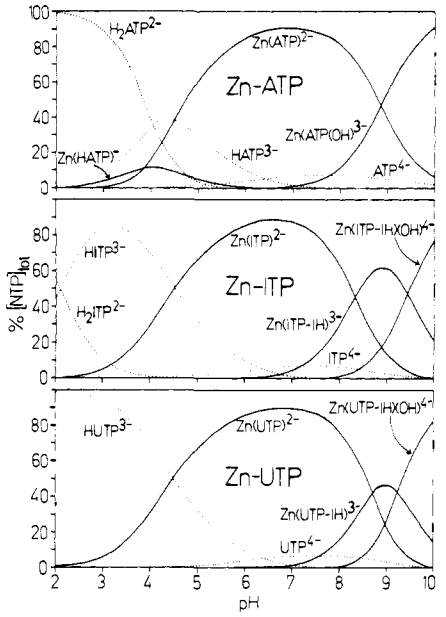


Figure 3. Same as Figure 2. Upper part: Zn^{2+} and ATP. Middle part: Zn^{2+} and ITP [concentrations of $Zn(HITP)^-$ and $(ITP\text{-}1H)^{5-}$ are <0.4 and <1.4%]. Lower part: Zn^{2+} and UTP [concentration of $(UTP\text{-}1H)^{5-}$ is <0.9%].

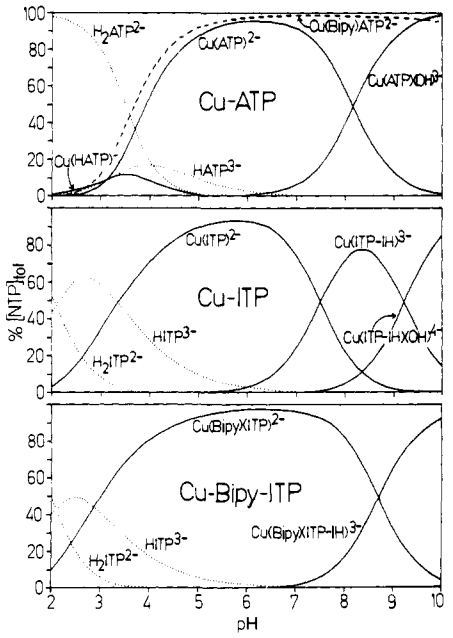


Figure 4. Same as Figure 2 (computed with the constants listed in ref 15 and 16). Upper part: Cu^{2+} and ATP; broken line, ternary system with 2,2′-bipyridyl [concentrations in the ternary system of $Cu(HATP)^-$, $Cu(ATP)^{2-}$, $Cu(ATP)(OH)^{3-}$, and $Cu(Bipy)(HATP)^-$ are <0.4, ≤0.9, ≤2.4, and ≤16.7%, respectively]. Middle part: Cu^{2+} and ITP [concentrations of $Cu(HITP)^-$, ITP^{4-}, and $(ITP\text{-}1H)^{5-}$ are <0.6, <1.6, and <0.2%, respectively]. Lower part: ternary system, Cu^{2+}, 2,2′-bipyridyl, and ITP [concentrations of $Cu(HITP)^-$, $Cu(ITP)^{2-}$, $Cu(ITP\text{-}1H)^{3-}$, $Cu(ITP\text{-}1H)(OH)^{4-}$, $Cu(Bipy)(HITP)^-$, ITP^{4-}, and $(ITP\text{-}1H)^{5-}$ are ≤0.03, ≤1.0, <0.8, <2.2, ≤1.3, ≤1.2, and ≤1.3%, respectively].

show that the concentration of the several species with a given nucleotide is rather independent on the kind of metal ion, in accord with the general trend in stability of $M(NTP)^{2-}$,[12] except the complexes $M(NTP\text{-}1H)^{3-}$ and $M(NTP\text{-}1H)(OH)^{4-}$ differ significantly in the pH of their formation as well as in their concentration. Figure 3 presents the influence of a given metal ion on complex formation with different nucleotides, and Figure 4 compares binary and ternary systems. Further comparisons between the several parts of Figures 2-4 show that the ITP and GTP systems behave rather similarly; the same is true for the UTP and TTP, or ATP and CTP systems. For the Mg^{2+} systems a similar distribution may be anticipated as for the Mn^{2+} systems.

In all the M^{2+} nucleotide systems in the physiological pH range from 7 to 8 the complex $M(NTP)^{2-}$ dominates, but also in all the systems (where structurally possible) at least some of the base deprotonated species, $M(NTP\text{-}1H)^{3-}$, is present. In contrast, the hydroxylated species, $M(NTP\text{-}1H)(OH)^{4-}$, occurs only in minor concentrations even with Cu^{2+} and Zn^{2+}.

Acknowledgments. The potentiometric titrations and the spectrophotometric measurements, as well as their evaluations, were performed with the skillful technical assistance of Miss R. Baumbusch and Miss S. Strub. The computer was made available by the Rechenzentrum der Universität Basel. These supports as well as the one by a research grant from the Schweizerischen Nationalfonds zur Förderung der wissenschaftlichen Forschung are gratefully acknowledged.

References and Notes

(1) M. Dixon and E. Webb, *Enzymes*, 1, 1 (1964).
(2) G. L. Eichhorn in "Inorganic Biochemistry", Vol. 2, G. L. Eichhorn, Ed., Elsevier, Amsterdam, London, New York, 1973, p 1191.
(3) (a) R. M. Izatt, J. J. Christensen, and J. H. Rytting, *Chem. Rev.*, **71**, 439 (1971); (b) R. Phillips, *ibid.*, **66**, 501 (1966).
(4) A. T. Tu and M. J. Heller in "Metal Ions in Biological Systems", Vol. 1, H. Sigel, Ed., Marcel Dekker, New York, N.Y., 1974, p 1.
(5) C. Miller Frey and J. Stuehr, ref 4, p 51.
(6) H. Sigel, ref 4, Vol. 2, 1973, p 63.
(7) H. Sigel, *Angew. Chem.*, in press.
(8) C. F. Naumann and H. Sigel, *J. Am. Chem. Soc.*, **96**, 2750 (1974).
(9) G. P. P. Kuntz, Th. A. Glassman, C. Cooper, and T. J. Swift, *Biochemistry*, **10**, 843 (1971); **11**, 538 (1972).
(10) T. A. Glassmann, J. Suchy, and C. Cooper, *Biochemistry*, **12**, 2430 (1973).
(11) Y.-F. Lam, G. P. P. Kuntz, and G. Kotowycz, *J. Am. Chem. Soc.*, **96**, 1834 (1974).
(12) H. Sigel and D. B. McCormick, *Acc. Chem. Res.*, **3**, 201 (1970).
(13) P. W. Schneider, H. Brintzinger, and H. Erlenmeyer, *Helv. Chim. Acta*, **47**, 992 (1964).
(14) E. Walaas, *Acta Chem. Scand.*, **12**, 528 (1958).
(15) D. H. Buisson and H. Sigel, *Biochim. Biophys. Acta*, **343**, 45 (1974).
(16) H. Sigel, D. H. Buisson, and B. Prijs, *Bioinorgan. Chem.*, in press.
(17) H. Sigel, *Eur. J. Biochem.*, **3**, 530 (1968).
(18) M. J. Clarke and H. Taube, *J. Am. Chem. Soc.*, **96**, 5413 (1974). I am grateful to Dr. Clarke and Professor Taube for a preprint.
(19) Abbreviations: Bipy, 2,2′-bipyridyl; M = general bivalent metal ion; NTP = nucleoside 5′-triphosphate, i.e., ATP, ITP, GTP, CTP, UTP, or TTP.
(20) H. Sigel, *Helv. Chim. Acta*, **48**, 1513 (1965).
(21) H. Brintzinger, *Helv. Chim. Acta*, **44**, 1199 (1961).
(22) H. A. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc.*, **71**, 2703 (1949).
(23) The constants for the 2,2′-bipyridyl-Mn^{2+} nucleotide complexes could not be determined due to the low stability of $Mn(Bipy)^{2+}$.
(24) J. C. Sari and J. P. Belaich, *J. Am. Chem. Soc.*, **95**, 7491 (1973).
(25) A. M. Fiskin and M. Beer, *Biochemistry*, **4**, 1289 (1965).
(26) H. Sternlicht, D. E. Jones, and K. Kustin, *J. Am. Chem. Soc.*, **90**, 7110 (1968).
(27) (a) M. S. Zetter, H. W. Dodgen, and J. P. Hunt, *Biochemistry*, **12**, 778 (1973); (b) C. M. Frey and J. E. Stuehr, *J. Am. Chem. Soc.*, **94**, 8898 (1972); (c) C. M. Frey, J. L. Banyasz, and J. E. Stuehr, *ibid.*, **94**, 9198 (1972).
(28) This is in accord with the values of ΔpK_A observed for Cu^{2+} NTP systems in different concentrations.[17]
(29) (a) H. Sigel, C. F. Naumann, and B. Prijs, *Eur. J. Biochem.*, **46**, 589 (1974); (b) C. F. Naumann, B. Prijs, and H. Sigel, *ibid.*, **41**, 209 (1974); (c) H. Sigel, K. Becker, and D. B. McCormick, *Biochim. Biophys. Acta*, **148**, 655 (1967).
(30) A. Fulton and L. E. Lyons, *Aust. J. Chem.*, **21**, 419 (1968); T. Montenay-Garestier and C. Héiène, *Biochemistry*, **10**, 300 (1971).
(31) H. Sigel and B. Prijs, *Helv. Chim. Acta*, **50**, 2357 (1967); H. Sigel and H. Brintzinger, *ibid.*, **47**, 1701 (1964).