Ternary Complexes in Solution[1] as Models for Enzyme-Metal Ion-Substrate Complexes. Comparison of the Coordination Tendency of Imidazole and Ammonia toward the Binary Complexes of Mn(II), Co(II), Ni(II), Cu(II), Zn(II), or Cd(II) and Uridine 5′-Triphosphate or Adenosine 5′-Triphosphate

Nityananda Saha[2] and Helmut Sigel*

Contribution from the Institute of Inorganic Chemistry, University of Basel, CH-4056 Basel, Switzerland. *Received October 29, 1981*

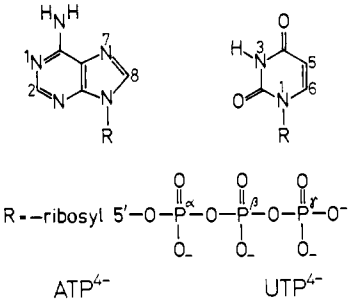
Abstract: For an improved understanding of the driving forces leading to mixed-ligand complexes in biological systems, ternary complexes of the type $M(NTP)(L)^{2-}$, where M^{2+} = Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, or Cd^{2+}, NTP^{4-} = ATP^{4-} or UTP^{4-}, and L = imidazole (Im) or ammonia, have been studied by potentiometric pH titrations (I = 0.1 M, $NaNO_3$; 25 °C). Imidazole and ammonia are the simplest models for the corresponding ligating groups occurring in proteins; hence, together with the mentioned nucleoside 5′-triphosphates one may mimic enzyme-metal ion-substrate complexes. Their stability was quantified by $\Delta(\log K_M) = \log K^{M(NTP)}_{M(NTP)(L)} - \log K^{M}_{M(L)}$ to learn more about the factors that govern the selectivity in the formation of such complexes; this difference is the equilibrium constant for $M(NTP)^{2-} + M(L)^{2+} \rightleftharpoons M(NTP)(L)^{2-} + M^{2+}$. It becomes thus evident that the mixed-ligand complexes containing UTP are about 0.1-0.3 log unit more stable than those with ATP. This result confirms recent conclusions on the participation of the adenine moiety (at N-7, aside from the coordination to the phosphate residue) in the formation of the binary $M(ATP)^{2-}$ complexes; rough estimations on the extent of the resulting macrochelate formation in the $M(ATP)^{2-}$ complexes could also be made. Both binary complexes, $M(UTP)^{2-}$ and $M(ATP)^{2-}$, discriminate between imidazole and ammonia; i.e., the ternary imidazole complexes show an enhanced stability. Furthermore, by calculating *apparent* stability constants for pH 7, it becomes clear that in the physiological pH range only the imidazole groups are easily accessible for the formation of mixed-ligand complexes; for ammonia and amino groups the competition of the proton is rather large. Indeed, only the ternary $M(NTP)(Im)^{2-}$ complexes are formed in significant concentrations at pH 7, as is demonstrated for some of the systems by plotting the distribution of the complex species in dependence on pH.

Metal ion complex formations are among the prominent interactions in nature,[3-5] and the imidazole residue is an important and versatile binding site in biological systems,[6-8] while nucleoside 5′-triphosphates (NTP)[9] are equally important as substrates for many enzymic reactions.[10-13] It is therefore not surprising that already several years ago, e.g., for pyruvate kinase, an intermediate ternary metal ion complex that involves an imidazole residue of the protein and the phosphate moiety of ATP[9] has been suggested.[14]

Aside from some few favorable cases where both ligands coordinate rather strongly to the metal ion,[15,16] solution studies of mixed-ligand complexes containing nucleoside 5′-triphosphates have so far been strongly hampered for the following reasons: (i) metal ions facilitate the dephosphorylation of nucleoside 5′-triphosphates,[17-19] (ii) there are difficulties already in obtaining reliable stability constants for the binary NTP complexes,[16] and (iii) the tendency of nucleoside 5′-triphosphates to self-stack, a reaction that is also metal ion promoted,[20] complicates the situation even more.

After getting familiar with these difficulties in a systematic way,[16-20] we learned also to handle them by restricting the time of the potentiometric titrations to minimize the dephosphorylation reactions and by working at such concentrations where the monomeric NTP complexes dominate strongly. These latter conditions also *disfavor* the dephosphorylation because this reaction proceeds, at least with purine 5′-triphosphates,[18] via dimers; in the case of pyrimidine 5′-triphosphates, monomeric metal ion

Chart I



(1) This is part 41 of the series "Ternary Complexes in Solution". (a) Part 40: Saha, A.; Sigel, H. *Inorg. Chim. Acta* **1982**, *66*, L29-L30. (b) Part 39: Gampp, H.; Sigel, H.; Zuberbühler, A. D. *Inorg. Chem.* **1982**, *21*, 1190-1195.

(2) Done during a leave of absence from the Department of Chemistry of the University of Calcutta to the University of Basel.

(3) Eichhorn, G. L., Ed. "Inorganic Biochemistry"; Elsevier: New York, 1973; Vol. 1 and 2.

(4) Sigel, H., Ed. "Metal Ions in Biological Systems"; Marcel Dekker: New York, 1973-1982; Vol. 1-14.

(5) Wood, J. M. *Naturwissenschaften* **1975**, *62*, 357-364.

(6) Sundberg, R. J.; Martin, R. B. *Chem. Rev.* **1974**, *74*, 471-517.

(7) Sigel, H.; Fischer, B. E.; Prijs, B. *J. Am. Chem. Soc.* **1977**, *99*, 4489-4496.

(8) Sigel, H. *Inorg. Chem.* **1980**, *19*, 1411-1413.

(9) Abbreviations: ATP, adenosine 5′-triphosphate; Im, imidazole; L, general (monodentate) ligand; M, general metal ion; Nta, nitrilotriacetate; NTP, nucleoside 5′-triphosphate; UTP, uridine 5′-triphosphate.

(10) Spiro, T. G. "Phosphate Transfer and Its Activation by Metal Ions; Alkaline Phosphatase"; Chapter 17 of ref 3.

(11) Cooperman, B. S. *Met. Ions Biol. Syst.* **1976**, *5*, 79-125. Cf. ref 4.

(12) Mildvan, A. S. *Adv. Enzymol. Relat. Areas* Mol. *Biol.* **1979**, *49*, 103-26.

(13) Sigel, H., Ed. "Nucleotides and Derivatives: Their Ligating Ambivalency"; 1979; Vol. 8 of ref 4.

(14) Mildvan, A. S.; Cohn, M. *J. Biol. Chem.* **1966**, *241*, 1178-1193.

(15) Sigel, H.; Naumann, C. F. *J. Am. Chem. Soc.* **1976**, *98*, 730-739.

(16) Sigel, H. *J. Inorg. Nucl. Chem.* **1977**, *39*, 1903-1911.

(17) Amsler, P. E.; Sigel, H. *Eur. J. Biochem.* **1976**, *63*, 569-581.

(18) Sigel, H.; Amsler, P. E. *J. Am. Chem. Soc.* **1976**, *98*, 7390-7400.

(19) Sigel, H.; Hofstetter, F., submitted for publication.

(20) Scheller, K. H.; Hofstetter, F.; Mitchell, P. R.; Prijs, B.; Sigel. H. *J. Am. Chem. Soc.* **1981**, *103*, 247-260.

0002-7863/82/1504-4100$01.25/0

Table I. Logarithms of the Stability Constants of Several Binary $M(L)^{2+}$ and Ternary $M(NTP)(L)^{2-}$ Complexes (I = 0.1 M, $NaNO_3$; 25 °C)[a]

ligand (L)	M^{2+}	log $K^{M}_{M(L)}$	log $K^{M(UTP)}_{M(UTP)(L)}$	log $K^{M(ATP)}_{M(ATP)(L)}$
imidazole[b]	Mn^{2+}	1.25 ± 0.03	1.27 ± 0.09	1.05 ± 0.10
	Co^{2+}	2.40 ± 0.02	2.04 ± 0.03	1.85 ± 0.06
	Ni^{2+}	3.03 ± 0.01	2.63 ± 0.03	2.44 ± 0.02
	Cu^{2+}	4.21 ± 0.01	3.84 ± 0.02	3.53 ± 0.02
	Zn^{2+}	2.51 ± 0.01	2.54 ± 0.02	2.41 ± 0.02
	Cd^{2+}	2.71 ± 0.01	2.33 ± 0.03	2.03 ± 0.04
NH_3[c]	Mn^{2+}	1.27 ± 0.10	[e]	1.01 ± 0.15
	Co^{2+}	2.08 ± 0.03 (6)[d]	≤1.9	≤1.9
	Ni^{2+}	2.74 ± 0.06 (9)[d]	≤2.5	≤2.3
	Cu^{2+}	4.18 ± 0.03 (17)[d]	3.60 ± 0.12	3.4 ± 0.2
	Zn^{2+}	2.41 ± 0.09 (3)[d]	≤2.3	≤2.3
	Cd^{2+}	2.67 ± 0.03 (9)[d]	≤2.3	≤2.2[f]

[a] The errors given are 3 times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. [b] $pK^{H}_{H(Im)}$ = 7.04 ± 0.01. [c] $pK^{H}_{H(NH_3)}$ = 9.38 ± 0.01. [d] Average of the constants listed in ref 23 and 24 for I = 0–2 M and 20–30 °C; a view on the constants given in the literature shows that the influence of ionic strength and temperature is small. The range of error given is the standard error of the mean value resulting from averaging the values of the literature; the number in parentheses gives the number of available constants. [e] No (limiting) value could be determined for this system because $pK^{H}_{Mn(UTP)}$ = 9.45,[26] i.e., the formation of Mn(UTP)-$(NH_3)^{2-}$ through deprotonation of $H(NH_3)^{+}$ and the deprotonation at N-3 in $Mn(UTP)^{2-}$ occur in the same pH range. In the Mn^{2+}/ATP/NH_3 system, a determination was possible because $pK^{H}_{Mn(ATP)(H_2O)}$ = 10.7.[26] [f] Estimation: log $K^{Cd(ATP)}_{Cd(ATP)(NH_3)} \simeq 2.0 \pm 0.2$.

complexes are the intermediates.[18,19]

The most simple ligand that allows us to elucidate the binding properties of the imidazole residue in mixed-ligand complexes is imidazole (Im) itself. Ammonia was also included in the present study for comparison and to mimic mixed-ligand complexes containing amino residues. We have therefore measured the stability of the ternary complexes formed by Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, or Cd^{2+}, the two mentioned monodentate ligands (L), and uridine 5′-triphosphate (UTP) or adenosine 5′-triphosphate (ATP) (Chart I). Such ternary complexes, like, e.g., Zn-$(ATP)(Im)^{2-}$, are obviously simple models for natural enzyme–M^{2+}–nucleoside 5′-triphosphate complexes.

However, despite this apparent simplicity, discriminating properties become evident: the ternary complexes with imidazole are more stable than those with ammonia, and in certain especially favored cases equilibrium 1 is even slightly displaced to the right

$$M(NTP)^{2-} + M(L)^{2+} \rightleftharpoons M(NTP)(L)^{2-} + M^{2+} \quad (1)$$

side. Selectivity is further observed due to the different properties of UTP^{4-} and ATP^{4-}; the formation of the ternary ATP complexes is in general somewhat inhibited, compared with the formation of the ternary $M(UTP)(L)^{2-}$ complexes. This is because with several metal ions a significant percentage of $M(ATP)^{2-}$ exists as a macrochelate; i.e., the metal ion is coordinated not only to the triphosphate residue but also to N-7 of the purine moiety,[20] and hence, the second ligand has less access to the coordination sphere of the metal ion.

Experimental Section

Materials. The nitrate salts of the metal ions, the disodium salt of EDTA, nitric acid, NaOH (Titrisol), imidazole, and NH_4NO_3 (all these reagents were of pa grade) were from Merck AG, Darmstadt. The titer of the NaOH used for the titrations was determined with potassium hydrogen phthalate (Merck AG); the exact concentrations of the stock solutions of imidazole (titrated in the presence of an excess of HNO_3) and NH_4NO_3 were measured by titrations with NaOH. The concentrations of the metal ion stock solutions were determined with EDTA. The disodium salt of ATP ($C_{10}H_{14}N_5O_{13}P_3{\cdot}Na_2{\cdot}3H_2O$ krist reinst) and the trisodium salt of UTP ($C_9H_{12}N_2O_{15}P_3{\cdot}Na_3{\cdot}2H_2O$ reinst; min 98%) were purchased from Serva Feinbiochemica GmbH, Heidelberg. The amount of free phosphate initially present was 2% for ATP and 4% for UTP (determined as described in ref 21). To account for this and to be able to prepare metal ion–NTP solutions of exactly a 1:1 ratio, we also determined by potentiometric pH titrations the molecular weight of the purchased triphosphates. For ATP we measured 602 ± 2, which is in excellent agreement with 605.2, the value given by the company for the above formula; for UTP we determined 568 ± 2, which is also in fair agreement with 586.2 given by the company for the above formula (the difference corresponds to the molecular weight of a H_2O molecule).

Potentiometric Measurements. The pH titrations were carried out with a Metrohm potentiograph E336 and a Metrohm macro EA 121 glass electrode. The buffers (pH 4.64, 7.00, and 9.00) used for calibrating the potentiograph were from Metrohm AG, Herisau, Switzerland. The direct pH-meter readings were used in the calculations for the acidity constants.

The acidity constant $K^{H}_{H(L)}$ of $H(imidazole)^{+}$ was determined by titrating 50 mL of aqueous 0.85 mM HNO_3 and $NaNO_3$ (I = 0.1 M; 25 °C) in the presence and absence of 0.7 mM imidazole under N_2 with 1 mL of 0.05 M NaOH. The acidity constant of NH_4^{+} (0.7 mM) was determined in exactly the same way, but the aqueous HNO_3 was only 0.15 mM. $K^{H}_{H(L)}$ was calculated within the range between 3% and 97% neutralization, where possible.

The conditions for the determination of the stability constants $K^{M}_{M(Im)}$ of the binary imidazole complexes (I = 0.1 M; 25 °C) were the same as for the acidity constant, but (a part of) $NaNO_3$ was replaced by $M(NO_3)_2$ with ratios of M^{2+}:Im between 14:1 and 50:1; with Cu^{2+} also ratios as low as 7:1 have been used. $K^{Mn}_{Mn(NH_3)}$ was determined correspondingly with a Mn^{2+}:NH_3 ratio of 50:1. Under these conditions practically only the 1:1 complexes form; i.e., the concentration of the species $M(L)_m^{2+}$ with $m \geq 2$ can be neglected. Hence, the stability constants $K^{M}_{M(L)}$ were computed by taking into account the species H^{+}, $H(L)^{+}$, L, M^{2+}, and $M(L)^{2+}$;[22] indeed, the individually calculated constants showed no dependence on pH or on the excess of M^{2+}. Data were usually collected from 10% complex formation to the beginning of hydrolysis of M^{2+}_{aq}, which was evident from the titrations without imidazole. The constants for the binary ammonia complexes of the other metal ions were taken from the literature (see Table I).[23-25]

The stability constants $K^{M(NTP)}_{M(NTP)(L)}$ of the ternary complexes were determined from solutions as described above, but with $[L]_{tot}$ = 0.7 mM and $[M^{2+}]_{tot} = [NTP]_{tot}$ = 2.0 mM; with Cu^{2+} also $[Cu^{2+}]_{tot} = [NTP]_{tot}$ = 1.0 mM was used. As the coordination tendency between L and $Mn(NTP)^{2-}$ is relatively small, in these experiments $[Mn]_{tot} = [NTP]_{tot}$ = 10.0 mM had to be used to obtain a significant depression of the buffer region of $H(L)^{+}$/L. For prevention of dephosphorylation[17-19] as far as possible, the nucleotide (NTP^{4-})[26] was added as the last reagent to a reaction solution, and the automatic titration was completed within 10–15 min, depending on the system.

The stability of the binary $M(NTP)^{2-}$ complexes is high;[16] therefore to a first approximation complete complex formation can be assumed.[16,26] This means $[M(NTP)^{2-}]$ is close to 2×10^{-3} M under the given experimental conditions for M^{2+} = Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, or Cd^{2+}, in addition to $[Cu(NTP)^{2-}] \simeq 10^{-3}$ M. Under these conditions the metal ion facilitated self-association of the nucleoside 5′-triphosphates is small and about 95% or more of $M(NTP)^{2-}$ is present in the monomeric form.[20]

(21) Buisson, D. H.; Sigel, H. *Biochim. Biophys. Acta* **1974**, *343*, 45–63.
(22) Griesser, R.; Sigel, H. *Inorg. Chem.* **1970**, *9*, 1238–1243.
(23) Sillén, L. G.; Martell, A. E. "Stability Constants of Metal-Ion Complexes"; The Chemical Society, London (a) Special Publication, 1964; No. 17. (b) *Ibid.*, Supplement 1, 1971; No. 25.
(24) Smith, R. M.; Martell, A. E. "Critical Stability Constants"; Plenum Press: New York and London, 1976; Vol. 4.
(25) Banerjea, D.; Kaden, T. A.; Sigel, H. *Inorg. Chem.* **1981**, *20*, 2586–2590.
(26) Sigel, H. *J. Am. Chem. Soc.* **1975**, *97*, 3209–3214.

With the assumption that the self-stacking tendency of ATP^{4-} is promoted by Mn^{2+} similarly as by Mg^{2+} (cf. ref 20), in the 10^{-2} M solutions about 93% of $Mn(ATP)^{2-}$ will be present as monomers; with UTP^{4-} and its complexes there is no such problem under the given conditions, as the self-stacking tendency is much smaller.[20]

Data for the stability constants $K^{M(NTP)}{}_{M(NTP)(L)}$ were usually collected from a 5% (or 10%) formation degree on, but more important, they were collected only in this pH range where about 90% (or more) of $M(NTP)^{2-}$ are formed[16,26] and where the formation of the base-ionized $M(UTP-H)^{3-}$ and $M(UTP-H)(OH)^{4-}$ does not yet occur.[26,27] Hence, the constants $K^{M(NTP)}{}_{M(NTP)(L)}$ were computed by taking into account the species H^+, $H(L)^+$, L, $M(NTP)^{2-}$, and $M(NTP)(L)^{2-}$ and by evaluating the difference in NaOH consumption between two solutions, i.e., one with and one without L. This procedure (which equals procedure II of ref 15) is superior to the initially more attractive method (procedure I of ref 15) where log $\beta^{M}{}_{M(NTP)(L)}$ is calculated by taking into account all 10 or 12 possible species. As demonstrated and discussed in ref 15, this is due to the fact that in procedure II (which leads also to a lower standard deviation) most systematic errors connected with the binary M^{2+}/NTP system cancel; indeed, the constants determined in this study again showed no systematic variation with pH.

All the equilibrium constants listed were calculated from at least four independent pairs of titration curves.

Results and Discussion

The stability constants of the ternary metal ion complexes containing ATP^{4-} or UTP^{4-} (NTP^{4-}) and one of the monodentate ligands (L), imidazole (Im), or ammonia (NH_3) were calculated for eq 2 from the data obtained from potentiometric pH titrations

$$N(NTP)^{2-} + L \rightleftharpoons M(NTP)(L)^{2-} \quad (2)$$

$$K^{M(NTP)}{}_{M(NTP)(L)} = [M(NTP)(L)]/([M(NTP)][L])$$

(I = 0.1 M, $NaNO_3$; 25 °C). Similarly, the constants of the binary complexes (eq 3) were also determined or taken from the literature.[23-25]

$$M^{2+} + L \rightleftharpoons M(L)^{2+} \quad (3)$$

$$K^{M}{}_{M(L)} = [M(L)]/([M][L])$$

The results are listed in Table I. The acidity constants of $H(Im)^+$ and $H(NH_3)^+$ and the stability constants of the binary imidazole complexes are in excellent agreement with our previous determinations and the average values obtained from the constants given in the literature (see Table I of ref 25). From the mixed-ligand systems only the stability constant, log $K^{Cu(ATP)}{}_{Cu(ATP)(Im)}$ = 3.48 ± 0.09. has been determined previously;[21] this result agrees also favorably with the present value of 3.53 ± 0.02 log units.[28] In many of the ammonia systems only the upper limit of the stability constants of the ternary complexes could be determined due to the formation of $M(ATP)(OH)^{3-}$ and $M(UTP-H)^{3-}$.[26,27]

1. Comparison of the Stability of the Ternary UTP and ATP Complexes. In Figure 1 a representative set of experimental data obtained from the potentiometric pH titrations is shown. The pH of the reaction solution is plotted against the neutralization degree for the $H(Im)^+$ system in the presence and absence of Zn^{2+}/NTP or Cu^{2+}/NTP. As the formation of ternary complexes shifts the buffer region of $H(Im)^+$/Im to lower pH values, it is immediately obvious from Figure 1, i.e., without any mathematical evaluation of the data, that the Cu^{2+} complexes are more stable than the Zn^{2+} complexes. This is interesting because Cu^{2+} has commonly a smaller coordination number (usually 4) than Zn^{2+} (usually 6);[23,24] hence the nucleotides are expected to saturate the coordination sphere of Cu^{2+} to a larger extent (see also section 2), leaving thus fewer binding sites for the coordination of the second ligand.

However, more important is the observation (Figure 1) that the deprotonation of $H(Im)^+$ occurs with Cu^{2+} or Zn^{2+}/UTP at a lower pH than with Cu^{2+} or Zn^{2+}/ATP; hence, the ternary

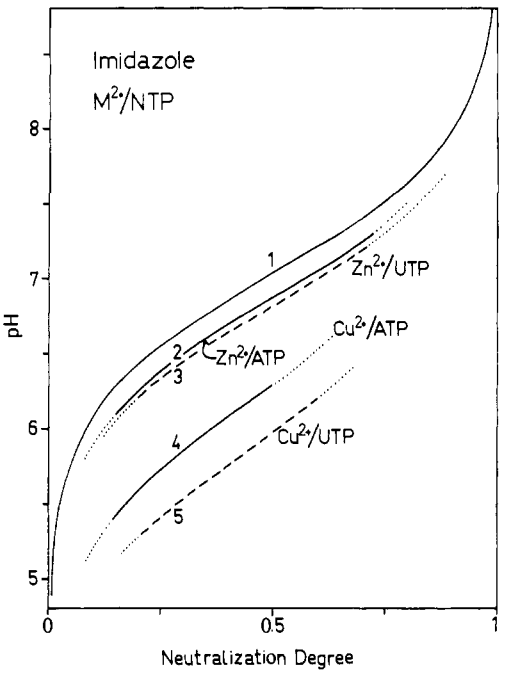
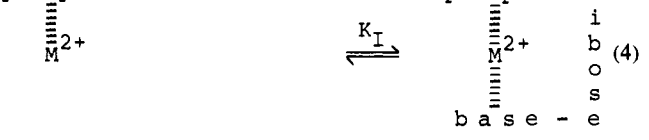


Figure 1. Dependence of neutralization degree upon pH during potentiometric pH titration of $H(imidazole)^+$ without (1) and with M^{2+}/ATP (2, 4) or M^{2+}/UTP (broken curves: 3, 5) in aqueous solution (I = 0.1 M, $NaNO_3$; 25 °C). The dotted-line portions extended toward lower degrees of neutralization indicate uncertainty due to incomplete formation of $M(NTP)^{2-}$; the extensions toward higher degrees of neutralization indicate uncertainty due to hydrolysis or deprotonation of UTP complexes at N-3 (see Experimental Section). Concentrations of the reaction solutions are (1) $[H(Im)^+] = 7 \times 10^{-4}$ M and (2-5) $[M^{2+}] = [NTP] = 2 \times 10^{-3}$ M. The volume of the reaction solutions was 50 mL; the titration was carried out with 0.05 M NaOH.

$M(UTP)(Im)^{2-}$ complexes are more stable than the $M(ATP)(Im)^{2-}$ complexes. This conclusion is confirmed for all the metal ions studied by the stability constants listed in Table I, and it holds also, as far as this can be judged, for the corresponding ternary complexes with ammonia.

This result is a reflection of the *intra*molecular, and therefore concentration independent, equilibrium 4 between an "open"



(4)

$$K_I = \frac{[M(NTP)^{2-}{}_{cl}]}{[M(NTP)^{2-}{}_{op}]} = \frac{K^{M}{}_{M(NTP)}}{K^{M}{}_{M(NTP)_{op}}} - 1 \quad (5)$$

isomer, $M(NTP)^{2-}{}_{op}$, and a "closed" species, $M(NTP)^{2-}{}_{cl}$, occurring for binary $M(NTP)^{2-}$ complexes.[20] It has been shown recently[20,29,30] for $M(ATP)^{2-}$ complexes that the macrochelated isomer is formed by a simultaneous coordination of the metal ion to the phosphate residue and to N-7 of the base moiety (see Chart I); the extent of the formation of $M(ATP)^{2-}{}_{cl}$ depends strongly on the metal ion involved.[20] No such intramolecular macrochelate formation was observed for $M(UTP)^{2-}$ complexes.[20] Therefore, the coordination sphere of the metal ions coordinated to UTP^{4-} is somewhat less saturated than the coordination sphere of the metal ions coordinated to ATP^{4-}.

Hence, the present results are a completely *independent* confirmation of the earlier conclusion[20,29,30] that for $M(ATP)^{2-}$ complexes macrochelates and equilibrium 4 are important while

(27) Sigel, H. *Eur. J. Biochem.* **1968**, *3*, 530-537.

(28) This result is very gratifying because the former value was calculated[21] by taking into account the species H^+, $H(Im)^+$, $H_2(ATP)^{2-}$, $H(ATP)^{3-}$, ATP^{4-}, $Cu(ATP)^{2-}$, $Cu(Im)^{2+}$, $Cu(Im)_2{}^{2+}$, Cu^{2+}, and $Cu(ATP)(Im)^{2-}$. Hence, this confirms also the conclusions about the evaluation procedures I and II, which are indicated in the Experimental Section (for details, see ref 15).

(29) Mariam, Y. H.; Martin, R. B. *Inorg. Chim. Acta* **1979**, *35*, 23-28.

(30) Martin, R. B.; Mariam, Y. H. *Met. Ions Biol. Syst.* **1979**, *8*, 57-124; cf. ref 4.

Table II. Comparison of the Coordination Tendency of Imidazole or Ammonia toward $M(NTP)^{2-}$ and $M(aq)^{2+}$ according to Eq 7, Which Corresponds to Equilibrium 1 ($I = 0.1$ M, $NaNO_3$; 25 °C)[a]

	$\Delta(\log K_M)$[b]					
system	Mn^{2+}	Co^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}	Cd^{2+}
$M^{2+}/UTP^{4-}/Im$	+0.02	−0.36	−0.40	−0.37	+0.03	−0.38
$M^{2+}/UTP^{4-}/NH_3$				−0.6	≤−0.1	≤−0.4
$M^{2+}/ATP^{4-}/Im$	−0.20	−0.55	−0.59	−0.68	−0.10	−0.68
$M^{2+}/ATP^{4-}/NH_3$	−0.3			−0.8	≤−0.1	
$M^{2+}/Nta^{3-}/Im$		+0.02	+0.02	+0.25	+0.16	
$M^{2+}/Nta^{3-}/NH_3$		−0.26	−0.20	−0.39	~−0.1	

[a] The corresponding data for $M(Nta)^-$ are given for comparison and taken from Table I of ref 25. [b] Calculated from the results listed in Table I. If no value is given for $\Delta(\log K_M)$, no interpretable value may be calculated from the upper limits listed in Table I.

they play no role for $M(UTP)^{2-}$ complexes,[20] i.e., $[M(ATP)^{2-}_{cl}] \gg [M(UTP)^{2-}_{cl}]$. In other words, the differences between the stability constants, $\log K^{M(UTP)}_{M(UTP)(L)} - \log K^{M(ATP)}_{M(ATP)(L)}$ (= 0.13 to 0.31), reflect clearly the presence of the "closed" $M(ATP)^{2-}$ isomers. One may even go one step further; namely, by using eq 5 (for details, see ref 20) and the mentioned differences, one may estimate the percentage of the "closed" isomers of the $M(ATP)^{2-}$ complexes. In doing so one must be aware, however, that ternary complexes may well be formed with both isomers of $M(ATP)^{2-}$ and *not* only with $M(ATP)^{2-}_{op}$ (although this latter species is certainly favored for mixed-ligand complex formation); therefore, the calculated percentages must be considered only as a rough (and rather too low) estimation. Nevertheless, according to the present estimation, depending on the kind of metal ion, about 25–50% of the binary $M(ATP)^{2-}$ complexes exist in the "closed" form; this is in the order of the 10–80% obtained previously (see Table VIII of ref 20).

In any case the present results (Table I and Figure 1) demonstrate clearly that the tendency of binary ATP and UTP complexes to form mixed-ligand complexes is somewhat different. This could also, among others,[20] be one of the tools used by nature to achieve selectivity.

2. Comparison of the Stability of the Binary and Ternary Complexes. With the constants of eq 2 and 3, the position of equilibrium 1 may now be quantified. This means the corresponding equilibrium constant $\Delta(\log K_M)$ (eq 6) may be calculated

$$10^{\Delta(\log K_M)} = [M(NTP)(L)][M]/([M(NTP)][M(L)]) \quad (6)$$

with eq 7,[31,32] and thus the stability of the ternary complexes is

$$\Delta(\log K_M) = \log K^{M(NTP)}_{M(NTP)(L)} - \log K^{M}_{M(L)} = \log K^{M(L)}_{M(L)(NTP)} - \log K^{M}_{M(NTP)} \quad (7)$$

characterized in relation to the stability of the corresponding binary complexes.

An attempt to obtain statistical (st) values[31] for $\Delta(\log K_M)$ (eq 7) gives for octahedral (oh) coordination spheres and ternary complexes with the tridentate UTP^{4-} and the monodentate L $\Delta(\log K_{M/st/oh}) = -0.3$, while for those with ATP^{4-} $\Delta(\log K_{M/st/oh}) = -0.3$ to -0.5.[33] For the Cu^{2+} systems a statistical value is more difficult to assess,[31] but assuming a square-planar (sp) coordination sphere, one obtains for a tridentate coordination of UTP^{4-} $\Delta(\log K_{Cu/st/sp}) = -0.6$; for ATP^{4-} with its larger denticity, the value must be smaller. Another approach,[35] which accounts in an indirect way at least partly for steric restrictions within the coordination sphere, solvation effects, etc., is based on the experimental data (exptl) obtained for the binary complexes of imidazole and ammonia. One obtains for the octahedral complexes $\Delta(\log K_{M/oh/exptl}) \simeq -1.0$ and for the Cu^{2+} systems $\Delta(\log K_{Cu/exptl}) \simeq -1.5$. This latter approach appears as somewhat more realistic for the present comparisons.

The values obtained for $\Delta(\log K_M)$ (eq 7) in this work are listed in Table II, together with some related data from the literature.[25] When the reasonings of the preceding paragraph about the values expected for $\Delta(\log K_M)$ are taken into account, it is evident from Table II that the ternary $M(UTP)(Im)^{2-}$ complexes are certainly more stable than expected. With Mn^{2+} and Zn^{2+}, $\Delta(\log K_M)$ values are observed that are even slightly *positive* or at least close to 0, i.e., that equilibrium 1 is about in balance and the coordination tendency of imidazole toward $M(aq)^{2+}$ and $M(UTP)^{2-}$ is about equal. The $\Delta(\log K_M)$ values of the $M(ATP)(Im)^{2-}$ complexes are between 0.13 and 0.31 log unit smaller than those of the $M(UTP)(Im)^{2-}$ complexes; this is the result of the higher denticity of ATP^{4-}, as discussed in section 1.

The relatively high stability of the $M(UTP)(Im)^{2-}$ complexes is in accordance with the earlier observations[7,31,32,36] that mixed-ligand complexes consisting of a heteroaromatic N base and an O donor ligand are especially favored, a result that was also confirmed for ternary imidazole complexes.[8,25] This increased stability depends on the π-accepting qualities of the heteroaromatic N ligand.[37] The present observations agree herewith because ammonia has no π-accepting properties and the $M(NTP)(NH_3)^{2-}$ complexes are indeed less stable than the corresponding $M(NTP)(Im)^{2-}$ complexes, at least in those cases where the comparison can be made (Table II).

A similar observation has been made before in a study[25] dealing with the coordination tendency of imidazole and ammonia toward M(nitrilotriacetate)$^-$ complexes; the corresponding $\Delta(\log K_M)$ values are also listed in Table II: they are *positive* for the ternary $M^{2+}/Nta^{3-}/Im$ systems and *negative* for the corresponding NH_3 systems. A comparison of these data with those of the NTP^{4-} complexes shows that the $\Delta(\log K_M)$ values for the NTP^{4-} systems are shifted toward smaller values. This could originate from the differences in charge, solvation, and steric arrangements.[38]

(31) Sigel, H. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 394–402.

(32) Sigel, H. "Coordination Chemistry-20" Banerjea, D. Ed.; published by IUPAC through Pergamon Press: Oxford and New York; 1980, pp 27–45.

(33) In an octahedral coordination sphere, UTP^{4-} coordinates via the three phosphate groups, hence $\Delta(\log K_{M/st/oh}) = \log (3:1)/(6:1) = -0.3$.[31] For a fourfold coordination as possible with ATP^{4-} by an additional involvement of the base moiety (cf. ref 20, 30, 34) $\Delta(\log K_{M/st/oh}) = \log (2:1)/(6:1) = -0.5$. This latter statistical value would, however, be the lower limit for the ATP^{4-} systems because only a part of the $M(ATP)^{2-}$ complexes are fourfold coordinated, as discussed in the previous section.

(34) The suggested structure for a tetracoordinated ATP^{4-} complex has in its inner sphere the N-7 and the β,γ-phosphate groups coordinated, while the α group (see Chart I) is only outersphere bound, i.e., via H_2O.[30]

(35) With the assumption of a tridentate coordination of NTP^{4-}, a basis for the expected size of $\Delta(\log K_M)$ may be obtained by comparing the coordination tendency of L to M^{2+} and $M(L)_3^{2+}$, i.e., by calculating the difference $\log K^{M(L)_3}_{M(L)_4} - \log K^{M}_{M(L)}$. This difference is −1.42 and −1.54 log units for Ni^{2+}/Im and NH_3, −1.73 and −1.66 log units for Cd^{2+}/Im and NH_3, and −2.09 and −2.04 log units for Cu^{2+}/Im and NH_3.[24] As the properties of the binary Im and NH_3 systems are obviously rather similar, one may generalize the matter and expect (exptl) for octahedral coordination spheres (Ni^{2+} and Cd^{2+}), by taking into account also that $M(L)^{2+}$ has only one possibility for the dissociation of L while there are four in $M(L)_4^{2+}$ (this corresponds to 0.6 log unit), that $\Delta(\log K_{M/oh/exptl}) \simeq -1.0$, while for the Cu^{2+} systems $\Delta(\log K_{Cu/exptl}) \simeq -1.5$.

(36) Sigel, H. *Chimia* **1967**, *21*, 489–500.

(37) (a) Huber, P. R.; Griesser, R.; Sigel, H. *Inorg. Chem.* **1971**, *10*, 945–947. (b) Huber, P. R.; Sigel, H. *Z. Naturforsch., B. Anorg. Chem., Org. Chem.* **1972**, *27*, 1319–23. (c) Walker, F. A.; Sigel, H.; McCormick, D. B. *Inorg. Chem.* **1972**, *11*, 2756–2763. (d) Fischer, B. E.; Sigel, H. *Ibid.* **1979**, *18*, 425–428.

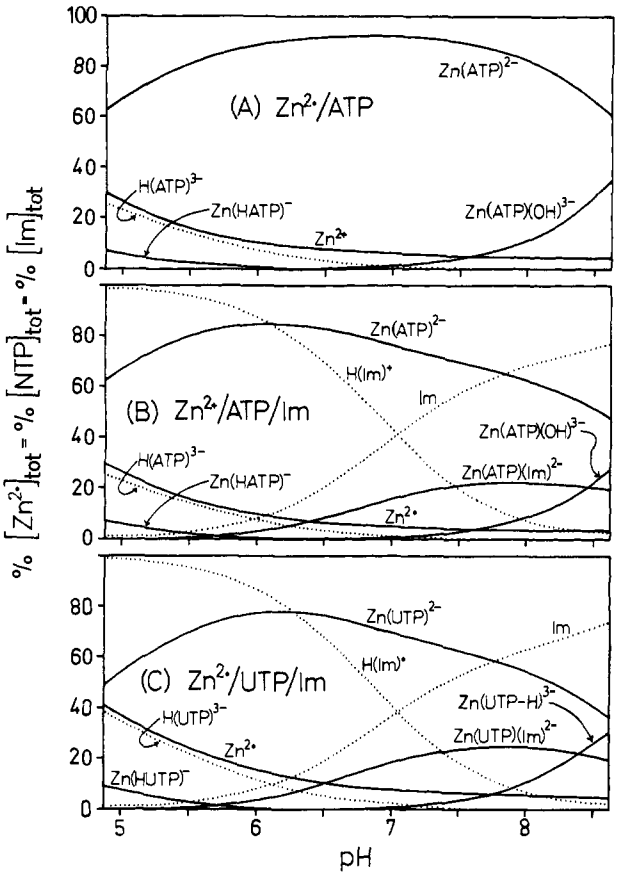


Figure 2. Effect of pH on the concentrations of the species present in an aqueous solution of (A) Zn^{2+}/ATP, (B) Zn^{2+}/ATP/Im, or (C) Zn^{2+}/UTP/Im. Results are given as the percentage of the total Zn^{2+} (= total ATP, UTP, or Im) present (I = 0.1 M; 25 °C), computed with the potentiometrically determined constants of Table I and the additional constants given below, for concentrations of 2×10^{-3} M for each reactant. The dotted lines indicate the free ligand species and the solid lines Zn^{2+} or its complexes. (A) Zn^{2+} and ATP: calculated with $pK^{H}_{H_2(ATP)}$ = 4.06,[39] $pK^{H}_{H(ATP)}$ = 6.42,[16] $K^{Zn}_{Zn(HATP)}$ = 2.67,[39] log $K^{Zn}_{Zn(ATP)}$ = 5.16 (av of ref 16), and $pK^{H}_{Zn(ATP)(H_2O)}$ = 8.87;[26] $[H_2(ATP)^{2-}] \leq 3.6\%$, $[ATP^{4-}] \leq 5.4\%$. (B) Zn^{2+}, ATP, and Im: calculated with log $K^{Zn(Im)}_{Zn(Im)(ATP)}$ = 5.06 (eq 7) and the estimated value log $K^{Zn(Im)}_{Zn(Im)(HATP)} \simeq$ log $K^{Zn}_{Zn(HATP)}$ + Δ(log K_{Zn}) = 2.67 (cf. ref 39) −0.10 (Table II) = 2.57 and the constants given for the preceding part; $[Zn(Im)^{2+}] < 1.8\%$, $[Zn(HATP)(Im)^{-}] < 0.03\%$, $[H_2(ATP)^{2-}] \leq 3.6\%$, $[ATP^{4-}] \leq 5.6\%$. (C) Zn^{2+}, UTP, and Im: calculated with $pK^{H}_{H(UTP)}$ = 6.45,[16] pK^{H}_{UTP} = 9.70,[26] log $K^{Zn}_{Zn(UTP)}$ = 4.75,[16] $pK^{H}_{Zn(UTP)}$ = 8.71,[26] $pK^{H}_{Zn(UTP\cdot H)(H_2O)}$ = 9.24,[26] log $K^{Zn(Im)}_{Zn(Im)(UTP)}$ = 4.78 (eq 7), and the estimated values log $K^{Zn}_{Zn(HUTP)} \simeq$ log $K^{Zn(bpy)}_{Zn(bpy)(HUTP)} \simeq$ log $K^{Zn(bpy)}_{Zn(bpy)(HITP)}$ = 2.46 (cf. ref 16 and 40), and log $K^{Zn(Im)}_{Zn(Im)(HUTP)} \simeq$ log $K^{Zn}_{Zn(HUTP)}$ + Δ(log K_{Zn}) = 2.46 + 0.03 (Table II) = 2.49; $[Zn(Im)^{2+}] \leq 2.4\%$, $[Zn(HUTP)(Im)^{-}] < 0.07\%$, $[UTP^{4-}] < 8.4\%$, $[(UTP\cdot H)^{5-}] < 0.6\%$, $[Zn(UTP\cdot H)(OH)^{4-}] < 6.7\%$. The acidity constant of the species $Zn(UTP)(Im)^{2-}$ for deprotonation at N-3 of the uridine moiety is not known; with the estimate $pK^{H}_{Zn(UTP)(Im)} \simeq 9.0$ (based on previous results),[26] one calculates for $[Zn(UTP\cdot H)(IM)^{3-}]$ 2.4% and 6% at pH 8 and 8.5, respectively.

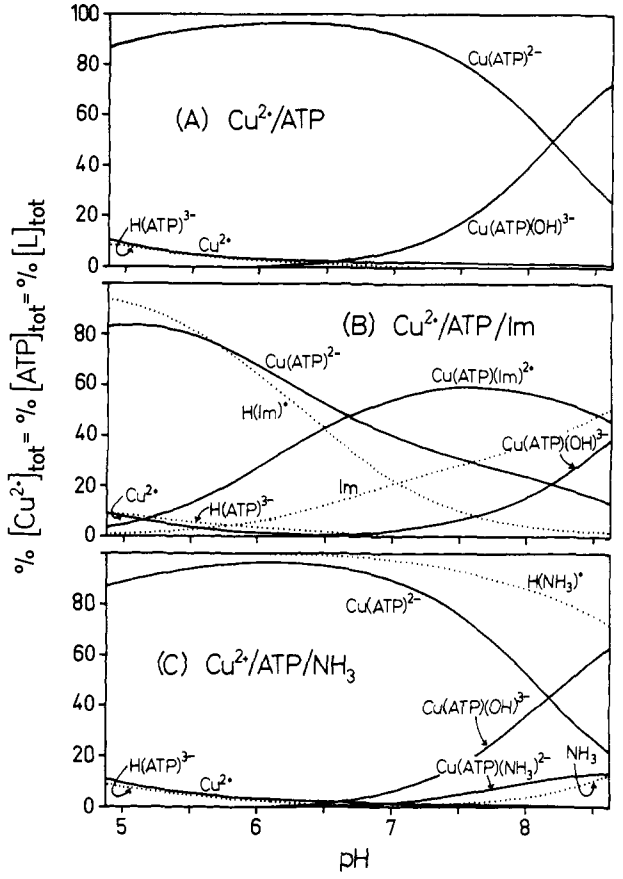


Figure 3. Effect of pH on the concentrations of the species present in an aqueous solution of (A) Cu^{2+}/ATP, (B) Cu^{2+}/ATP/Im, or (C) Cu^{2+}/ATP/NH_3. Details as in Figure 2. (A) Cu^{2+} and ATP: calculated with $pK^{H}_{H_2(ATP)}$ = 4.06,[39] $pK^{H}_{H(ATP)}$ = 6.42,[16] log $K^{Cu}_{Cu(HATP)}$ = 3.12,[39] log $K^{Cu}_{Cu(ATP)}$ = 6.21 (average from ref 16), and $pK^{H}_{Cu(ATP)(H_2O)}$ =8.17;[21] $[Cu(HATP)^{-}] < 2.4\%$, $[H_2(ATP)^{2-}] < 1.3\%$, $[ATP^{4-}] \leq 1.5\%$. (B) Cu^{2+}, ATP, and Im: calculated with log $K^{Cu(Im)}_{Cu(Im)(ATP)}$ = 5.53 (eq 7) and the estimated value log $K^{Cu(Im)}_{Cu(Im)(HATP)} \simeq$ log $K^{Cu}_{Cu(HATP)}$ + Δ(log K_{Cu}) = 3.12 (cf. ref 39) − 0.68 (Table II) = 2.44 and the constants given for the preceding part; $[Cu(Im)^{2+}] < 3.1\%$, $[Cu(HATP)^{-} \leq 2.2\%$, $[Cu(HATP)(Im)^{-}] \leq 0.1\%$, $[H_2(ATP)^{2-}] < 1.4\%$, $[ATP^{4-}] \leq 3.0\%$. (C) Cu^{2+}, ATP, and NH_3: calculated with log $K^{Cu(NH_3)}_{Cu(NH_3)(ATP)}$ = 5.43 (eq 7) and the estimated value log $K^{Cu(NH_3)}_{Cu(NH_3)(HATP)} \simeq$ log $K^{Cu}_{Cu(HATP)}$ + Δ(log K_{Cu}) = 3.12 (cf. ref 39) − 0.78 (Table II) = 2.34 and the constants given under (A); $[Cu(NH_3)^{2+}] \leq 1.4\%$, $[Cu(HATP)^{-}] < 2.4\%$, $[Cu(HATP)(NH_3)^{-}] < 0.0001\%$, $[H_2(ATP)^{2-}] < 1.3\%$, $[ATP^{4-}] < 1.9\%$.

Figures 2 and 3 have been designed[39,40] to demonstrate the influence of imidazole or ammonia on the distribution of the complex species in M^{2+}/NTP systems. The concentrations of the reactants used in the calculations are 2×10^{-3} M and always equal to each other; consequently an increase in the concentration, e.g., of the monodentate ligand, will also rise the concentration of the mixed-ligand complex. It is evident from Figure 2 that even with these low reactant concentrations in the physiological pH range considerable amounts of ternary Zn(NTP)(Im) complexes are formed.

A comparison of the middle parts of Figures 2 and 3 reveals that an increase in log $K^{Zn(ATP)}_{Zn(ATP)(Im)}$ from 2.41 by about 1.1 log units to log $K^{Cu(ATP)}_{Cu(ATP)(Im)}$ = 3.53 favors the formation of the ternary complex at pH 7 from about 16% to 55%. In the Mn^{2+}/ATP/Im system, which has the smallest stability constant (log $K^{Mn(ATP)}_{Mn(ATP)(Im)}$ = 1.05), $Mn(ATP)(Im)^{2-}$ exists under these conditions at pH 7 only 1%; at pH 8 this amount increases to 1.8%, while at 10 times larger reactant concentrations (i.e., 2×10^{-2} M) the corresponding numbers are 8.6 and 14%. It is obvious that favorable steric arrangements in proteins (e.g., through chelate formation) may favor the coordination of an imidazole group considerably.

From the lower part of Figure 3 it follows that ternary complexes with ammonia are formed in the physiological pH range only in low concentrations. The Cu^{2+} complexes are most stable (cf. Table I), but still $Cu(ATP)(NH_3)^{2-}$ reaches at pH 7 only about 1.8% (see also the following section).

(38) For example, one would expect that the solvation of $M(NTP)^{2-}$ is more intense than that of $M(Nta)^{-}$, because the ethylene groups of Nta^{3-} will oppose solvation by water while the oxygens of the phosphate chain will facilitate it under formation of hydrogen bonds; actually these might even be formed with coordinated water molecules. The access of a second ligand to the coordination sphere of a metal ion already bound to NTP^{4-} or Nta^{3-} is thus expected to be different, i.e., to be more difficult in $M(NTP)^{2-}$.

(39) Taqui Khan, M. M.; Martell, A. E. *J. Am. Chem. Soc.* **1966**, *88*, 668–671.

(40) Chaudhuri, P.; Sigel, H. *J. Am. Chem. Soc.* **1977**, *99*, 3142–3150.

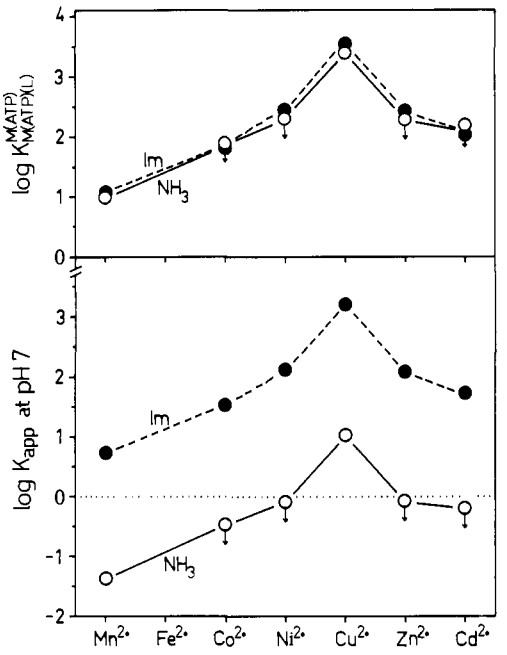


Figure 4. Logarithms of the stability constants, log $K^{M(ATP)}_{M(ATP)(L)}$ (upper), for several ternary $M(ATP)(L)^{2-}$ complexes with L = imidazole (●) or ammonia (○) (see Table I), and logarithms of the corresponding *apparent* constants, log K_{app} (lower), calculated with eq 8 for pH 7.0.

3. Discriminating Properties and General Conclusions. In section 1 we have already seen that the tendency of $M(UTP)^{2-}$ and $M(ATP)^{2-}$ to undergo mixed-ligand complex formation is different, owing to the partial coordination of the adenine moiety in the binary $M(ATP)^{2-}$ complexes. This observation adds a third dimension to the already described[20] differences between these two nucleotides and their interaction with metal ions. This means ATP^{4-} complexes are favored over UTP^{4-} complexes in the formation of macrochelates and in the promotion of the self-stacking tendency through metal ions, while regarding the formation of mixed-ligand complexes with simple monodentate N ligands, we see now that $M(UTP)^{2-}$ is favored. The interaction between both types of binary $M(NTP)^{2-}$ complexes and negatively charged ligands like acetate or HPO_4^{2-} is most probably small, as the corresponding results[25] with $M(Nta)^-$ complexes suggest.

In addition, both types of $M(NTP)^{2-}$ complexes discriminate between imidazole and ammonia, as we have seen in section 2; the coordination of imidazole is favored due to its π-accepting properties.[37a,b] However, this observation should *not* be confused with the observation evident from Figure 3 that under identical conditions the formation degree in the physiological pH range of $Cu(ATP)(NH_3)^{2-}$ is much smaller than that of $Cu(ATP)(Im)^{2-}$. This latter result is only to a small extent due to the differences in the stability constants of these two ternary complexes (see upper part of Figure 4); the reason originates in the different basicity of imidazole and ammonia.

In case of ammonia the competition between protons and metal ions to coordinate at the nitrogen is quite high in the physiological pH range, while with the smaller proton affinity of imidazole this aspect is nearly negligible. *Apparent* stability constants [41,42] may be calculated with eq 8, to account for the competition between

$$\log K_{app} = \log K^{M}_{M(L)} - \log (1 + [H^+]/K^{H}_{H(L)}) \quad (8)$$

metal ions and protons for a given binding site. In Figure 4 the stability constants, log $K^{M(ATP)}_{M(ATP)(L)}$, and the corresponding *apparent* constants, log K_{app}, are plotted for the ternary ATP^{4-} complexes. For the ternary UTP^{4-} complexes, the corresponding figure would be very similar. It may also be added that the coordination tendency of ammonia and alkylamines are quite alike,[43] so that one may assume that the present data mimic the coordination tendency of amino groups in biological systems quite well. Furthermore, estimations[41] about the stability of such ternary complexes formed with Fe^{2+} may also be obtained from Figure 4.

From a comparison of the upper with the lower part of Figure 4, it is evident that the stability of the ternary ammonia complexes at pH 7 is drastically decreased while that of the imidazole complexes remains nearly unaltered. To conclude, imidazole and imidazole residues are much more easily accessible in biological systems for the formation of (binary and) ternary complexes than are ammonia and its related amino groups. Hence, imidazole groups are twofold favored for a metal ion coordination: (i) the competition of the proton is low (Figure 4), and (ii) the formation of mixed-ligand complexes that also involve O donors is facilitated (section 2). Indeed, this conclusion agrees well with the actual observation that the imidazole group is a very common binding site in nature often occurring in combination with O donor (or O donorlike) groups.[44] It seems now safe to predict that this combination will also be observed in many enzyme–metal ion ion–substrate complexes.

It should again be pointed out in this connection that ternary $M(NTP)(Im)^{2-}$ complexes are indeed formed in appreciable amounts in the physiological pH range already under low reactant concentrations (2×10^{-3} M), as is evident from Figures 2 and 3. That similar ternary complexes occur also in biological systems seems rather certain, especially as some of the reactant concentrations are sometimes quite high in nature: for example, the chromaffin granules, which store catecholamines in the adrenal medulla, contain both substantial amounts of metal ions and high concentrations of ATP (~0.1 M).[45]

Acknowledgment. Part of the work was performed with the technical assistance of Madeleine Imhof and Rita Baumbusch. The computers were made available by the Rechenzentrum der Universität Basel (Univac 1100/81). This support, a research grant from the Swiss National Science Foundation, and a fellowship to N.S. from the Amt für Ausbildungsbeiträge des Kantons Basel Stadt are gratefully acknowledged.

Registry No. Mn, 7439-96-5; Co, 7440-48-4; Ni, 7440-02-0; Cu, 7440-50-8; Zn, 7440-66-6; Cd, 7440-43-9; ATP, 56-65-5; UTP, 63-39-8; Im, 288-32-4; NH_3, 7664-41-7.

(41) Sigel, H.; McCormick, D. B. Acc. *Chem. Res.* **1970**, *3*, 201–208.
(42) Sigel, H. *Met. Ions Biol. Syst.* **1979**, *8*, 125–158. Cf. ref 4.
(43) Ilcheva, L.; Bjerrum, J. *Acta Chem. Scand. A* **1976**, *30*, 343–350.
(44) See, e.g., Table VI of ref 7.
(45) (a) Kirshner, N.; Kirshner, A. G. *Philos. Trans. R. Soc. London, Ser. B* **1971**, *261*, 279–289. (b) Winkler, H. *Neuroscience* **1976**, *1*, 65–80. (c) Frausto da Silva, J. J. R.; Williams, R. *J. P. Nature (London)* **1976**, *263*, 237–239. (d) Granot, J.; Rosenheck, K. *FEBS Lett.* **1978**, *95*, 45–48.