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Stability and Structure of Mixed-Ligand Metal Ion Complexes That Contain Ni^{2+}, Cu^{2+}, or Zn^{2+}, and Histamine, as well as Adenosine 5′-Triphosphate (ATP^{4-}) or Uridine 5′-Triphosphate (UTP^{4-}): An Intricate Network of Equilibria

Bernd Knobloch,[a] Ariel Mucha,[a, b] Bert P. Operschall,[c] Helmut Sigel,[c] Małgorzata Jeżowska-Bojczuk,[b] Henryk Kozłowski,[b] and Roland K. O. Sigel*[a]

Abstract: With a view on protein–nucleic acid interactions in the presence of metal ions we studied the "simple" mixed-ligand model systems containing histamine (Ha), the metal ions Ni^{2+}, Cu^{2+}, or Zn^{2+} (M^{2+}), and the nucleotides adenosine 5′-triphosphate (ATP^{4-}) or uridine 5′-triphosphate (UTP^{4-}), which will both be referred to as nucleoside 5′-triphosphate (NTP^{4-}). The stability constants of the ternary $M(NTP)(Ha)^{2-}$ complexes were determined in aqueous solution by potentiometric pH titrations. We show for both ternary-complex types, $M(ATP)(Ha)^{2-}$ and $M(UTP)(Ha)^{2-}$, that intramolecular stacking between the nucleobase and the imidazole residue occurs and that the stacking intensity is approximately the same for a given M^{2+} in both types of complexes: The formation degree of the intramolecular stacks is estimated to be 20 to 50%. Consequently, in protein–nucleic acid interactions imidazole–nucleobase stacks may well be of relevance. Furthermore, the well-known formation of macrochelates in binary M^{2+} complexes of purine nucleotides, that is, the phosphate-coordinated M^{2+} interacts with N7, is confirmed for the $M(ATP)^{2-}$ complexes. It is concluded that upon formation of the mixed-ligand complexes the M^{2+}–N7 bond is broken and the energy needed for this process corresponds to the stability differences determined for the $M(UTP)(Ha)^{2-}$ and $M(ATP)(Ha)^{2-}$ complexes. It is, therefore, possible to calculate from these stability differences of the ternary complexes the formation degrees of the binary macrochelates: The closed forms amount to (65±10)%, (75±8)%, and (31±14)% for $Ni(ATP)^{2-}$, $Cu(ATP)^{2-}$, and $Zn(ATP)^{2-}$, respectively, and these percentages agree excellently with previous results obtained by different methods, confirming thus the internal validity of the data and the arguments used in the evaluation processes. Based on the overall results it is suggested that $M(ATP)^{2-}$ species, when bound to an enzyme, may exist in a closed macrochelated form only, if no enzyme groups coordinate directly to the metal ion.

Keywords: chelates · complex stabilities · isomers · metalloenzymes · protein–nucleic acid interactions

1. Introduction

Alessio and co-workers[1] have recently categorized the role of the metal ion in anticancer compounds into five classes. The following four of these are also of relevance for enzymes and ribozymes:[2–5] i) The metal ion has a structural role and is instrumental for the three-dimensional shape of the protein or the nucleic acid; ii) the metal ion has a functional role and is binding to the biological target, that is, to the active site in the protein or RNA; iii) the metal ion is a carrier for the active ligands, that is, the substrates; and iv) the metal ion itself is the active catalyst. Especially, but not only, point i) is often connected with further noncovalent interactions,[3,6–8] among which hydrophobic and/or π-stacking interactions are especially prominent.[6,9,10] This view is also supported by the π-stacks of histidine–phenylalanine (imidazole–phenyl) in a fern plastocyanine,[7,8] by the tryptophan–tryptophan (indole–indole) interactions in Trpzip β hairpin formation[11] or by model studies[12–14] including a Cu^{II}–metallocyclophane probe for guanosine 5′-monophosphate in which π-stacking between the anthracene moieties and the guanine residue occurs.[15]

It is becoming more and more evident that large nucleic acids like ribozymes[4,16–18] and riboswitches[19] change their three-dimensional structure upon binding metal ions, metal ion complexes, or other small metabolites. For example, the

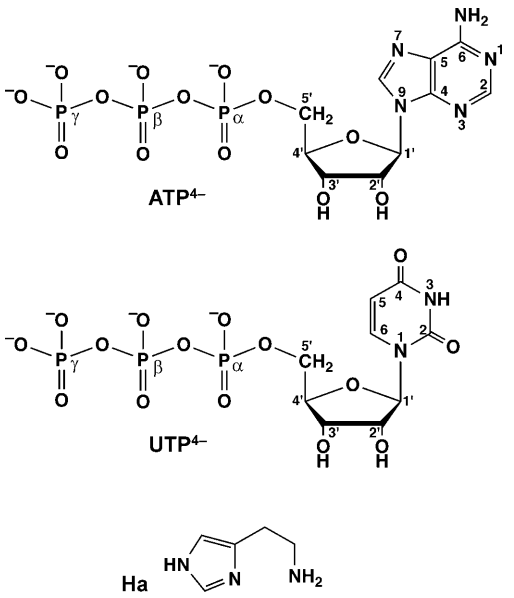
[a] Dr. B. Knobloch, Dr. A. Mucha, Prof. Dr. R. K. O. Sigel
Institute of Inorganic Chemistry, University of Zürich
Winterthurerstrasse 190, 8057 Zürich (Switzerland)
Fax: (+41) 44-635-6802
E-mail: roland.sigel@aci.uzh.ch

[b] Dr. A. Mucha, Prof. Dr. M. Jeżowska-Bojczuk, Prof. Dr. H. Kozłowski
Department of Bioinorganic and Biomedical Chemistry
Faculty of Chemistry, University of Wrocław
F. Joliot Curie 14, 50-383 Wrocław (Poland)

[c] Dipl.-Ing. B. P. Operschall, Prof. Dr. H. Sigel
Department of Chemistry, Inorganic Chemistry, University of Basel
Spitalstrasse 51, 4056 Basel (Switzerland)

corrin moiety of coenzyme B_{12} induces the structural change within the *btuB* riboswitch.[20] Furthermore, for the ribosome it is known[21] that some metal ions are located at the interface between the protein and nucleic acid parts, and have ligating sites from both partners in their coordination sphere.[4,21] Other prominent examples are nucleic acid polymerases[22–25] and repair enzymes, which also involve metal ions at the nucleic acid–protein interface.[26] In all of the above cases, it is crucial to know both the local metal ion geometries and the affinities to understand structure and function of these molecules.[5,27–29]

With the above observations in mind we decided to study the "simple" mixed-ligand systems containing histamine and adenosine 5′-triphosphate (ATP^{4-}) or uridine 5′-triphosphate (UTP^{4-}) (Scheme 1),[24,30] as well as one each of the divalent

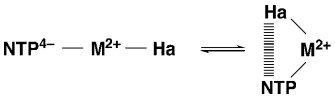


Scheme 1. Chemical structures of the nucleoside 5′-triphosphates (NTP^{4-}) used in this study, that is, of adenosine 5′-triphosphate (ATP^{4-}) and uridine 5′-triphosphate (UTP^{4-}); the structures are shown in their dominating *anti* conformation,[24,30] together with the labeling system of the triphosphate chain. The ligand histamine (Ha) is shown in the orientation allowing the formation of a six-membered chelate.

metal ions (M^{2+}) Ni^{2+}, Cu^{2+}, or Zn^{2+}. We concentrated on these three metal ions because they are of relevance in many proteins and enzymes.[2] Histamine with its imidazole group is found in body tissues, is released during allergic responses,[31] and is a derivative of the amino acid histidine. The nucleotides ATP^{4-} or UTP^{4-} are representatives of purine or pyrimidine nucleotides, respectively,[24] and also important substrates in many enzymatic reactions. The nucleoside 5′-triphosphates (NTP^{4-}) were selected because of their high metal ion complex stability compared with those formed by nucleoside monophosphates.[30,32] In this study, we answer the following questions:

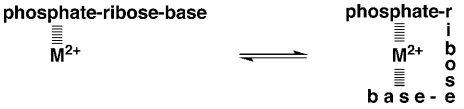
i) Is the intensity of the phosphate-metal ion interaction in the $M(NTP)^{2-}$ complexes affected by the additional coordination of histamine (Ha), which gives rise to the formation of ternary $M(NTP)(Ha)^{2-}$ complexes?

ii) Is there any indication for an intramolecular stacking interaction between the imidazole residue of Ha and the nucleobase moieties of the NTPs as indicated in Equilibrium (1)?—If so, can the formation degrees be determined?



(1)

iii) Does the additional coordination of Ha affect the position of the well-known intramolecular equilibrium, as it occurs in binary purine-nucleotide complexes[24,30,32,33] for which the phosphate-coordinated metal ion interacts to a certain extent with N7 of the purine moiety? This interaction is expressed in Equilibrium (2):



(2)

Can anything be concluded about the formation degrees of these macrochelates?

We will show that the answer to all these questions is yes and that the results obtained fit well into the picture that emerges from previous studies of related systems.[34–39] These previous data will be compared and discussed in Sections 2.1 and 2.3.

2. Results and Discussion

Adenine derivatives undergo self-association as a result of the aromatic-ring stacking of their nucleobases.[40] Therefore, all potentiometric pH titrations (25 °C; $I = 0.1$ M, $NaNO_3$) were carried out at ligand concentrations close to 1 mM or below. Under these conditions self-stacking of adenine nucleotides is negligible[41] and, therefore, the properties of the monomeric species were investigated.

2.1. Definition of equilibrium constants and corresponding results: The acidity constants (K_a) of protonated ligands (L), according to Equilibrium (3a), are given in this study as the negative logarithms of their definition (pK_a) provided in Equation (3b).

$$H(L)^+ \rightleftharpoons L + H^+ \tag{3a}$$

$$K^{H}_{H(L)} = [L][H^+]/[H(L)^+] \tag{3b}$$

Similarly, the stability constants of M^{2+} complexes, according to Equilibrium (4a), are listed in the form of the loga-

rithm of the corresponding formation constant [log$K$; Eq. (4b)].

$$M^{2+} + L \rightleftharpoons M(L)^{2+} \quad (4a)$$

$$K^{M}_{M(L)} = [M(L)^{2+}]/([M^{2+}][L]) \quad (4b)$$

The acidity constants of twofold protonated histamine (Ha) have now been determined (Table 1, entries 1 and 2).

Table 1. Negative logarithms of the acidity constants, pK_a [Eq. (3)], of the ligands used in this study and as determined by potentiometric pH titrations in water at 25 °C and I = 0.1 M.[a]

No.	Equilibrium	pK_a	Ref.
1	$H_2(Ha)^{2+} \rightleftharpoons H(Ha)^{+} + H^{+}$	6.08 ± 0.02	–[b]
2	$H(Ha)^{+} \rightleftharpoons Ha + H^{+}$	9.88 ± 0.02	–[b]
3[c]	$H_3(ATP)^{-} \rightleftharpoons H_2(ATP)^{2-} + H^{+}$	1.7 ± 0.1	[43]
4[d]	$H_2(ATP)^{2-} \rightleftharpoons H(ATP)^{3-} + H^{+}$	4.00 ± 0.01	[44]
5	$H(ATP)^{3-} \rightleftharpoons ATP^{4-} + H^{+}$	6.47 ± 0.01	[44]
6[c]	$H_2(UTP)^{2-} \rightleftharpoons H(UTP)^{3-} + H^{+}$	2.0 ± 0.1	[45]
7	$H(UTP)^{3-} \rightleftharpoons UTP^{4-} + H^{+}$	6.48 ± 0.02	[46][f]
8[e]	$UTP^{4-} \rightleftharpoons (UTP-H)^{5-} + H^{+}$	9.57 ± 0.02	[46]

[a] So-called practical (or mixed) acidity constants are listed (see Section 4.2).[42] The error limits given are three times the standard error of the mean value (3σ) or the sum of the probable systematic errors, whichever is larger. For entries 1 and 2 the ionic strength, I, was kept constant with $NaNO_3$; in all other instances either also $NaNO_3$ or $NaClO_4$ had been used. [b] This study. [c] In $H_3(ATP)^{-}$ and $H_2(UTP)^{2-}$ two protons are at the triphosphate chain and the first one is released according to entries 3 and 6; for the second one, which is located at the terminal γ-phosphate group (Scheme 1), entries 5 and 7 hold. [d] The proton is released from the (N1)H^{+} site (Scheme 1). [e] The (N3)H unit is deprotonated (Scheme 1). [f] See also refs. [44,47].

The acidity constants of the (partly) protonated nucleoside 5′-triphosphates, which are considered in this study, were taken from the literature (Table 1, entries 3–8).[42–47]

The measured "practical" acidity constants for $H_2(Ha)^{2+}$ (Table 1, entries 1, 2) can easily be transformed into their corresponding concentration constants (see Section 4.2):[42] These results, that is, $pK^{H}_{H_2(Ha)} = (6.08 \pm 0.02) - (0.02 \pm 0.02) = 6.06 \pm 0.03$ and $pK^{H}_{H(Ha)} = (9.88 \pm 0.02) - (0.02 \pm 0.02) = 9.86 \pm 0.03$, are in excellent agreement with previous determinations: The NIST database[48] lists $pK^{H}_{H_2(Ha)} = 6.11 \pm 0.06$ and $pK^{H}_{H(Ha)} = 9.81 \pm 0.07$, and the IUPAC database,[49] including a review,[50] gives $pK^{H}_{H_2(Ha)} = 6.07 \pm 0.02$ and $pK^{H}_{H(Ha)} = 9.81 \pm 0.02$.

Our results, as determined by potentiometric pH titrations, regarding the stabilities of the ternary complexes composed of M^{2+}, NTP^{4-}, and Ha are given in entries 6, 7 (ATP^{4-}) and 11, 12 (UTP^{4-}) of Table 2, together with the stability constants of the corresponding binary complexes.[44,50–54] The stability constants of the binary M^{2+}/Ha complexes are from a critical IUPAC collection[50] (and in accord with data bases)[48,49] and the M^{2+}/NTP^{4-} data have recently been verified in a different context[32,51] (see also ref. [44]).

To the best of our knowledge from the six mixed-ligand systems studied now, only two have been investigated before, both in independent studies.[35,36] The present overall stability constant of the $Zn(ATP)(Ha)^{2-}$ complex, $\log \beta^{Zn}_{Zn(ATP)(Ha)} = 10.08 \pm 0.09$ [Table 2, entry 6; see also Equilibrium scheme (5)], is 0.67 ± 0.12 log units smaller than the value determined earlier, $\log \beta^{Zn}_{Zn(ATP)(Ha)} = 10.75 \pm 0.08$ (25 °C; I = 0.1 M, KNO_3).[36] A possible reason for this discrepancy is that in the latter study the stability constant has been corrected for the competition of K^{+} binding. On the other hand, the agreement of the values for the Cu(ATP)$(Ha)^{2+}$ complex is excellent: $\log \beta^{Cu}_{Cu(ATP)(Ha)} = 15.29 \pm 0.09$ (Table 2, entry 6) versus $\log \beta^{Cu}_{Cu(ATP)(Ha)} = 15.35 \pm 0.05$ (25 °C; I = 0.1 M, KNO_3).[36] The log β values of the other study[35] are lower by about 1 and 2 log units for the Cu^{2+} and Zn^{2+} complex, respectively (25 °C; I = 0.1 M, KCl), if compared with the present results. These earlier values[35] ought to be ignored because the indicated discrepancies can hardly be explained by the formation of chlorido complexes alone.

Comparison of the values listed in entry 7 of Table 2 for the reaction between $M(ATP)^{2-}$ and Ha shows similar trends: The stability constants that follow from the work of Arena et al.[36,55] are $\log K^{Zn(ATP)}_{Zn(ATP)(Ha)} = 5.52 \pm 0.09$ and $\log K^{Cu(ATP)}_{Cu(ATP)(Ha)} = 8.85 \pm 0.06$. If compared with our values

Table 2. Logarithms of the stability constants for the binary [Eq. (4)] and mixed ligand complexes [see Equilibrium scheme (5)] containing M^{2+}/Ha/ATP or UTP, together with the acidity constants for some related equilibria (aqueous solution; 25 °C; I = 0.1 M).[a,b]

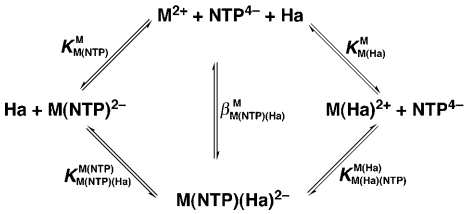
No.	Equilibrium	log K for the complexes with M^{2+} =			Ref.
		Ni^{2+}	Cu^{2+}	Zn^{2+}	
1	$M^{2+} + Ha \rightleftharpoons M(Ha)^{2+}$	6.82 ± 0.05	9.56 ± 0.06	5.21 ± 0.05	[50]
2	$M(Ha)^{2+} + Ha \rightleftharpoons M(Ha)_2^{2+}$	5.03 ± 0.03	6.50 ± 0.10	4.92 ± 0.05	[50]
3[c]	$M^{2+} + H(ATP)^{3-} \rightleftharpoons M(H;ATP)^{-}$	2.86 ± 0.11	3.59 ± 0.08	2.86 ± 0.11	[44][d]
4	$M^{2+} + ATP^{4-} \rightleftharpoons M(ATP)^{2-}$	4.86 ± 0.05	6.34 ± 0.03	5.16 ± 0.06	[44][d]
5	$M(ATP)(H_2O)^{2-} \rightleftharpoons M(ATP)(OH)^{3-} + H^{+}$	−9.41 ± 0.08	−8.17 ± 0.02	−8.87 ± 0.04	[53][e]
6	$M^{2+} + ATP^{4-} + Ha \rightleftharpoons M(ATP)(Ha)^{2-}$	10.83 ± 0.08	15.29 ± 0.09	10.08 ± 0.09	–[f,g]
7	$M(ATP)^{2-} + Ha \rightleftharpoons M(ATP)(Ha)^{2-}$	5.97 ± 0.09	8.95 ± 0.09	4.92 ± 0.07	–[f]
8[h]	$M^{2+} + H(UTP)^{3-} \rightleftharpoons M(H;UTP)^{-}$	2.51 ± 0.25	2.80 ± 0.08	2.73 ± 0.09	[44][d]
9	$M^{2+} + UTP^{4-} \rightleftharpoons M(UTP)^{2-}$	4.47 ± 0.03	5.87 ± 0.03	5.01 ± 0.03	[44][d]
10	$M(UTP)^{2-} \rightleftharpoons M(UTP-H)^{3-} + H^{+}$	−9.10 ± 0.03	−8.0 ± 0.2	−8.71 ± 0.04	[53][i]
11	$M^{2+} + UTP^{4-} + Ha \rightleftharpoons M(UTP)(Ha)^{2-}$	10.89 ± 0.08	15.43 ± 0.12	10.09 ± 0.06	–[f,g]
12	$M(UTP)^{2-} + Ha \rightleftharpoons M(UTP)(Ha)^{2-}$	6.42 ± 0.09	9.56 ± 0.12	5.08 ± 0.05	–[f]

[a] The error limits given are three times the standard error of the mean value (3σ); for entries 1 and 2 the error limits of ref. [50] are listed. The error limits of derived data were calculated according to the error propagation after Gauss. [b] For entries 1 and 2 the ionic strength, I, was kept constant with KNO_3;[50] in the present work $NaNO_3$ was used (entries 6, 7, 11, and 12); in all other instances either $NaNO_3$ or $NaClO_4$ had been employed. [c] For the structures of the $M(H;ATP)^{-}$ species ref. [44] should be consulted. [d] See also ref. [51]. [e] The value for $Cu(ATP)(OH)^{3-}$ is from ref. [54]. [f] This work. [g] The values listed in this row refer to $\log \beta^{M}_{M(NTP)(Ha)}$; see Equation (6) and the Equilibrium scheme (5). [h] In the $M(H;UTP)^{-}$ complexes the proton and M^{2+} are at the triphosphate chain.[44,51] [i] The value for $Cu(UTP-H)^{3-}$ was calculated from the information provided in ref. [53]; see also ref. [52].

from Table 2, $\log K^{Zn(ATP)}_{Zn(ATP)(Ha)} = 4.92 \pm 0.07$ and $\log K^{Cu(ATP)}_{Cu(ATP)(Ha)} = 8.95 \pm 0.09$, one sees that there is a relatively large discrepancy for the Zn^{2+} system (0.60 ± 0.11 log units) and a perfect agreement for the Cu^{2+} one (0.10 ± 0.11 log units), that is, the differences are of a similar size as observed above for the overall stability constants $\log \beta$. The corresponding values from the other earlier study[35] are again far off and need to be ignored. It should be emphasized, however, that all our equilibrium constants are within themselves conclusive, as we shall see later in Section 2.4 and 2.5.

To mediate a feeling for the formation degree of the various complex species in the physiological pH range of about 7.5, distribution curves are shown in Figure 1 for the M^{2+}/ATP/Ha systems.[56–58] It is evident that with Cu^{2+} the mixed-ligand complex dominates strongly; in the Ni^{2+} system the ternary complex still occurs in significant concentrations, whereas in the Zn^{2+} system the formation degree of $Zn(ATP)(Ha)^{2-}$ is relatively small.

2.2. Evaluation of the stabilities of the ternary complexes: A mixed-ligand or ternary complex of the kind considered here is composed of a metal ion and two different ligands. There are various ways to quantify the stabilities of such ternary complexes.[9,59,60] Here we consider the Equilibrium scheme (5), which holds as long as labile metal ions, having a fast ligand exchange rate, are considered, that is, $M(NTP)(Ha)^{2-}$ and $M(Ha)(NTP)^{2-}$ are two expressions describing the same species.



(5)

From the Equilibrium scheme (5) it follows that the overall stability constant $\log \beta^{M}_{M(NTP)(Ha)}$ [Eq. (6)] is connected by the two equilibria [Eqs. (7) and (8)] seen in the lower part of the equilibrium scheme:

$$\log \beta^{M}_{M(NTP)(Ha)} = [M(NTP)(Ha)^{2-}]/([M^{2+}][NTP^{4-}][Ha]) \quad (6)$$

$$\log K^{M(NTP)}_{M(NTP)(Ha)} = \log \beta^{M}_{M(NTP)(Ha)} - \log K^{M}_{M(NTP)} \quad (7)$$

$$\log K^{M(Ha)}_{M(Ha)(NTP)} = \log \beta^{M}_{M(NTP)(Ha)} - \log K^{M}_{M(Ha)} \quad (8)$$

A simple way[30] to quantify the stability of the mixed-ligand complex is to compare, for example, the affinity of Ha towards $M(NTP)^{2-}$ with the affinity towards $M(aq)^{2+}$. This is expressed by the stability differences given in Equations (9a)–(9c):[60]

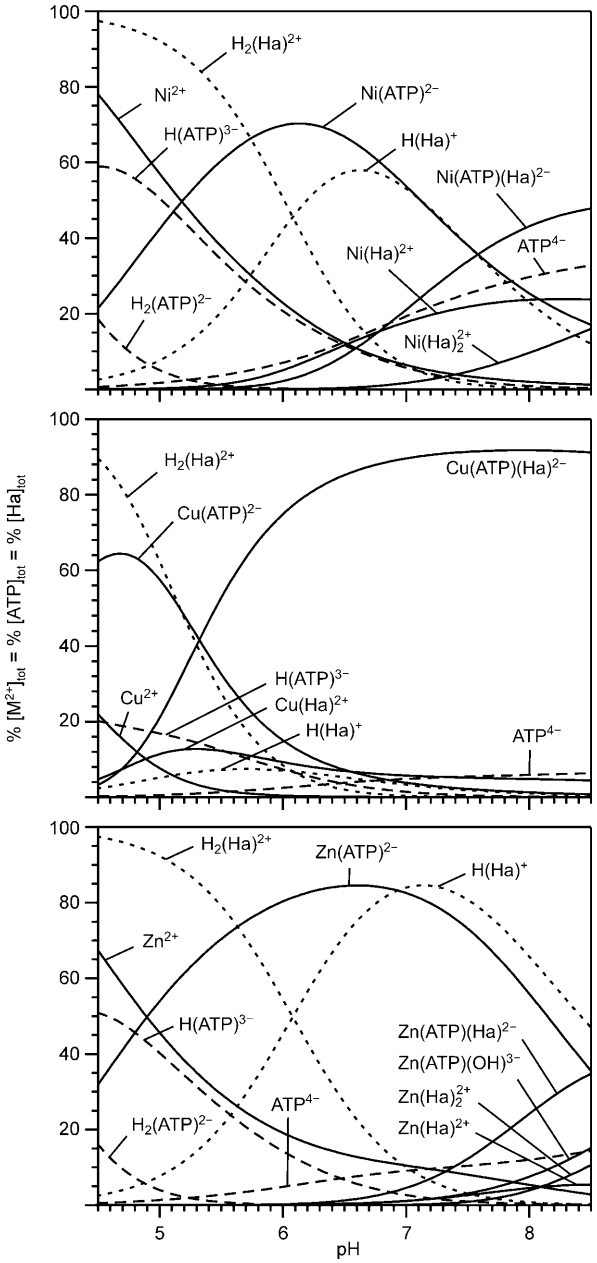


Figure 1. Influence of pH on the concentration of the species present in an aqueous solution of M^{2+}, ATP and Ha. The results are given as the percentage of the total M^{2+}, ATP or Ha present (=0.6 mM each); they were calculated with the equilibrium constants listed in Tables 1 and 2, the pK_a values of $M(H_2O)_n^{2+}$ for Ni^{2+} (9.4),[56] Cu^{2+} (7.5),[56] and Zn^{2+} (9.0),[57] and the software 'Speciation', which is part of the 'Aqua Solution Software'.[58] Written in parentheses below are the maximum concentrations of those species that occur only in small amounts in the pH range 4.5 to 8.5. They are, therefore, omitted from the diagrams. Upper part: Ni^{2+}: $Ni(H;ATP)^{-}$ (0.5% at pH 4.5), $Ni(ATP)(OH)^{3-}$ (2.1% at pH 8.5), $Ni(OH)^{-}$ (0.15% at pH 8.5) and Ha (0.5% at pH 8.5). Middle part: Cu^{2+}: $Cu(ATP)(OH)^{3-}$ (1.6%), $Cu(OH)^{-}$ (0.08%), $Cu(Ha)_2{}^{2+}$ (3.8%) and Ha (0.02%), all at pH 8.5. Lower part: Zn^{2+}: $Zn(H;ATP)^{-}$ (0.73% at pH 4.5), $Zn(OH)^{+}$ (0.9% at pH 8.5) and Ha (2.0% at pH 8.5).

$$\Delta \log K_{M/NTP/Ha} = \log K^{M(NTP)}_{M(NTP)(Ha)} - \log K^{M}_{M(Ha)} \quad (9a)$$

$$= \log K^{M(Ha)}_{M(Ha)(NTP)} - \log K^{M}_{M(NTP)} \quad (9b)$$

$$= \log \beta^{M}_{M(NTP)(Ha)} - \log K^{M}_{M(NTP)} - \log K^{M}_{M(Ha)} \quad (9c)$$

Since $\Delta \log K_{M/NTP/Ha}$ is the difference between stability constants, it has to be a constant itself and it quantifies the position of Equilibrium (10):

$$M(NTP)^{2-} + M(Ha)^{2+} \rightleftharpoons M(NTP)(Ha)^{2-} + M^{2+} \quad (10)$$

Based on experience[48,49] and statistics, *negative* values for $\Delta \log K_{M/NTP/Ha}$ are expected.[30,59,60] For example, $\Delta \log K_{2:2/stat/oh} = -0.38$ is expected for a regular octahedral coordination sphere and two different bidentate ligands.[30,60] Correspondingly, for a Jahn–Teller distorted octahedral coordination sphere of Cu^{2+} (see ref. [61]) the statistical value $\Delta \log K_{2:2/stat/Cu} \approx -0.9$ was proposed.[59] Finally, if one considers a regular octahedral coordination sphere and the equatorial binding of a tridentate (NTP^{4-}) and a bidentate ligand (Ha), one obtains the statistical value $\Delta \log K_{3:2/stat/oh} = -0.78$, because there are 12 possibilities for the coordination of the bidentate Ha to a regular octahedral coordination sphere, whereas only two possibilities remain if an NTP^{4-} is already equatorially bound; evidently, the statistical factor of 2/12 corresponds to the mentioned −0.78 log units.

The quantification of the stability of the ternary complexes, according to Equation (9), is summarized in Table 3 and is of interest regarding the first question raised in the Introduction. It is evident that the results, especially for the $M(UTP)(Ha)^{2-}$ complexes, indicate an *increase* in complex stability compared to the above statistical considerations. This is especially evident for the $Cu(UTP)(Ha)^{2-}$ complex, with $\Delta \log K_{Cu/UTP/Ha} = 0.00 \pm 0.13$ (Table 3, column 5, row 5), which is the most simple case, and which is open for an interpretation because in the equatorial (part of the) coordination sphere of Cu^{2+}, Ha and UTP^{4-} are both expected to coordinate in a bidentate manner. Hence, the stability enhancement of about 0.9 log units (based on the statistically expected value; see above) may be attributed i) to the combination of a heteroaromatic N base (the imidazole residue) and O donor sites in the coordination sphere of Cu^{2+}, a combination well known to give rise to enhanced complex stabilities due to the formation of π backbonds,[60,62,63] plus ii) some intramolecular aromatic-ring stacking between the pyrimidine and imidazole residues of UTP^{4-} and Ha, respectively. In all the other cases, these effects also exist, but are partly hidden by other effects resulting from a higher density of the NTPs in more complicated coordination spheres, as well as by macrochelate formation [Eq. (2)] in the case of the $M(ATP)^{2-}$ complexes (see below).

Table 3. Stability constants of some ternary $M(NTP)(Ha)^{2-}$ [Eq. (7)] and binary $M(Ha)^{2+}$ complexes [Eq. (4)], together with the stability differences according to Equation (9) (25 °C; $I = 0.1$ M).[a]

NTP^{4-}	M^{2+}	$\log K^{M(NTP)}_{M(NTP)(Ha)}$	$\log K^{M}_{M(Ha)}$	$\Delta \log K_{M/NTP/Ha}$
ATP^{4-}	Ni^{2+}	5.97 ± 0.09	6.82 ± 0.05	−0.85 ± 0.10
	Cu^{2+}	8.95 ± 0.09	9.56 ± 0.06	−0.61 ± 0.11
	Zn^{2+}	4.92 ± 0.07	5.21 ± 0.05	−0.29 ± 0.09
UTP^{4-}	Ni^{2+}	6.42 ± 0.09	6.82 ± 0.05	−0.40 ± 0.10
	Cu^{2+}	9.56 ± 0.12	9.56 ± 0.06	0.00 ± 0.13
	Zn^{2+}	5.08 ± 0.05	5.21 ± 0.05	−0.13 ± 0.07

[a] The values in columns 4 and 3 are from rows 1 and 7, 12 in Table 2, respectively. For the error limits see footnote [a] of Table 2.

2.3. Conclusions regarding macrochelate formation and aromatic-ring stacking: Comparison of the stability differences $\Delta \log K_{M/UTP/Ha}$ and $\Delta \log K_{M/ATP/Ha}$, as given in Table 3, reveals that the stabilities of the ternary $M(UTP)(Ha)^{2-}$ complexes are on average by about 0.4 log units more favored than those of the $M(ATP)(Ha)^{2-}$ complexes. Why? The only obvious difference between ATP^{4-} and UTP^{4-} is that in the $M(ATP)^{2-}$ species macrochelates can form [Eq. (2)], that is, that the phosphate-coordinated metal ion interacts in addition with N7 of the adenine moiety. This macrochelate formation is well established for all kinds of purine nucleotides and becomes evident from enhanced complex stabilities.[24,30,41,44,51,64] In addition, it has also been proven to occur by using various spectroscopic techniques (including crystal structures) as summarized recently.[32]

However, there are two more pertinent points, which are also well established in the literature: i) The release of the purine-N7 site from the coordination sphere of the metal ion upon formation of mixed ligand complexes and ii) the formation of intramolecular aromatic-ring stacks involving NTPs. These two points need to be discussed here in somewhat more detail.

The "weak" point in macrochelate formation of $M(ATP)^{2-}$ complexes [Eq. (2)] is the coordination of N7 of the adenine residue. Indeed, N7 release in aqueous solution is well known in the presence of bidentate ligands, such as 2,2′-bipyridine (Bpy), 1,10-phenanthroline (Phen), and L-tryptophanate, as evidenced by ^{1}H NMR studies of the corresponding ternary Mg^{2+}, Zn^{2+}/ATP complexes,[65] and the solid state.[66–68] The same is indicated, also by ^{1}H NMR spectroscopy (Zn^{2+}, Cd^{2+}, Pb^{2+}), for other amino acids including leucinate.[69] Furthermore, simple monodentate ligands like OH^-, for example, in the $Zn(ATP)(OH)^{3-}$ and $Cd(ATP)(OH)^{3-}$ complexes, promote N7 release, as was established by ^{1}H NMR shift experiments.[70] This also holds for other monodentate ligands, as they occur in the $Cd(ATP)(NH_3)^{2-}$ and $M(ATP)(imidazole)^{2-}$ species in which $M^{2+} = Zn^{2+}$ or Cd^{2+}, as established by ^{1}H NMR spectroscopy.[71]

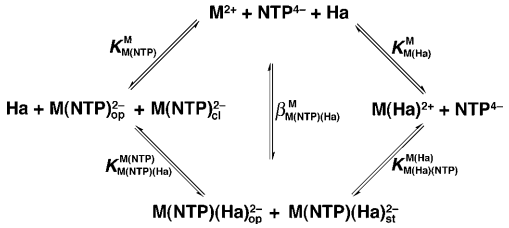
The release of N7 from the M^{2+} coordination sphere in $M(ATP)(imidazole)^{2-}$ for $M^{2+} = Mn^{2+}$, Co^{2+}, Zn^{2+}, or Cd^{2+} was also indirectly proven to occur by a careful analysis of stability data.[71] Further indirect evidence for N7 release was obtained from the M^{2+}-facilitated hydrolysis of ATP: The formation of the $M(ATP)(OH)^{3-}$ species (for Zn^{2+}, Cu^{2+}, or Cd^{2+}) inhibits the dephosphorylation reaction as this depends on N7 binding to M^{2+}.[72] The same is true for the peroxidase-like activity of $Cu(ATP)^{2-}$, which results in a

degradation of the nucleobase residue, as long as N7 is coordinated, and, therefore, upon formation of $Cu(ATP)(OH)^{3-}$ the peroxidase-like reaction is inhibited.[73,74] To conclude, based on the summarized observations it is evident that N7 will also be released from the M^{2+} coordination sphere upon formation of the $M(ATP)(Ha)^{2-}$ species. In fact, this is confirmed by the results described below (see Sections 2.4 and 2.5).

Intramolecular stack formation in $M(Arm)(NTP)^{2-}$ (Arm = Bpy or Phen) is well established and, in aqueous solution, is commonly associated with an increase in complex stability,[6] if compared to a corresponding ternary complex with the same metal ion-coordination sphere, but no possibility to undergo stacking (see for example refs. [13,14]). For example, the ternary $M(Phen)(ATP)^{2-}$ complexes show a formation degree for the intramolecular stack of 90% or more, that is, independent of the metal ion, be it Mg^{2+}, Ca^{2+}, Cu^{2+}, or Zn^{2+}.[6] Such intramolecular stacks have also been shown to occur in solution by ^1H NMR shift experiments,[6] for example, for $Mg(Phen)(ATP)^{2-}$ or Zn(Bpy or Phen)$(ATP)^{2-}$,[65] as well as by spectrophotometric measurements (charge-transfer bands) of the $M(Bpy)(ATP\text{ or }ITP)^{2-}$ complexes (ITP^{4-} = inosine 5′-triphosphate) with Co^{2+}, Ni^{2+}, Cu^{2+}, or Zn^{2+} (ref. [75]) and they also occur in the solid state.[66,68,76]

Regarding protein–nucleic acid interactions stacks and/or hydrophobic interactions have been demonstrated to occur (^1H NMR spectroscopy) between the indole or isopropyl residue and the purine moiety in $M(ATP)(Trp\text{ or }Leu)^{3-}$ species.[6,69] However, there is also evidence that, for $M(ATP)(imidazole)^{2-}$ complexes, intramolecular stacking between the purine moiety and the imidazole ring occurs to some extent (^1H NMR/Zn^{2+}, Cd^{2+}).[77] Interestingly, for the Ni(ATP)(histidinate)$^{3-}$ complex, intramolecular stack formation between the purine moiety and the imidazole ring was also anticipated.[39] Important, for the present context is, however, the conclusion, based on thermodynamic parameters (ΔH^0, ΔS^0), that in $Zn(ATP)(histamine)^{2-}$ intramolecular stacking occurs.[36] Hence, we have to conclude that the intramolecular Equilibrium (1) actually exists.

2.4. Relevance of macrochelate formation for the reduced stability of the $M(ATP)(Ha)^{2-}$ complexes and extent of stacking in $Cu(UTP)(Ha)^{2-}$: What is the reason for the observation that $\Delta \log K_{M/UTP/Ha} > \Delta \log K_{M/ATP/Ha}$ (see Table 3, column 5)? Evidently, one cannot postulate that in the $M(UTP)(Ha)^{2-}$ complexes the intramolecular stacks are of a higher stability than in $M(ATP)(Ha)^{2-}$. If there were a difference, it would have to be the other way round (see also below). Hence, the lower stability of the $M(ATP)(Ha)^{2-}$ complexes has to be a result of the release of N7 from the coordination sphere of M^{2+} upon formation of the mixed ligand complexes. That is, upon formation of $M(ATP)(Ha)^{2-}$ the N7–M^{2+} bond needs to be broken and the energy needed for this process goes on the account of the stability of the ternary complex. Rather than performing further ^1H NMR (or other spectroscopic) measurements, we decided for a rigorous analytical evaluation of the available stability data, because N7 release upon mixed-ligand complex formation, as well as stacking between a nucleobase residue and the imidazole moiety, has already been established beyond any doubt (see the summaries above in Section 2.3). NMR measurements would only prove something that we already know, but would not allow a quantitative evaluation, because the limiting chemical shifts (for complete stacking or complete macrochelate formation) cannot be measured, but only assumptions can be made.[65,77,78] In contrast, a rigorous evaluation of the stability data should allow quantitative conclusions about the formation degrees of the species and reveal the intricate network of equilibria that exists between the binary and ternary systems (Scheme 2).



Scheme 2. An extended version of the Equilibrium scheme (5), which now also displays the isomers of the intramolecular Equilibria (1) and (2). It needs to be emphasized that the isomeric open (op) or macrochelated/closed (cl) $M(NTP)^{2-}_{op}$ and $M(NTP)^{2-}_{cl}$ complexes [Eq. (2)] are interlinked by the concentration-independent, intramolecular and dimensionless equilibrium constant K_I; with $K_I = [M(NTP)^{2-}_{cl}]/[M(NTP)^{2-}_{op}] = 0$ the situation for the $M(UTP)^{2-}$ species is described. For the isomeric equilibrium involving stack (st) formation, $[M(NTP)(Ha)^{2-}_{op}] \rightleftharpoons [M(NTP)(Ha)^{2-}_{st}]$ [Eq. (1)], the analogous reasoning holds. See text in Sections 2.4 and 2.5.

At this point it may be helpful to recall that the formation degrees estimated for the intramolecular stacks in $Zn(Bpy)(UTP)^{2-}$ and $Zn(Bpy)(ATP)^{2-}$ amounted to 40 and 55%, respectively.[65] This means, there is a difference, but, in this instance, not a very significant one. It is expected that the replacement of the two-ring Bpy by the single-ring imidazole (in Ha) should make the stacking interaction even more similar, because the two-ring purine and the single-ring pyrimidine each have as a partner only the single-ring imidazole. Indeed, it has previously been shown[71] that the stacking intensity for $M(ATP)(imidazole)^{2-}$ and $M(UTP)(imidazole)^{2-}$, for $M^{2+} = Mn^{2+}$, Co^{2+}, Zn^{2+}, or Cd^{2+}, is identical within the error limits. We postulate that the same is true for the $M(ATP)(Ha)^{2-}$ and $M(UTP)(Ha)^{2-}$ complexes.

The proof of this postulate, as given below, follows closely the earlier analysis developed by Martin (for details see ref. [71]). The basic reasoning of the procedure is: i) The difference in the stabilities of the binary $M(ATP)^{2-}$ and $M(UTP)^{2-}$ complexes reflects the extent of the N7–M^{2+} interaction[44,51] and is expressed by the term $\log \Delta_{Binary}$, which equals the difference of the two corresponding log stability constants (Table 2). ii) The difference in the stabilities of the ternary $M(ATP)(Ha)^{2-}$ and $M(UTP)(Ha)^{2-}$ complexes, that

is, $\log \Delta_{Ternary}$, reflects also the intensity of the N7–M^{2+} interaction, but with a reverse sign because the bond needs now to be broken, plus a possible effect due to a different extent of stacking. iii) If the stacking interactions in $M(ATP)(Ha)^{2-}$ and $M(UTP)(Ha)^{2-}$ equal each other, that is, their contribution cancels, then the stability differences $\log \Delta_{Binary}$ and $\log \Delta_{Ternary}$ must be identical, yet with an opposite sign.

Table 4. Comparison of the stability constants of the binary $M(ATP)^{2-}$ and $M(UTP)^{2-}$ complexes as well as of the corresponding ternary $M(ATP)(Ha)^{2-}$ and $M(UTP)(Ha)^{2-}$ complexes containing histamine (Ha) (aqueous solution; 25 °C; $I = 0.1$ M).[a]

M^{2+}	$\log K^{M}_{M(ATP)}$	$\log K^{M}_{M(UTP)}$	$\log \Delta_{Binary}$[b]	$\log K^{M(ATP)}_{M(ATP)(Ha)}$	$\log K^{M(UTP)}_{M(UTP)(Ha)}$	$\log \Delta_{Ternary}$[b]	$\Delta \log \Delta_{Ha}$[c]
Ni^{2+}	4.86 ± 0.05	4.47 ± 0.03	0.39 ± 0.06	5.97 ± 0.09	6.42 ± 0.09	−0.45 ± 0.13	−0.06 ± 0.14
Cu^{2+}	6.34 ± 0.03	5.87 ± 0.03	0.47 ± 0.04	8.95 ± 0.09	9.56 ± 0.12	−0.61 ± 0.15	−0.14 ± 0.17
Zn^{2+}	5.16 ± 0.06	5.01 ± 0.03	0.15 ± 0.07	4.92 ± 0.07	5.08 ± 0.05	−0.16 ± 0.09	−0.01 ± 0.11

[a] The values in columns 2, 3, 5, and 6 are from rows 4, 9, 7, and 12 in Table 2, respectively. For the error limits see footnote [a] of Table 2. [b] Differences between the values in the two columns to the left. [c] $\Delta \log \Delta_{Ha} = \log \Delta_{Binary} + \log \Delta_{Ternary}$.

The data of the corresponding evaluation are summarized in Table 4. It is evident that the absolute values of $\log \Delta_{Binary}$ and $\log \Delta_{Ternary}$ are identical within their error limits. In other words, the differences between these values, which are given as $\Delta \log \Delta_{Ha}$ in column 8 of Table 4, are zero within their error limits. This confirms the above postulate that the extent of stacking in the two ternary complexes, $M(ATP)(Ha)^{2-}$ and $M(UTP)(Ha)^{2-}$, is identical or at least very similar.

What is the formation degree of the intramolecular stack in these $M(NTP)(Ha)^{2-}$ complexes? Unfortunately, there is only a single case for which an estimation is possible: For the $Cu(UTP)(Ha)^{2-}$ complex $\Delta \log K_{Cu/UTP/Ha} = 0.00 \pm 0.13$ (Table 3) holds and as discussed above in the final paragraph of Section 2.2, this increased stability is attributed to i) the combination of Arm-N/O donors at the Cu^{2+} sites and ii) stack formation between the pyrimidine/imidazole rings. Because for the ternary complex Cu(4-aminomethylimidazole)(pyrocatecholate) $\Delta \log K_{Cu/Ami/Pyr} = -0.35 \pm 0.05$ holds[62] and because this complex (in which no stacking is possible for steric reasons) has the same pattern of coordinating atoms towards Cu^{2+}, the difference $\Delta\Delta \log K = \Delta \log K_{Cu/UTP/Ha} - \Delta \log K_{Cu/Ami/Pyr} = (0.00 \pm 0.13) - (-0.35 \pm 0.05) = 0.35 \pm 0.14$ (3σ) reflects the stability enhancement owing to intramolecular stack formation in $Cu(UTP)(Ha)^{2-}$ according to Equilibrium (1). Note, pyrocatecholate is here a good model for the bidentate O-donor binding of the triphosphate residue of UTP^{4-} to Cu^{2+}, because in ternary Cu^{2+} complexes of Bpy, pyrocatecholate and methylphosphonylphosphate, CH_3–$P(O)_2^-$–O–PO_3^{2-} (a diphosphate analogue) behave alike.[79] By following known routes[13,14,80] the intramolecular, dimensionless equilibrium constant K_I can now be calculated, $K_I = 10^{\Delta\Delta \log K} - 1 = 1.24 \pm 0.72$, and from this follows the formation degree of the stack in $Cu(UTP)(Ha)^{2-}$ amounting to 55 ± 15 %. This result is in excellent agreement with the 40 % given above for $Zn(Bpy)(UTP)^{2-}$,[65] which also contains a one-ring/one-ring interaction. It is further in the same order, as observed by using ^{1}H NMR spectroscopy for $M(ATP)(imidazole)^{2-}$ complexes for which stack formation varies between 15 and 55 %.[77]

2.5. Extent of macrochelate formation in the $M(ATP)^{2-}$ complexes, based on the stabilities of the ternary $M(NTP)(Ha)^{2-}$ species: The consequence of the above evaluation (see also ref. [71]) regarding $\Delta \log \Delta_{Ha} \approx 0$ (Table 4) is that the differences between the differences defined by Equation (9), which are listed in Table 3 (column 5), reflect the extent of the N7–M^{2+} interaction, and thus the formation degree of the macrochelate [Eq. (2)], in the $M(ATP)^{2-}$ complexes. Hence, the definition given in Equation (11) holds:

$$\Delta\Delta \log K_{M/UTP,ATP/Ha} = \Delta \log K_{M/UTP/Ha} - \Delta \log K_{M/ATP/Ha} \quad (11)$$

If we define $\Delta\Delta \log K_{M/UTP,ATP/Ha}$ as the logarithm of the stability enhancement *SE*, we obtain, based on Equation (9), Expression (12):

$$\log SE = \left(\log K^{M(UTP)}_{M(UTP)(Ha)} - \log K^{M}_{M(Ha)}\right) - \left(\log K^{M(ATP)}_{M(ATP)(Ha)} - \log K^{M}_{M(Ha)}\right) \quad (12a)$$

$$= \log K^{M(UTP)}_{M(UTP)(Ha)} - \log K^{M(ATP)}_{M(ATP)(Ha)} \quad (12b)$$

From Equation (12b), the Expression (13) follows:

$$10^{\log SE} = \frac{[M(UTP)(Ha)^{2-}]}{[M(UTP)^{2-}][Ha]} \cdot \frac{[M(ATP)^{2-}][Ha]}{[M(ATP)(Ha)^{2-}]} \quad (13a)$$

$$= \frac{[M(UTP)(Ha)^{2-}][M(ATP)^{2-}]}{[M(UTP)^{2-}][M(ATP)(Ha)^{2-}]} \quad (13b)$$

Considering that in Equation (13b) the term $[M(ATP)^{2-}]$ encompasses the total analytical concentration of $M(ATP)^{2-}$, that is, of the open (op) and closed (cl) isomers seen in Equilibrium (1), we obtain Equation (14):

$$10^{\log SE} = \frac{[M(UTP)(Ha)^{2-}]\left(\left[M(ATP)^{2-}_{op}\right] + \left[M(ATP)^{2-}_{cl}\right]\right)}{[M(UTP)^{2-}][M(ATP)(Ha)^{2-}]} \quad (14)$$

Considering further, as we saw above in Section 2.4, that the extent of the intramolecular stacking in the two $M(ATP)(Ha)^{2-}$ and $M(UTP)(Ha)^{2-}$ complexes is the same, their stabilities are the same and thus their concentrations cancel and because $M(UTP)^{2-}$ represents $M(ATP)^{2-}_{op}$ Equa-

tion (14) reduces to Equation (15):

$$10^{\log SE} = \frac{\left[M(ATP)^{2-}_{op}\right] + \left[M(ATP)^{2-}_{cl}\right]}{\left[M(ATP)^{2-}_{op}\right]} \quad (15a)$$

$$= 1 + \frac{\left[M(ATP)^{2-}_{cl}\right]}{\left[M(ATP)^{2-}_{op}\right]} \quad (15b)$$

Since the ratio $\left[M(ATP)^{2-}_{cl}\right]/\left[M(ATP)^{2-}_{op}\right]$ reflects the position of the intramolecular Equilibrium (2), it also defines its dimension-less equilibrium constant, K_I, as given in Equation (16):

$$K_I = \frac{\left[M(ATP)^{2-}_{cl}\right]}{\left[M(ATP)^{2-}_{op}\right]} = 10^{\log SE} - 1 \quad (16)$$

Finally, with K_I known, the percentage of the closed or macrochelated species in Equilibrium (2) follows from Equation (17):

$$\%M(ATP)^{2-}_{cl} = 100K_I/(1 + K_I) \quad (17)$$

Application of the indicated procedure yields the results summarized in Table 5. Clearly, the formation degree of the macrochelated species is remarkable for all three $M(ATP)^{2-}$ complexes studied. Of further interest is a comparison of the present results, obtained by means of data from ternary complexes, with previous results obtained by different methods. This overview is provided in Table 6.[81,82]

Table 5. Extent of intramolecular macrochelate formation in $M(ATP)^{2-}$ complexes based on a comparison of the stability constants of the corresponding ternary $M(UTP)(Ha)^{2-}$ and $M(ATP)(Ha)^{2-}$ complexes (aqueous solution; 25 °C; $I = 0.1$ M).[a]

M^{2+}	$\log K^{M(UTP)}_{M(UTP)(Ha)}$ (Table 2, row 12)	$\log K^{M(ATP)}_{M(ATP)(Ha)}$ (Table 2, row 7)	$\log SE$ [Eq. (12b)]	K_I [Eq. (16)]	$\%M(ATP)^{2-}_{cl}$ [Eq. (17)]
Ni^{2+}	6.42 ± 0.09	5.97 ± 0.09	0.45 ± 0.13	1.82 ± 0.83	65 ± 10
Cu^{2+}	9.56 ± 0.12	8.95 ± 0.09	0.61 ± 0.15	3.07 ± 1.41	75 ± 8
Zn^{2+}	5.08 ± 0.05	4.92 ± 0.07	0.16 ± 0.09	0.45 ± 0.29	31 ± 14

[a] For the reasoning behind this evaluation see text in Sections 2.4 and 2.5 for details. For the error limits see footnote [a] of Table 2.

Comparison of the various results shows that the present percentages for $M(ATP)^{2-}_{cl}$ agree within the error limits excellently with the previous results from the literature (Table 6). This observation is very satisfying because it confirms the correctness and inner consistency of our stability data. Furthermore, it confirms that the various assumptions made during the evaluation process, including that the intramolecular stack formation of $M(ATP)(Ha)^{2-}$ and $M(UTP)(Ha)^{2-}$ occurs to about the same extent, are correct.

Table 6. Comparison of the extent of intramolecular macrochelate formation in $M(ATP)^{2-}$ complexes [Eq. (2)] as determined by various methods: The results are given as $\%M(ATP)^{2-}_{cl}$ [Eq. (17)] (aqueous solution; 25 °C; $I = 0.1$ M).[a]

M^{2+}	Present results[b]	Via binary complexes[c]	Various methods
Ni^{2+}	65 ± 10	56 ± 6	59[d]/60[e]/59[f]
Cu^{2+}	75 ± 8	67 ± 3	68 ± 4[g]
Zn^{2+}	31 ± 14	28 ± 10	26 ± 5[h]

[a] For the error limits see footnote [a] of Table 2. [b] From Table 5, column 6. [c] These values were calculated based on the stability constants determined for binary complexes[44] by potentiometric pH titrations (see also refs. [30,51,64]). [d] Calculated from the stability constants given in ref. [81] (15 °C; $I = 0.1$ M, KNO_3): $\log K^{Ni}_{Ni(ATP)} = 4.79$ and $\log K^{Ni}_{Ni(ATP)op} = 4.40$ (based on $Ni(CTP)^{2-}$ and $Ni(H;P_3O_{10})^{2-}$) (see pages 81, 95 and 105 in ref. [81]) give $K_I = 1.455$. [e] Calculated from $K_I = 1.5$ given in ref. [82], based on stability constants data (15 °C; $I = 0.1$ M, KNO_3). [f] From kinetic data in ref. [82] $K_I = 1.429$ follows and this yields the above percentage (15 °C; $I = 0.1$ M, KNO_3). [g] Based on potentiometric pH titrations of binary complexes.[45] [h] Calculated via the stability of ternary M(NTP)(imidazole)$^{2-}$ complexes.[71]

3. Conclusions

What have we learned from studying the stabilities of the ternary M/ATP or UTP/Ha systems?

i) The results show that intramolecular stacks in $M(ATP)(Ha)^{2-}$ and $M(UTP)(Ha)^{2-}$ form and that their formation degree is, for a given metal ion, very similar. For protein/nucleic acid interactions, this may be of relevance, as the number of potentially available nucleobase residues for such an adduct formation (with indole, phenyl or imidazole residues) is rather large. This view is supported by the known intramolecular stacking occurring in $M(ATP)(imidazole)^{2-}$ (ref. [77]) and $M(ATP)(tryptophanate)^{3-}$ (refs. [6,65,69]) complexes, as well as by the described[26] hydrophobic interaction, which occurs between three tyrosine residues (of the pol α RB69 DNA polymerase) and DNA to form a tight-fitting binding pocket at the minor groove side. Similarly, for T7 RNA polymerase it was shown[25] that during translocation a tyrosine residue stacks with nucleobases of DNA and RNA. A related example emphasizes further the general importance of stacking interactions, that is, in *Anabaena* flavodoxin is the isoalloxazine moiety of flavin mononucleotide (FMN) sandwiched by the aromatic rings of hydroxyphenyl (tyrosine) and indole (tryptophan) residues.[6,83]

ii) The formation degrees of the intramolecular stacks could only be determined for a single case, $\%M(UTP)(Ha)^{2-}_{stack} = (55 \pm 15)\%$ (Section 2.4). However, it is estimated to be generally between about 20 to 50%, based on results obtained for $M(ATP)(imidazole)^{2-}$ complexes.[77] Clearly, such interactions are weak because at 25 °C a formation degree of 20% ($\log SE = 0.1$) corresponds to a change in free energy (ΔG^0) of only -0.57 kJ mol^{-1} and 50% ($\log SE = 0.3$) correspond to -1.71 kJ mol^{-1}.[84] This means, that if such percentages of a substrate are needed to be in the correct conformation/orientation for allowing the enzyme to proceed with a reaction, the amount of energy in-

volved is small. On the other hand, in certain adducts, as indicated in i), the number of interactions may be substantial.

iii) An interesting conclusion that follows from the overall results is that an imidazole residue coordinated to a metal ion may still undergo stacking interactions with another suitable aromatic-ring system. A similar situation was recently observed in the solution structure of a metal-modified DNA duplex containing imidazole–Ag^+–imidazole base pairs.[85] In this context it is worthwhile to recall that, for example, in a fern plastocyanin an imidazole-phenyl (histidine/phenylalanine) stack was observed.[7,8]

iv) Another remarkable (though indirect) observation is the fact that the N7–M^{2+} bond present in the binary $M(ATP)^{2-}$ macrochelates is broken upon formation of mixed ligand complexes as follows from the internal validity of the data in Section 2.4 (Table 4). This conclusion is in accord with the previous results summarized in Section 2.3, that is, the release of N7 in $M(ATP)^{2-}$ complexes by the coordination of, for example, OH^- or NH_3, as shown by using NMR spectroscopy.[70,71] Consequently, these results suggest that, when bound to an enzyme, the complexes may exist in a closed macrochelated form only, if no enzyme groups coordinate directly to the metal ion—outersphere interactions may be allowed.

v) Although not studied here, different types of macrochelates may form. For example, based on a combination of several methods, it was previously concluded for $Ni(ATP)^{2-}$ that about 30% form the macrochelate with N7 being innersphere coordinated, 25% being N7 outersphere bound (i.e., with a water molecule between Ni^{2+} and N7), and 45% exist as $Ni(ATP)^{2-}_{op}$, that is, in the open form of the Equilibrium (2).[44,64,86] For $Zn(ATP)^{2-}$ also some outersphere binding was suggested, whereas Cu^{2+} only binds to N7 innersphere.[64,86]

4. Experimental Section

4.1. Materials: The disodium salt of adenosine 5′-triphosphate, the trisodium salt of uridine 5′-triphosphate, histamine dihydrochloride, and the disodium salt of 1,2-diaminoethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (Na_2H_2EDTA) were purchased from Sigma–Aldrich Co. (St. Louis, USA) with a minimum purity of 99%. Nitric acid and the nitrate salts of Ni^{2+}, Cu^{2+}, and Zn^{2+} were from Merck KGaA (Darmstadt, Germany), and potassium hydrogen phthalate (all *pro analysi)* from Fluka AG (Buchs, Switzerland). All other materials, including the buffer solutions and ultrapure, CO_2-free water, were the same as used previously.[47]

The concentrations of the NaOH and $M(NO_3)_2$ stock solutions were determined as described.[87] The aqueous stock solutions of histamine (Ha) were freshly prepared daily, and their exact concentrations were determined in each experiment by evaluation of the corresponding titration pair, that is, the differences in NaOH consumption between solutions with and without histamine (see below). Aqueous stock solutions of the nucleoside 5′-triphosphates were also freshly prepared daily and their concentrations determined by photometry by using the molar absorption coefficients ($M^{-1}cm^{-1}$) of $\varepsilon_{259}=15400$ for ATP (pH 7.0) and of $\varepsilon_{262}=10000$ for UTP (pH 11), respectively.[88] The NTP stock solutions were adjusted to pH 8.1 with sodium hydroxide and then mixed with equimolar amounts of $M(NO_3)_2$ ($M^{2+}=Ni^{2+}$, Cu^{2+}, Zn^{2+}) directly before use to minimize dephosphorylation[72] of the nucleoside 5′-triphosphates (see also below).

4.2. Potentiometric pH titrations: All titrations were performed with the same equipment as used previously and calibrated as described.[47] The acidity constants determined at $I=0.1$ M ($NaNO_3$) and 25 °C are so-called practical, mixed, or Brønsted constants, which may be converted into the corresponding concentration constants by subtracting 0.02 from the listed pK_a values.[42] It should be noted that the ionic product of water (K_w) and the mentioned conversion term do not enter into our calculations, because the differences in NaOH consumption between solutions with and without histamine are evaluated.[42,89] The presented stability constants of the complexes are, as usual, concentration constants.

4.3. Determination of equilibrium constants: The acidity constants $K^{H}_{H_2(Ha)}$ and $K^{H}_{H(Ha)}$ of $H_2(Ha)^{2+}$ [Eq. (3)] were determined by titrating aqueous HNO_3 (50 mL, 0.2 mM) (25 °C; $I=0.1$ M, $NaNO_3$) under N_2 with up to 1.5 mL NaOH (0.06 M) in the presence and absence of $H_2(Ha)^{2+}$ (0.60 mM). A second set of titrations was performed using a $H_2(Ha)^{2+}$ concentration of 0.30 mM. It should be emphasized that the calculated acidity constants showed no dependence on the histamine concentration. The experimental data were evaluated with a curve-fitting procedure by using a Newton–Gauss non-linear least-squares program by employing the differences in NaOH consumption, every 0.1 pH unit, between the two titrations, that is, with and without histamine. The acidity constants of $H_2(Ha)^{2+}$ were calculated in the pH range 5.5 to 10.5, corresponding to 21% neutralization (initial) for the equilibrium $H_2(Ha)^{2+}/H(Ha)^+$ and 81% (final) for $H(Ha)^+$/Ha. The final results for the acidity constants of $H_2(Ha)^{2+}$ are the averages of the values from ten independent pairs of titrations.

The stability constants of the $M(NTP)(Ha)^{2-}$ complexes with $M^{2+}=Ni^{2+}$, Cu^{2+}, or Zn^{2+} and $NTP^{4-}=ATP^{4-}$ or UTP^{4-} were determined under the conditions used for the acidity constants, but now $NaNO_3$ was partly replaced by a 1:1 mixture of M^{2+} and NTP^{4-}, which exists to the largest part as $M(NTP)^{2-}$ (25 °C; $I=0.1$ M). The ratios between M^{2+}/NTP^{4-} and histamine in the various titrations were 2.6:1, 1.7:1, 1.3:1, and 1.05:1 for Ni^{2+}/ATP^{4-} and Cu^{2+}/ATP^{4-}; 2.6:1 and 1.5:1 for Zn^{2+}/ATP^{4-}; 2.6:1, 1.7:1, 1.3:1, and 1.05:1 for Ni^{2+}/UTP^{4-}; 1.7:1, 1.3:1, and 1.05:1 for Cu^{2+}/UTP^{4-}; and 2.6:1 and 1.7:1 for Zn^{2+}/UTP^{4-}. For all systems it holds that the calculated stability constants showed no dependence on the excess of M^{2+}/NTP^{4-} used.

The data were collected every 0.1 pH unit and the stability constants $\beta^{M}_{M(NTP)(Ha)}$ [Eqs. (5) and (6)] were calculated by taking into account the species H^+, $H_2(NTP)^{2-}$, $H(NTP)^{3-}$, NTP^{4-}, $H_2(Ha)^{2+}$, $H(Ha)^+$, Ha, M^{2+}, $M(NTP)^{2-}$, $M(Ha)^{2+}$, $M(Ha)^{2+}_2$, and $M(NTP)(Ha)^{2-}$.[90,91] The acidity constants of $H_2(ATP)^{2-}$ and $H_2(UTP)^{2-}$, as well as the stability constants of the metal ion complexes of $M(ATP)^{2-}$, $M(UTP)^{2-}$, $M(Ha)^{2+}$, and $M(Ha)^{2+}_2$, needed for the evaluation were taken from the literature (see Tables 1 and 2, vide infra). In addition, the data were evaluated for $K^{M(NTP)}_{M(NTP)(Ha)}$ [Eqs. (4) and (5)], that is, by attributing the protons released from $H_2(Ha)^{2+}/H(Ha)^+$ to the formation of the ternary complex; thus only the species H^+, $H_2(Ha)^{2+}$, $H(Ha)^+$, Ha, $M(NTP)^{2-}$, and $M(NTP)(Ha)^{2-}$ were considered.[34,69] The evaluations commenced at a formation degree of the $M(ATP)^{2-}$ species of at least 85%, while the upper limit was given by the onset of the deprotonation of a water ligand in $M(ATP)(H_2O)^{2-}_n$ or by deprotonation of (N3)H in $M(UTP)^{2-}$ species, both values appear in the literature.[53,54] For a comparison of the two evaluation methods ref. [90] should be consulted.

Representative examples for the pH ranges employed by both evaluation methods are 6.6–8.1 (Ni^{2+}), 5.0–6.1 (Cu^{2+}), and 7.5–8.0 (Zn^{2+}). The results obtained by the two (rather different) evaluation methods were quite similar and overlapping within their error limits; therefore, we decided to use the averaged values as the final results. Finally, it should be mentioned that the accessible pH range for the evaluation of the $Zn(ATP)(Ha)^{2-}$ and $Zn(UTP)(Ha)^{2-}$ complexes is rather small. In these instances only the second evaluation procedure was employed[90] and thus the corresponding stability constants should be considered as estimates only. However, at the same time it needs to be emphasized that in the evaluations in Sections 2.4 and 2.5 only stability constant differences are employed and thus any systematic errors cancel.

The final results for the stability constants of the $M(NTP)(Ha)^{2-}$ complexes are the averages of at least three independent titrations, usually four or five. There was no indication of a metal ion-promoted hydrolysis of the nucleoside triphosphates during the time required for a titration experiment (in the maximum about 30 min).

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