the Cr(III) analogues (Table IV). However, these differences are explained readily by the extra contribution to $\Delta H^{\ddagger}$ of 17 kJ mol^{-1}, which is required to break the shorter Cr–Cl bond of the pentakis(methylamine) complex compared to that of the pentaammine complex. When this contribution is taken into account, the values of $\delta(\Delta G^{\ddagger})$ are −39 kJ mol^{-1} and −34 kJ mol^{-1}, respectively, for Co(III) and Cr(III). These two numbers are approximately equal within the experimental errors of the kinetic studies. Moreover, it is clear that the major contribution to the differences in the behavior of Co(III) and Cr(III) is the much greater value of $\Delta H^{\ddagger}$ for $[Cr(NH_2CH_3)_5Cl]^{2+}$ as opposed to that obtained for $[Co(NH_2CH_3)_5Cl]^{2+}$. While the errors in $\Delta H^{\ddagger}$ are large, especially for the Co(III) complexes, the trend clearly points to a common dissociative conjugate base mechanism operating for both Co(III) and Cr(III).

Conclusions. A reexamination of the available evidence points to I_d mechanisms operating for the spontaneous aquations of both $[Co^{III}(NH_2R)_5X]^{n+}$ and $[Cr^{III}(NH_2R)_5X]^{n+}$ complexes, with similar activated states for the two metal ions. The kinetic differences are mainly attributable to the effects of π-bonding and the ionic radii of the metal ions in the ground state, rather than any inherent differences in the activated states. Similarly, the limiting S_N1CB mechanism applies equally well to the base hydrolyses of both Co(III) and Cr(III). It is also likely that the kinetic behavior of Rh(III), which is intermediate between Co(III) and Cr(III), may be due to ground-state rather than activated-state differences, and this aspect is currently being explored.

In hindsight, the enthalpy and entropy of activation give more mechanistic information than is generally recognized. It has been argued often that because $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are not generally sensitive to whether or not Co(III) or Cr(III) is the metal ion involved in the substitution reaction, they are not useful for mechanistic assignments. However, the analysis described here indicates that these arguments were based on the false premise that the mechanisms of spontaneous aquations of Co(III) and Cr(III) are different.

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Comparison of the Stabilities of Monomeric Metal Ion Complexes Formed with Adenosine 5′-Triphosphate (ATP) and Pyrimidine-Nucleoside 5′-Triphosphates (CTP, UTP, TTP) and Evaluation of the Isomeric Equilibria in the Complexes of ATP and CTP

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Under experimental conditions where the self-association of the nucleoside 5′-triphosphates (NTPs) ATP, CTP, UTP, and TTP is negligible, potentiometric pH titrations were carried out to determine the stabilities of the $M(H{\cdot}NTP)^-$ and $M(NTP)^{2-}$ complexes, with $M^{2+} = Mg^{2+}$, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+} ($I = 0.1$; 25 °C). These experiments were evaluated and combined with previous results from the same laboratory to obtain a large number of experimental data. The aim of this procedure was to generate reliable stability constants, especially for $M(NTP)^{2-}$, with a clearly defined error range. The stability of most of the $M(ATP)^{2-}$ complexes was significantly larger than that of the corresponding complexes formed with the pyrimidine-nucleoside 5′-triphosphates (PNTPs); this increased stability was attributed, in agreement with previous research, to the formation of macrochelates. The percentage of the macrochelated isomers in the $M(ATP)^{2-}$ systems was quantified by employing the difference $\log K^{M}_{M(ATP)} - \log K^{M}_{M(PNTP)}$: with the exception of $Ca(ATP)^{2-}$, which exists only as a phosphate-coordinated species, all mentioned $M(ATP)^{2-}$ complexes form to different extents macrochelates, $M(ATP)^{2-}_{cl}$ (e.g., $Cu(ATP)^{2-}_{cl}$ and $Zn(ATP)^{2-}_{cl}$ are formed to 67 ± 2% and 28 ± 7%, respectively). When earlier results of spectrophotometric studies are taken into account, it becomes evident that of the 56 ± 4% of $Ni(ATP)^{2-}_{cl}$ about 25% exists in the form of an outer-sphere macrochelate; i.e., a water molecule is between N-7 and the phosphate-coordinated Ni^{2+}. Similar reasoning and ^1H NMR shift measurements indicate that probably 11 ± 6% of $Mg(ATP)^{2-}$ is also present in aqueous solution in the form of such an outer-sphere-macrochelated isomer. Careful analysis of all available data indicates that inner- and outer-sphere forms of $M(ATP)^{2-}$ occur for Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, and Cd^{2+} in comparable amounts; $Cu(ATP)^{2-}$ forms no outer-sphere species to any significant extent, and $Ca(ATP)^{2-}$ exists only in the open, phosphate-coordinated form. Of all $M(PNTP)^{2-}$ complexes only $Cu(CTP)^{2-}$ forms a base-back-bound species (32 ± 6%); this base back-binding is confirmed by UV difference spectroscopy. In a detailed analysis the isomeric equilibria occurring with $M(H{\cdot}NTP)^-$ complexes are evaluated; estimates for the formation degree of the isomers carrying the proton at the nucleic base residue or at the γ-phosphate group are given by taking into account also macrochelate formation where appropriate. As M^{2+}/NTP complexes are the substrates for many enzymic reactions, some possible biological implications of these results regarding selectivity are indicated.

Enzymic reactions involving[2] ATP and other NTPs are metal ion dependent,[3-8] the metal–NTP complexes being usually the substrates. This dependence explains the interest of many coordination chemists in these complexes; considering the metabolic importance of ATP, their efforts are also well justified. In the case of ATP, the most basic site at the adenine residue is N-1,[9]

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(2) Abbreviations: Ado, adenosine; AMP, adenosine 5′-monophosphate; ATP, adenosine 5′-triphosphate; CTP, cytidine 5′-triphosphate; Cyt, cytidine; dien, diethylenetriamine (=1,4,7-triazaheptane); M^{2+}, general divalent metal ion; NTP, nucleoside 5′-triphosphate; PNTP, pyrimidine-nucleoside 5′-triphosphate, i.e. CTP, UTP, or TTP; UTP, uridine 5′-triphosphate; TTP, thymidine 5′-triphosphate. The phosphate groups in the NTPs are labeled α, β, and γ, where the last refers to the terminal phosphate group.

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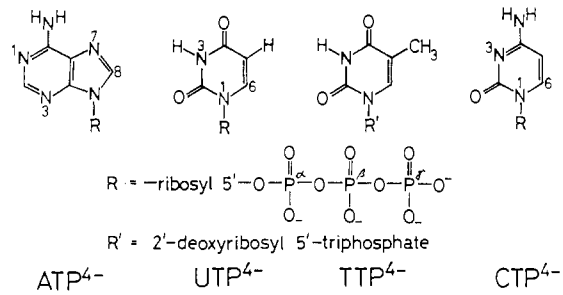

Figure 1. Structures of the nucleoside 5'-triphosphates (NTP^{4-}) used in this study.

while metal ions when already bound to the phosphate chain may also interact with N-7.[10] This N-7 interaction occurs in 1:1 complexes with transition-metal ions and Zn^{2+} or Cd^{2+},[11-13] whereas Mg^{2+} does not undergo *direct* interaction with the adenine ring system.[10,11]

However, knowing the composition of the complexes and certain sites of interaction does not entail knowing their structures: there is indeed much confusion in the literature about the structure of $M(ATP)^{2-}$ complexes. Part of this confusion was resolved[11] by showing that metal ions promote the self-association of ATP, which occurs via stacking of the purine residues. For example, if one wants to learn something on the properties of $Mg(ATP)^{2-}$ or $Zn(ATP)^{2-}$, one has to work at concentrations smaller than 5×10^{-3} or 10^{-3} M, respectively;[11] only under these conditions is about 96% or more of the complexes present in their monomeric form.

An evaluation[11] of the literature that takes into account only those studies[14-17] in which the monomeric forms strongly dominated and compares the percentages of the macrochelated isomers calculated from stability constants determined by potentiometric pH titrations with the percentages obtained from UV spectrophotometric or NMR studies indicates that for some $M(ATP)^{2-}$ complexes larger percentages are obtained with the first-mentioned method. In a previous spectrophotometric study[17] of $Ni(ATP)^{2-}$ this apparent discrepancy was resolved by postulating that three isomers exist in equilibrium: (i) an "open" isomer, with the metal ion only coordinated to the phosphate chain; (ii) an isomer in which the metal ion is also coordinated to N-7; (iii) an outer-sphere isomer, i.e. a species with a water molecule between N-7 and the metal ion. Clearly, the last two isomers would contribute to the increased stability as found by the potentiometric pH titrations, while in the UV measurements, which are based on the electronic perturbation resulting from the metal ion coordinating to the base moiety, only the isomer with *direct* N-7 coordination will show up.

The previously mentioned, so-called increased stability of $M(ATP)^{2-}$ complexes rests on comparisons with the stability of the corresponding complexes of pyrimidine-nucleoside 5'-triphosphates (PNTPs; Figure 1). The observed stability difference, if any, is then used to calculate[11] the percentage of the macrochelated isomers. The validity of this difference, resulting from large numbers, is crucial for any comparison, as indicated in the preceding paragraph. Moreover, the conclusions regarding the existence of the outer-sphere-closed isomer are only *indirect*. As it is difficult to obtain *direct* evidence for this outer-sphere isomer in aqueous solution, the accuracy of the stability constants limits the value of any conclusions.

Hence, this study aims to provide a comprehensive set of stability constants obtained under the same conditions and with clearly defined error limits for the complexes formed between Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, or Cd^{2+} and ATP or the PNTPs as well. This aim is achieved by evaluating old[12,18-21] and new titration data; in fact, several of the constants were not measured before. The complexes of UTP^{4-} and TTP^{4-} serve well for a comparison with the $M(ATP)^{2-}$ complexes because with these two PNTPs the stability is governed only by the affinity of the phosphate chain for metal ions; the base moieties of these nucleotides in their neutral form (see Figure 1) are not suited for metal ion coordination.[11] This could be different with CTP^{4-}: N-3 is known to be an effective binding site in cytidine complexes.[9,17,22-24] Therefore, a careful evaluation of the stabilities of the $M(CTP)^{2-}$ complexes in comparison with those of $M(UTP)^{2-}$ and $M(TTP)^{2-}$ was necessary. There are indications for a metal ion–base back-binding in $Cu(CTP)^{2-}$, but all the other $M(CTP)^{2-}$ complexes showed within experimental error the same stabilities as those of the corresponding $M(UTP)^{2-}$ and $M(TTP)^{2-}$ complexes, thus enlarging and solidifying further the basis for comparisons with the $M(ATP)^{2-}$ complexes. In addition, the isomeric equilibria due to metal ion–base back-binding and different locations of the proton in the $M(H{\cdot}NTP)^{-}$ complexes are evaluated for the first time.

Experimental Section

Materials. Sodium salts of ATP, CTP, UTP, and TTP were purchased from Serva Feinbiochemica GmbH, Heidelberg, FRG, and from Sigma Chemical Co., St. Louis, MO. All constants for each individual metal ion complex were always determined by carrying out for a given nucleotide independent titrations with substances from both companies. The free orthophosphate initially present (determined as described previously)[25,26] was always ≤2.5%, except for TTP (Sigma, 3.4%, Serva, 9.5%). The aqueous stock solutions of the NTPs were freshly prepared daily, and the pH was adjusted to about 8.5; the exact concentration was newly determined each time (see below). All experiments with the NTPs were done in such a way that dephosphorylation, which is metal ion promoted,[25,26] was kept to a minimum (see also ref 21). There were no significant differences observed between the constants calculated from experiments employing NTPs from the different sources, though in the case of TTP the error limits were somewhat larger. There was also no difference between the data obtained from the earlier experiments carried out in the presence of $NaClO_4$ (cf. ref 18–20) and that from those using $NaNO_3$ (this work and ref 12 and 21) as background electrolyte.

The nitrate salts of Na^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+}, the disodium salt of EDTA, HNO_3, and NaOH (Titrisol) (all p.A.) were obtained from Merck AG, Darmstadt, FRG.

The titer of NaOH used for the titrations was determined with potassium hydrogen phthalate (Merck AG); the exact concentrations of the NTP solutions used in the titrations with metal ions (titrated in the presence of an excess of HNO_3; see below) were measured by titrations with NaOH. The concentrations of the stock solutions of the divalent metal ions were determined with EDTA.

Potentiometric pH Titrations. The pH titrations were carried out with a Metrohm E536 potentiograph and a Metrohm EA 121 macro glass electrode. The buffers (pH 4.64 and 7.00) used for calibration were also from Metrohm AG, Herisau, Switzerland. The direct pH meter readings were used in the calculations of the acidity constants.

The acidity constants $K^{H}_{H_2(NTP)}$ and $K^{H}_{H(NTP)}$ of $H_2(ATP)^{2-}$ and $H_2(CTP)^{2-}$ and $K^{H}_{H(NTP)}$ of $H(UTP)^{3-}$ and $H(TTP)^{3-}$ (the value for $K^{H}_{H_2(UTP)}$ was taken from ref 21) were determined by titrating 50 mL of aqueous 0.9 mM HNO_3 and $NaNO_3$ (I = 0.1; 25 °C) in the presence and absence of 0.5 mM (sometimes also 0.4 or 0.6 mM) NTP^{4-} under

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Table I. Negative Logarithms of the Acidity Constants of Several $H_2(NTP)^{2-}$ Species (Eq 1 and 2) As Determined by Potentiometric pH Titrations in Water at 25 °C and I = 0.1 ($NaNO_3$ or $NaClO_4$)[a]

$H_2(NTP)^{2-}$	$pK^H_{H_2(NTP)}$	$pK^H_{H(NTP)}$[b]	no. of titrations
$H_2(UTP)^{2-}$	2.0 ± 0.1[c,d]	6.45 ± 0.01	32
$H_2(TTP)^{2-}$	2.0[c]	6.52 ± 0.02	10
$H_2(CTP)^{2-}$	4.55 ± 0.03[e]	6.55 ± 0.02	22
$H_2(ATP)^{2-}$	4.00 ± 0.01[f]	6.47 ± 0.01	57

[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] These values correspond to the release of a proton from the terminal γ-phosphate group.[10] [c] Both protons in $H_2(UTP)^{2-}$ and in $H_2(TTP)^{2-}$ are located at the triphosphate chain, and one of them is at the terminal γ-phosphate group;[10] to this latter proton corresponds $pK^H_{H(NTP)}$. For $H_2(TTP)^{2-}$ it is assumed that $pK^H_{H_2(TTP)} \simeq pK^H_{H_2(UTP)}$. [d] From ref 21. [e] This value corresponds to the release of the proton from N-3 of the pyrimidine-ring residue.[10,12] [f] This value corresponds to the release of the proton from N-1 of the purine-ring system.[10]

N_2 with 1 mL of 0.05 M NaOH and by using the differences in NaOH consumption between two such titrations for the calculations. Another set of experiments was carried out with 0.7 mM HNO_3, 0.4 mM NTP^{4-}, and 0.038 M NaOH. $K^H_{H_2(ATP)}$ or $K^H_{H_2(CTP)}$ and $K^H_{H(NTP)}$ were calculated with a Hewlett-Packard 9825A calculator (connected with a 7470A plotter) by a curve-fit procedure using a Newton–Gauss nonlinear-least-squares program within the pH range determined by the lowest point of neutralization reached by the experimental conditions (usually about 25% neutralization for the equilibrium $H_2(NTP)^{2-}/H(NTP)^{3-}$) and about 98% neutralization (for the equilibrium $H(NTP)^{3-}/NTP^{4-}$).

The conditions for the determination of the stability constants $K^M_{M(H\cdot NTP)}$ and $K^M_{M(NTP)}$ of the binary $M(H\cdot NTP)^-$ and $M(NTP)^{2-}$ complexes (I = 0.1 ($NaNO_3$); 25 °C) were the same as for the determination of the acidity constants, but the solutions contained now in addition $M(NO_3)_2$, the $[M^{2+}]$:[NTP] ratios being always 1:1. In part of the experiments the concentration of HNO_3 was doubled, to reach lower pH values for a better determination of $K^M_{M(H\cdot NTP)}$, and then 2 mL of NaOH was used for the titration. As M^{2+} ions promote, though with a different effectiveness, the dephosphorylation of NTPs,[25,26] both reactants were mixed only in the last minute before the titration, and this titration was usually completed within 15 minutes; in this way dephosphorylation of the 5′-triphosphates was minimized. The stability constants were computed for each pair of titrations with a curve-fitting procedure[27] that became satisfactory by taking into account the species H^+, $H_2(NTP)^{2-}$, $H(NTP)^{3-}$, NTP^{4-}, M^{2+}, $M(H\cdot NTP)^-$, and $M(NTP)^{2-}$. The evaluation of the data in the upper pH range was stopped at that point where hydrolysis of M^{2+}(aq) begins; this point was always evident from the titrations of the solutions containing M^{2+} but no NTP.

Results and Discussion

To obtain reliable stability constants with well-defined error limits, a large number of experimental data are desirable. Therefore, old[12,18–21] and new (about half and half) potentiometric pH titrations carried out in the laboratory of H.S. during the past 20 years had been evaluated; of course, only titrations carried out under comparable conditions and with a minimum of self-association[11] were used. The stability constants were independent of the background electrolyte administered, be it $NaClO_4$ or $NaNO_3$ (I = 0.1; 25 °C).

For all four NTPs probes from two different sources (Serva and Sigma) had been titrated. Usually each M^{2+}/pyrimidine-nucleoside 5′-triphosphate (PNTP) system was titrated by three different persons; in the case of the ATP systems four different people were involved. On the average each M^{2+}/UTP, -CTP, or -ATP system was titrated about eight times; the M^{2+}/TTP titrations were carried out only three times due to the scarcity of this nucleotide. The exact numbers of titrations are given for each system together with the corresponding results in Table II (vide infra). The acidity constants for the UTP, CTP, and ATP systems are the result of at least 22 independent titrations; those for TTP were obtained from 10 titrations (for details see Table I).

1. Acidity Constants of the $H_2(NTP)^{2-}$ Species. In accordance with earlier results[10,12] the pH titrations reveal that for CTP and ATP in the pH range of about 3.2–8.2 the following two protonation equilibria occur:

$$H_2(NTP)^{2-} \rightleftharpoons H(NTP)^{3-} + H^+ \quad (1a)$$

$$K^H_{H_2(NTP)} = [H(NTP)^{3-}][H^+]/[H_2(NTP)^{2-}] \quad (1b)$$

$$H(NTP)^{3-} \rightleftharpoons NTP^{4-} + H^+ \quad (2a)$$

$$K^H_{H(NTP)} = [NTP^{4-}][H^+]/[H(NTP)^{3-}] \quad (2b)$$

The corresponding acidity constants are listed in Table I; they agree well with earlier work.[10,12,18–21,28,29]

The acidity constant $K^H_{H_2(NTP)}$ determined for the first buffer region is due to the release of a proton from the protonated base moiety, i.e., from N-3 in $H_2(CTP)^{2-}$ and from N-1 in $H_2(ATP)^{2-}$, whereas $K^H_{H(NTP)}$ reflects the removal of a proton from the terminal γ-phosphate group in all the $H(NTP)^{3-}$ species.[10,12] In $H_2(UTP)^{2-}$ and $H_2(TTP)^{2-}$ the second proton also binds to the phosphate chain; as this proton is rather acidic, the value for $K^H_{H_2(UTP)}$ is taken from recent[21] work (Table I).

The values for $pK^H_{H(NTP)}$ are identical within 0.1 log unit for all four NTPs, indicating that the base residue has no significant influence on the acidity of the proton at the terminal γ-phosphate group. Therefore, in parts 3 and 5 the stability constants of the complexes are directly compared without adjustment for the slight differences in the acidity of the terminal $-OPO_3H^-$ group. This comparison is further justified by the recent observation made with monophosphates (L), that the slope of plots of log K^M_{ML} vs. pK_a is rather small ($m \simeq 0.3$).[30] Moreover, any adjustments for the M^{2+}/CTP systems (part 3) lack significance, and those for the M^{2+}/ATP systems (part 5) would nearly cancel out as the acidity constant of $H(UTP)^{3-}$ is slightly below that of $H(ATP)^{3-}$, while those of $H(TTP)^{3-}$ and $H(CTP)^{3-}$ are somewhat above.

2. Stability Constants of M^{2+} Complexes of PNTPs and of ATP. The experimental data of the potentiometric pH titrations for the 1:1 M^{2+}/NTP systems are completely described by equilibria 1–4, if the evaluation is not carried into the pH range

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

$$K^M_{M(H\cdot NTP)} = [M(H\cdot NTP)^-]/[M^{2+}][H(NTP)^{3-}] \quad (3b)$$

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

$$K^M_{M(NTP)} = [M(NTP)^{2-}]/[M^{2+}][NTP^{4-}] \quad (4b)$$

where formation of hydroxo complexes[12,31] occurs.[32] The acidity constant of the connected equilibrium 5 may be calculated with

$$M(H\cdot NTP)^- \rightleftharpoons M(NTP)^{2-} + H^+ \quad (5a)$$

$$K^H_{M(H\cdot NTP)} = [H^+][M(NTP)^{2-}]/[M(H\cdot NTP)^-] \quad (5b)$$

eq 6. The results for all four NTPs are listed in Table II. In

$$pK^H_{M(H\cdot NTP)} = pK^H_{H(NTP)} + \log K^M_{M(H\cdot NTP)} - \log K^M_{M(NTP)} \quad (6)$$

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Table II. Logarithms of the Stability Constants of $M(H{\cdot}NTP)^-$ (Eq 3) and $M(NTP)^{2-}$ Complexes (Eq 4) As Determined by Potentiometric pH Titrations in Aqueous Solution, together with the Negative Logarithms of the Acidity Constants (Eq 5) of the Corresponding $M(H{\cdot}NTP)^-$ Complexes (25 °C; I = 0.1 ($NaNO_3$ or $NaClO_4$))[a]

NTP	M^{2+}	log $K^M_{M(H\cdot NTP)}$	log $K^M_{M(NTP)}$	$pK^H_{M(H\cdot NTP)}$[b]	no. of titrations
UTP	Mg^{2+}	2.72 ± 0.09	4.27 ± 0.02	4.90 ± 0.09	10
	Ca^{2+}	2.74 ± 0.09	3.94 ± 0.02	5.25 ± 0.09	10
	Mn^{2+}	2.70 ± 0.08	4.91 ± 0.05	4.24 ± 0.09	8
	Co^{2+}	2.55 ± 0.16	4.73 ± 0.04	4.27 ± 0.17	7
	Ni^{2+}	2.51 ± 0.17	4.47 ± 0.02	4.49 ± 0.17	7
	Cu^{2+}	2.80 ± 0.05	5.87 ± 0.02	3.38 ± 0.05	12
	Zn^{2+}	2.73 ± 0.06	5.01 ± 0.02	4.17 ± 0.06	7
	Cd^{2+}	2.89 ± 0.04	5.10 ± 0.02	4.24 ± 0.05	4
TTP	Mg^{2+}	*c*	4.23 ± 0.04		3
	Ca^{2+}	*c*	3.85 ± 0.01		3
	Mn^{2+}	*c*	5.01 ± 0.11		3
	Co^{2+}	*c*	4.78 ± 0.02		3
	Ni^{2+}	*c*	4.52 ± 0.07		3
	Cu^{2+}	*c*	5.83 ± 0.11		3
	Zn^{2+}	*c*	5.03 ± 0.06		3
	Cd^{2+}	*c*	5.09 ± 0.09		3
CTP	Mg^{2+}	2.27 ± 0.18	4.20 ± 0.05	4.62 ± 0.19	5
	Ca^{2+}	2.21 ± 0.28	3.85 ± 0.04	4.91 ± 0.28	6
	Mn^{2+}	3.1 ± 0.3	4.90 ± 0.02	4.75 ± 0.3	3
	Co^{2+}	2.95 ± 0.17	4.78 ± 0.05	4.72 ± 0.18	5
	Ni^{2+}	2.70 ± 0.25	4.52 ± 0.02	4.73 ± 0.25	5
	Cu^{2+}	3.80 ± 0.06	6.03 ± 0.03	4.32 ± 0.07	6
	Zn^{2+}	3.05 ± 0.07	5.03 ± 0.05	4.57 ± 0.09	5
	Cd^{2+}	3.15 ± 0.06	5.05 ± 0.04	4.65 ± 0.07	8
ATP	Mg^{2+}	2.42 ± 0.05	4.29 ± 0.02	4.60 ± 0.05	11
	Ca^{2+}	2.20 ± 0.05	3.91 ± 0.02	4.76 ± 0.05	11
	Mn^{2+}	2.74 ± 0.06	5.01 ± 0.05	4.20 ± 0.08	12
	Co^{2+}	2.82 ± 0.14	4.97 ± 0.06	4.32 ± 0.15	9
	Ni^{2+}	2.86 ± 0.07	4.86 ± 0.03	4.47 ± 0.08	8
	Cu^{2+}	3.59 ± 0.05	6.34 ± 0.02	3.73 ± 0.09	16
	Zn^{2+}	2.86 ± 0.07	5.16 ± 0.04	4.17 ± 0.08	10
	Cd^{2+}	3.04 ± 0.06	5.34 ± 0.02	4.17 ± 0.06	10

[a] The errors given are 2 times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. The values of the error limits for $pK^H_{M(H\cdot NTP)}$ were calculated according to the error propagation after the method of Gauss.[33] [b] These values were calculated according to eq 6 by using the acidity constants of Table I and the stability constants listed above. [c] The small number of experimental data were not good enough to determine these constants independently. However, several trials showed that the stability constants obtained for the $M(H{\cdot}UTP)^-$ complexes represent well, at least in a first approximation, also the stability of the $M(H{\cdot}TTP)^-$ complexes.

general the agreement with earlier available data[11,12,18-21,28,29] is reasonable.[33,34]

Table III. Comparison of the Stability of the $M(CTP)^{2-}$ Complexes with the Stability of Other $M(PNTP)^{2-}$ Complexes[a] Having Only a M^{2+}/Phosphate Coordination (25 °C; I = 0.1 ($NaNO_3$ or $NaClO_4$))

M^{2+}	log $K^M_{M(CTP)}$[b]	log $K^M_{M(PNTP)}$[a]	log Δ[c]
Mg^{2+}	4.20 ± 0.05	4.26 ± 0.02	0
Ca^{2+}	3.85 ± 0.04	3.92 ± 0.02	0
Mn^{2+}	4.90 ± 0.02	4.93 ± 0.04	0
Co^{2+}	4.78 ± 0.05	4.75 ± 0.02	0.03 ± 0.05
Ni^{2+}	4.52 ± 0.02	4.49 ± 0.02	0.03 ± 0.03
Cu^{2+}	6.03 ± 0.03	5.86 ± 0.02	0.17 ± 0.04
Zn^{2+}	5.03 ± 0.05	5.02 ± 0.02	0.01 ± 0.05
Cd^{2+}	5.05 ± 0.04	5.10 ± 0.02	0

[a] The stability of the $M(PNTP)^{2-}$ complexes is represented here by the stabilities of the $M(UTP)^{2-}$ and $M(TTP)^{2-}$ species (see text in part 3). The values given above are for each metal ion the arithmetic mean of the stability constants determined for $M(UTP)^{2-}$ and $M(TTP)^{2-}$ by taking into account the number of titrations carried out in each case (Table II). The errors given are 2 times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. [b] Values from Table II. [c] $\log \Delta = \log K^M_{M(CTP)} - \log K^M_{M(PNTP)}$; analogous to eq 9 in part 5.

It is interesting to consider the deprotonation of the $M(H{\cdot}UTP)^-$ complexes according to equilibrium 5 and to compare the corresponding acidity constants (Table II) with the value due to equilibrium 2 (Table I). Depending on the kind of metal ion, an acidification of about 1.2–3.1 log units occurs. This acidified proton must be located at the terminal γ-phosphate group as there is no other basic site available in UTP^{4-}. The same reasoning applies for the $M(H{\cdot}TTP)^-$ complexes (see footnote *c* in Table II). However, with the $M(H{\cdot}CTP)^-$ and $M(H{\cdot}ATP)^-$ complexes a further basic site is available: N-3 at the cytosine residue and N-1 at the adenine moiety. Consequently, the proton in $M(H{\cdot}NTP)^-$ may be located either at the terminal γ-phosphate group or at the base residue of the nucleotide, producing intramolecular equilibria between several isomeric complexes. These equilibria will be discussed in parts 6–8. Priority is now first given to possible isomers of the $M(NTP)^{2-}$ species.

3. Comparison of the Stability of the $M(CTP)^{2-}$ Complexes with That of the Corresponding $M(UTP)^{2-}$ and $M(TTP)^{2-}$ Species: Evidence for Macrochelate Formation in $Cu(CTP)^{2-}$. Besides having the triphosphate chain, CTP^{4-} has a pyridine nitrogen, N-3 (Figure 1), which might also participate in complex formation of this nucleotide. Indeed, spectrophotometric measurements[17] with Ni^{2+} and ^1H NMR shift experiments[22] with Cd^{2+} revealed that cytidine complexes form; in addition, the stability of the $Cu(Cyt)^{2+}$ complex is known.[23,24] Therefore, to evaluate the situation with CTP^{4-} the stability of the $M(CTP)^{2-}$ complexes must be carefully compared with the stability of complexes capable of only a triphosphate coordination.

The neutral base moiety of UTP^{4-} and TTP^{4-} has no obvious binding site for a metal ion interaction (see Figure 1),[35] and indeed ^1H NMR shift measurements with Mg^{2+}, Zn^{2+}, and Cd^{2+} confirmed that metal ions coordinate only to the triphosphate chain.[11] Hence, the stabilities of $M(UTP)^{2-}$ and $M(TTP)^{2-}$ represent the metal ion affinity of the phosphate chain. For the comparison with the stabilities of the $M(CTP)^{2-}$ complexes, the values of $M(UTP)^{2-}$ and $M(TTP)^{2-}$ from Table II were averaged by using the number of independent titrations as weighting factor; the results are listed in Table III.

(33) It is evident that the equilibrium constants given in Tables I and II are conditional constants for 25 °C and I = 0.1 M; i.e., they are valid for solutions in which the Na^+ concentration is about 0.1 M. As many researchers work under similar conditions, we have not corrected our data for the complex formation between Na^+ and NTP but have preferred to publish the direct experimental results to prevent any ambiguity. However, if desired, the competition of Na^+ for NTP^{4-} protonation or coordination can easily be taken into account. For example, for the "corrected" acidity constant, $K^H_{H(NTP)}(cor)$, holds the relation $K^H_{H(NTP)}(cor) = K^H_{H(NTP)}/(1 + [Na^+]K^{Na}_{Na(NTP)})$: with $[Na^+] = 0.1$ M and $K^{Na}_{Na(ATP)} = 15\ M^{-1}$ [34a,b] one calculates for the term $\log (1 + [Na^+]K^{Na}_{Na(NTP)}] = 0.40$; i.e., one has to *add* about 0.4 log unit to the $pK^H_{H(NTP)}$ values of Table I to obtain acidity constants free of Na^+ competition. Similarly, for a "corrected" stability constant, $K^M_{M(NTP)}(cor)$, holds the relation $K^M_{M(NTP)}(cor) = K^M_{M(NTP)}(1 + [Na^+]K^{Na}_{Na(NTP)})$, and therefore one also has to *add* about 0.4 log unit to the $\log K^M_{M(NTP)}$ values of Table II to obtain stability constants free of Na^+ competition. Of course, the size of the correction depends on the value used for the stability constant, $K^{Na}_{Na(NTP)}$, of the $Na(NTP)^{3-}$ complex.[34c] However, it must be strongly emphasized that the conclusions presented in this study are *not* affected by the above-mentioned problem, because the extent of macrochelation (eq 7) is calculated from *differences* between stability constants (eq 8 and 9), and the affinity of Na^+ toward a triphosphate chain is of course independent of the base moiety (to which Na^+ has certainly no affinity).

(34) (a) O'Sullivan, W. J.; Perrin, D. D. *Biochemistry* **1964**, *3*, 18–26. (b) Botts, J.; Chashin, A.; Young, H. L. *Biochemistry* **1965**, *4*, 1788–1796. (c) For example, use of $K^{Na}_{Na(ATP)} = 35\ M^{-1}$ [34d] gives a correction term of 0.65 log unit and in this case the corrected acidity constant $pK^H_{H(ATP)}(cor) = 7.12$ for $H(ATP)^{3-}$ results, a value in excellent agreement with that of 7.10 ± 0.05 given in ref 34d. This result demonstrates nicely the agreement of the basic experimental data in the two studies, but it shows also that the same value for $pK^H_{H(ATP)}(cor)$ is obtained *only* because the same stability constant for the $Na(ATP)^{3-}$ complex was used in the calculations. (d) Cali, R.; Musumeci, S.; Rigano, C.; Sammartano, S. *Inorg. Chim. Acta* **1981**, *56*, L11–L13.

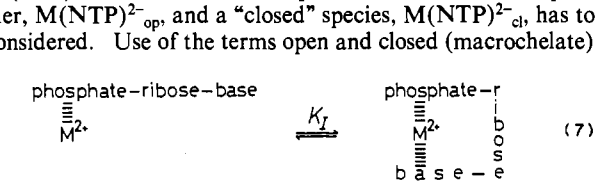
(35) The situation changes drastically if the proton from N-3 is released; this leads to an excellent binding site,[31] and under such conditions macrochelates are formed.[11]

Comparisons of the data given in Table III show that the differences in complex stability between the $M(CTP)^{2-}$ complexes and the $M(PNTP)^{2-}$ species are zero; i.e., the stability constants are identical within experimental error, the only exception being the data for $Cu(CTP)^{2-}$ (vide infra). That $Mg(CTP)^{2-}$, $Zn(CTP)^{2-}$, and $Cd(CTP)^{2-}$ show no increased stability agrees with earlier ^1H NMR shift measurements;[11] similarly, spectrophotometric[17] and kinetic studies[36,37] also gave no hint of any metal ion–base interaction in $Ni(CTP)^{2-}$. This observation may be explained by the conformation that nucleotides adopt in aqueous solution; i.e., by the relative orientation of the base and sugar rings about the N-1–C-1′ bond in pyrimidines.[10] Usually two main conformations occur, syn and anti, the latter being favored in aqueous solution.[10] In this anti conformation the N-1–C-6 bond of pyrimidines projects onto or near the ribose ring; hence, N-3 is directed away from the phosphate moiety.

Apparently, Cu^{2+} is the only metal ion with a large enough affinity for N-3 to force part of CTP^{4-} in $M(CTP)^{2-}$ into a syn conformation. $Cu(CTP)^{2-}$ is, by about 0.17 log unit, more stable than expected for a pure triphosphate coordination. Indeed, the affinity of Cu^{2+} for N-3 is quite pronounced as the following comparison of stability constants indicates: the stability of $Cu(Cyt)^{2+}$ ($\log K^{Cu}_{Cu(Cyt)} = 2.0$)[24] is considerably larger than that of $Cu(Ado)^{2+}$ with a N-7 coordination ($\log K^{Cu}_{Cu(Ado)} = 1.3$).[9,24] In fact, by using the difference between these latter stability constants (2.0 − 1.3 = 0.7) and the stability difference, which is due to the different extents of macrochelate formation, between $Cu(ATP)^{2-}$ and $Cu(CTP)^{2-}$ (6.34 − 6.03 = 0.3; Table II), it is possible to estimate the energy barrier between the syn and anti conformations of CTP^{4-}. As ATP^{4-} exists predominately in the anti conformation,[10] the overall value of 1.0 log unit (=0.7 + 0.3) represents an estimate for this barrier corresponding to about 6 kJ/mol.

In conclusion, Cu^{2+} is the only metal ion among those studied here able to form a macrochelate with CTP^{4-} by coordinating not only to the triphosphate chain, but *partly* also to N-3 of the pyrimidine moiety. This Cu^{2+}–base interaction in $Cu(CTP)^{2-}$ was confirmed by UV difference spectroscopy with a Cary 219 spectrophotometer in 2×10^{-4} M aqueous solutions as it leads to a perturbation of the CTP^{4-} spectrum (λ_{max} at 270 nm): one cell (1 cm) in the reference beam contained $Cu(ClO_4)_2$ and the other CTP^{4-}, whereas one cell in the sample beam contained the mixed system (at pH 6.0) and the other water; all four cells contained $NaClO_4$ to maintain $I = 0.1$ (25 °C). The difference spectrum shows a maximum near 291 nm and a minimum near 276 nm.

4. Estimate for the Extent of Macrochelate Formation in the $Cu(CTP)^{2-}$ Species. To quantify the metal ion–base interaction in $Cu(CTP)^{2-}$, the intramolecular equilibrium 7 between an "open" isomer, $M(NTP)^{2-}_{op}$, and a "closed" species, $M(NTP)^{2-}_{cl}$, has to be considered. Use of the terms open and closed (macrochelate)



(7)

does not imply a single fixed structure fitting each description. The term *open* refers to a family of conformations, though several structures may also occur in the closed form. The distinction between the terms should be viewed operationally: the greater stability constant observed with $Cu(CTP)^{2-}$ (and $M(ATP)^{2-}$; part 5) implies structures not occurring with $Cu(UTP)^{2-}$ and $Cu(TTP)^{2-}$ (or other $M(PNTP)^{2-}$ species); these additional structures are termed *closed*.

It was recently shown[17] that the dimensionless intramolecular equilibrium constant K_I can be deduced[37-40] from the experimentally accessible overall stability constant, $K^{M}_{M(NTP)}$ (eq 4; i.e., $K^{Cu}_{Cu(CTP)}$ of Table III), by using eq 8, provided the stability

$$K_I = \frac{[M(NTP)^{2-}_{cl}]}{[M(NTP)^{2-}_{op}]} = \frac{K^{M}_{M(NTP)}}{K^{M}_{M(NTP)_{op}}} - 1 \qquad (8)$$

constant, $K^{M}_{M(NTP)_{op}}$, of the "open" isomer $M(NTP)^{2-}_{op}$ is also known. As the acidity constants for $H(CTP)^{3-}$ and $H(UTP)^{3-}$ or $H(TTP)^{3-}$ are practically identical (see part 1), $K^{Cu}_{Cu(CTP)_{op}}$ will be well-described by the value of $K^{Cu}_{Cu(PNTP)}$ in Table III, yielding $K_I = 0.48 \pm 0.12$ for the isomeric $Cu(CTP)^{2-}_{cl}/Cu(CTP)^{2-}_{op}$ system.

From the given K_I value one calculates that 32 ± 6% of $Cu(CTP)^{2-}$ exists in the macrochelated form (eq 7); though significant, this formation degree is lower than the one observed for $Cu(ATP)^{2-}$, which is close to 70% (part 5). That the base back-binding in $Cu(CTP)^{2-}$ is rather weak is in accord with the Cu^{2+}-promoted dephosphorylation of CTP, where it is not reflected:[25,26] $Cu(CTP)^{2-}$, $Cu(UTP)^{2-}$, and $Cu(TTP)^{2-}$ behave quite alike in this respect, though these kinetic experiments were carried out at 50 °C and at this temperature the base back-binding might well be further reduced. In any case, a significant base back-binding as occurs for the N-6/N-7 site in $Cu(1,N^6\text{-etheno-ATP})^{2-}$ influences the dephosphorylation reaction significantly.[13,41,42]

5. Quantification of the Extent of Macrochelate Formation in $M(ATP)^{2-}$ Complexes. That several metal ions are able to form macrochelates with ATP^{4-} is well-known (see references listed in ref 11),[43] and the stability data given in Table II immediately confirm for a number of $M(ATP)^{2-}$ complexes an increased stability, if compared with the values listed for the pyrimidine-nucleoside 5′-triphosphate complexes.

Any evaluation of stability data according to eq 8 for the position of equilibrium 7 will depend on the accuracy of the ratio $K^{M}_{M(NTP)}/K^{M}_{M(NTP)_{op}}$. Hence, the error of the difference of the logarithms of these constants, as expressed in eq 9, is crucial. As

$$\log \Delta = \log K^{M}_{M(NTP)} - \log K^{M}_{M(NTP)_{op}} \qquad (9)$$

indicated already in part 4, $K^{M}_{M(NTP)_{op}}$ is not directly accessible, but the stability constants of the pyrimidine-nucleoside 5′-triphosphate complexes provide values for this constant. To make the basis for comparisons according to eq 9 as firm as possible for each metal ion, the stability constants of the $M(UTP)^{2-}$, $M(TTP)^{2-}$, and $M(CTP)^{2-}$ complexes have been averaged with the number of titrations (Table II) as weighting factors. The resulting stability constants are listed in Table IV under log $K^{M}_{M(PNTP)}$; these values characterize the stability of the open isomers in equilibrium 7, i.e., of a complex with phosphate coordination only. The single exception to this procedure is Cu^{2+}: due to the results described in parts 3 and 4 only the average of the stability constants for $Cu(UTP)^{2-}$ and $Cu(TTP)^{2-}$ was used; i.e., the value given in Table IV is the same as the corresponding one of Table III.

This procedure gives clear error limits for the crucial difference log Δ (eq 9),[45-48] and therefore the error limits for the dimen-

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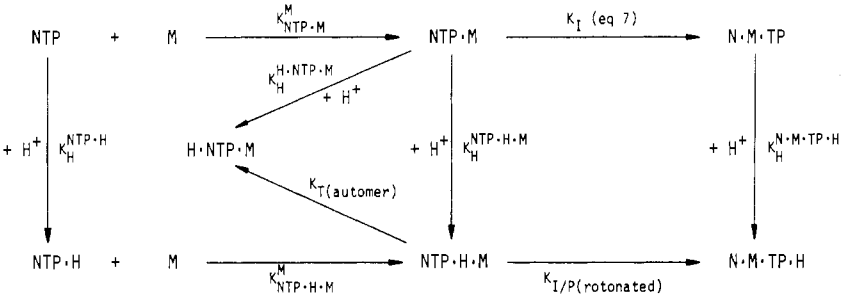
(37) Frey, C. M.; Stuehr, J. E. *J. Am. Chem. Soc.* **1978**, *100*, 139–145.
(38) Schwarzenbach, G.; Anderegg, G.; Schneider, W.; Senn, H. *Helv. Chim. Acta* **1955**, *38*, 1147–1170.
(39) Taylor, R. S.; Diebler, H. *Bioinorg. Chem.* **1976**, *6*, 247–264.
(40) (a) Sigel, H.; Scheller, K. H.; Rheinberger, V. M.; Fischer, B. E. *J. Chem. Soc., Dalton Trans.* **1980**, 1022–1028. (b) Sigel, H. *Angew. Chem.* **1982**, *94*, 421–432; *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 389–400.
(41) Scheller-Krattiger, V.; Sigel, H. *Inorg. Chem.* **1986**, *25*, 2628–2634.
(42) In all the mentioned cases, the reaction proceeds via a monomeric intermediate that is derived from $M(NTP)^{2-}$;[25,26,41] a comparison with Cu^{2+}/ATP is not possible, as here the reaction proceeds via a dimeric species.[25]
(43) The δ_0 values of the former ^1H NMR study[11] at pD 7.2 refer to the *monomeric* 1:1 Zn^{2+}/ATP system, in contrast to a recent claim made on page 1276 of ref 44. This means that these ^1H NMR measurements[11] prove the formation of a species having a N-7–Zn^{2+} (or –Cd^{2+}) interaction in $M(ATP)^{2-}$ (see also ref 12). These results must not be confused with the fact that at reactant concentrations $>10^{-3}$ M in a 1:1 Zn^{2+}/ATP system considerable self-association occurs (see Figure 7 in ref 11).
(44) Arena, G.; Calì, R.; Cucinotta, V.; Musumeci, S.; Rizzarelli, E.; Sammartano, S. *J. Chem. Soc., Dalton Trans.* **1983**, 1271–1278.

Table IV. Comparison of the Stability of the $M(ATP)^{2-}$ Complexes with the Stability of $M(PNTP)^{2-}$ Complexes[a] Having Only a M^{2+}/Phosphate Coordination and Extent of the Intramolecular Macrochelate Formation in the $M(ATP)^{2-}$ Complexes (25 °C; I = 0.1 ($NaNO_3$ or $NaClO_4$))

M^{2+}	log $K^{M}_{M(ATP)}$[b]	log $K^{M}_{M(PNTP)}$ = log $K^{M}_{M(ATP)_{op}}$[a,c]	log Δ (eq 9)	K_I (eq 7, 8)	% $M(ATP)^{2-}_{cl}$
Mg^{2+}	4.29 ± 0.02	4.24 ± 0.02	0.05 ± 0.03	0.12 ± 0.07	11 ± 6 (7 ± 6)[d]
Ca^{2+}	3.91 ± 0.02	3.90 ± 0.02	0.01 ± 0.03	0.02 ± 0.07	2 ± 6 (0 ± 7)[d]
Mn^{2+}	5.01 ± 0.05	4.93 ± 0.02	0.08 ± 0.06	0.20 ± 0.15	17 ± 10 (17 ± 12)[d]
Co^{2+}	4.97 ± 0.06	4.76 ± 0.02	0.21 ± 0.06	0.62 ± 0.24	38 ± 9 (40 ± 9)[d]
Ni^{2+}	4.86 ± 0.03	4.50 ± 0.02	0.36 ± 0.04	1.29 ± 0.19	56 ± 4 (57 ± 4)[d]
Cu^{2+}	6.34 ± 0.02	5.86 ± 0.02	0.48 ± 0.03	2.02 ± 0.20	67 ± 2
Zn^{2+}	5.16 ± 0.04	5.02 ± 0.01	0.14 ± 0.04	0.38 ± 0.13	28 ± 7 (28 ± 7)[d]
Cd^{2+}	5.34 ± 0.02	5.07 ± 0.02	0.27 ± 0.03	0.86 ± 0.12	46 ± 4 (43 ± 4)[d]

[a] For each metal ion the stability constants of all three pyrimidine-nucleoside 5′-triphosphate complexes have been averaged, with the exception for Cu^{2+}, where only the values for $Cu(UTP)^{2-}$ and $Cu(TTP)^{2-}$ have been used; see text in part 5. Otherwise the points given in footnote *a* of Table III apply also here. [b] Values from Table II. [c] See text in part 5. [d] The values in parentheses are obtained if for log $K^{M}_{M(ATP)_{op}}$ the values given under log $K^{M}_{M(PNTP)}$ of Table III are used; i.e., only the stability constants of the $M(UTP)^{2-}$ and $M(TTP)^{2-}$ complexes are considered. A comparison with the neighboring results should also mediate a feeling for the sensitivity of the calculations (eq 9) and for the reliability of the percentages listed. Regarding Cu^{2+}, see the text in part 5 and footnote *a*.



$$K^{NTP\cdot H}_{H} = \frac{[NTP\cdot H]}{[H][NTP]} \qquad K^{H\cdot NTP\cdot M}_{H} = \frac{[H\cdot NTP\cdot M]}{[H][NTP\cdot M]} \qquad K^{NTP\cdot H\cdot M}_{H} = \frac{[NTP\cdot H\cdot M]}{[H][NTP\cdot M]}$$

$$K^{N\cdot M\cdot TP\cdot H}_{H} = \frac{[N\cdot M\cdot TP\cdot H]}{[H][N\cdot M\cdot TP]} \qquad K^{M}_{NTP\cdot M} = \frac{[NTP\cdot M]}{[NTP][M]} \qquad K^{M}_{NTP\cdot H\cdot M} = \frac{[NTP\cdot H\cdot M]}{[M][NTP\cdot H]}$$

$$K_I = \frac{[N\cdot M\cdot TP]}{[NTP\cdot M]} = \frac{[M(NTP)^{2-}_{cl}]}{[M(NTP)^{2-}_{op}]} \quad \text{(eq 8)} \qquad K_{I/P} = \frac{[N\cdot M\cdot TP\cdot H]}{[NTP\cdot H\cdot M]} \qquad K_T = \frac{[H\cdot NTP\cdot M]}{[NTP\cdot H\cdot M]} = \frac{K^{H\cdot NTP\cdot M}_{H}}{K^{NTP\cdot H\cdot M}_{H}}$$

$$K^{NTP\cdot H}_{H} \cdot K^{M}_{NTP\cdot H\cdot M} = K^{M}_{NTP\cdot M} \cdot K^{NTP\cdot H\cdot M}_{H} \qquad K^{NTP\cdot H\cdot M}_{H} \cdot K_{I/P} = K_I \cdot K^{N\cdot M\cdot TP\cdot H}_{H}$$

Figure 2. Equilibrium scheme showing the interrelation between $M(H\cdot CTP)^-$ or $M(H\cdot ATP)^-$ complexes, their isomers, and other species present. The arrows indicate the direction for which the equilibrium constants are defined; for reasons of clarity charges are omitted. In NTP·M the metal ion is coordinated only at the triphosphate group, while N·M·TP symbolizes a macrochelate involving also the nucleoside-base residue (=$M(NTP)^{2-}_{cl}$ in eq 8; part 4). Similarly, in NTP·H·M the metal ion is at the triphosphate chain and the proton at the γ group; in N·M·TP·H the γ group also carries a proton, but the phosphate-coordinated metal ion now interacts in addition with the nucleoside base, forming a macrochelate. Accordingly, in H·NTP·M the proton is at the base residue and the metal ion at the phosphate; the other symbols in the scheme are correspondingly defined.

sionless equilibrium constants K_I (eq 7 and 8) and the percentages of the closed isomers are also well-defined.[49] As indicated in the introduction, the percentages given in Table IV for $M(ATP)^{2-}_{cl}$ are "overall" percentages comprising the macrochelate formed by inner-sphere coordination to N-7 as well as a possibly existing outer-sphere species with a water between the metal ion and N-7. A detailed evaluation of this situation is given in the Conclusions.

6. Sites of Protonation and Isomeric Equilibria for the M(H·ATP)⁻ and M(H·CTP)⁻ Species. As indicated in part 2, in these cases some complexes will carry the proton at the nucleic base residue and others at the γ-phosphate group. The interrelated equilibria are outlined in the scheme of Figure 2. There, the stability constant $K^{M}_{NTP\cdot M}$ refers to metal ion (M) binding at the deprotonated phosphate of a nucleotide (NTP) to give NTP·M (charges are omitted for clarity). Some of the open NTP·M complex may form a macrochelate (N·M·TP) where the metal ion bridges the phosphate and base moieties (part 5) as described by the intramolecular equilibrium constant K_I (see also eq 7). All three phosphate species, NTP, NTP·M, and N·M·TP, may become protonated at the γ-phosphate group to give NTP·H, NTP·H·M, and N·M·TP·H, respectively (Figure 2). Alternatively the open complex NTP·M may undergo protonation at the nucleic base to give H·NTP·M with the protonation constant $K^{H\cdot NTP\cdot M}_{H}$. The tautomeric equilibrium between NTP·H·M and H·NTP·M is described by the equilibrium constant K_T (Figure 2). Metal ion binding at NTP·H also gives NTP·H·M with the stability constant $K^{M}_{NTP\cdot H\cdot M}$. The species NTP·H·M may also form a macrochelate N·M·TP·H characterized by the intramolecular and dimensionless equilibrium constant $K_{I/P}$.

The observed stability constant, $K^{M}_{M(NTP)}$, for metal ion binding to the deprotonated nucleotide (eq 4) includes both "open" (NTP·M) and "closed" (N·M·TP) complexes, as shown across the top of the scheme in Figure 2 (see also eq 7 and 8 in part 4). From this reasoning follows (in analogy to eq 8) eq 10. Metalation

$$K^{M}_{M(NTP)} = \frac{[NTP\cdot M] + [N\cdot M\cdot TP]}{[NTP][M]} = K^{M}_{NTP\cdot M}(1 + K_I) \quad (10)$$

(45) It is not surprising that the difference log Δ follows the Irving–Williams sequence[46] (in contrast to columns 2 and 3 of Table IV), because this difference reflects the imidazole-like coordination of the divalent 3d metal ions to N-7 of the adenine residue (see also Conclusions).[47] Coordination of the divalent 3d ions to O donor ligands is much less dependent on the kind of metal ion;[48] indeed, it is longstanding experience that the stabilities of metal ion/phosphate complexes often do not strictly follow[48] the Irving–Williams sequence.

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(49) Earlier results[11] (based on measurements given in ref 19) for $M(ATP)^{2-}_{cl}$ (M^{2+} = Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}) are similar, though the values are throughout somewhat higher. It is clear that the present results are more reliable due to the greater number of titrations and the clearly defined error limits.

Table V. Estimates on the Formation Degree of the Species Occurring in the Isomeric Equilibria (See Figure 2) of $M(H\cdot ATP)^-$ and $M(H\cdot CTP)^-$ Complexes (25 °C; I = 0.1 ($NaNO_3$ or $NaClO_4$))[a]

$H(NTP)^{3-}$	M^{2+}	$\log (K^M_{M(H\cdot NTP)}/K^M_{NTP\cdot H\cdot M})$[b]	$K_{I/P} + K_T$[c]	% H·NTP·M[d]	% N·M·TP·H[d]
$H(ATP)^{3-}$	Mn^{2+}	0.04 ± 0.10	0.10 ± 0.25	0	9 ± 21
	Co^{2+}	0.27 ± 0.21	0.86 ± 0.91	0	46 ± 26
	Ni^{2+}	0.35 ± 0.18	1.24 ± 0.95	0	55 ± 19
	Cu^{2+}	0.79 ± 0.07	5.17 ± 1.00	51	33
	Zn^{2+}	0.13 ± 0.09	0.35 ± 0.29	0	26 ± 16
	Cd^{2+}	0.15 ± 0.07	0.41 ± 0.23	0	29 ± 12
$H(CTP)^{3-}$	Mn^{2+}	0.40 ± 0.31	1.51 ± 1.80	60 ± 29	0
	Co^{2+}	0.40 ± 0.23	1.51 ± 1.35	60 ± 21	0
	Ni^{2+}	0.19 ± 0.30	0.55 ± 1.08	35 ± 45	0
	Cu^{2+}	1.00 ± 0.08	9.00 ± 1.80	85	5
	Zn^{2+}	0.32 ± 0.09	1.09 ± 0.44	52 ± 10	0
	Cd^{2+}	0.26 ± 0.07	0.82 ± 0.30	45 ± 9	0

[a] As in Table II, the errors given are 2 times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. [b] $K^M_{NTP\cdot H\cdot M}$ is taken as $K^M_{M(H\cdot UTP)}$ (Table II); see text in part 7. [c] See eq 11. [d] About the assumptions made, see text in parts 7 and 8. The estimate for the concentration of the third isomer, the open phosphate-protonated NTP·H·M species, follows from the difference to 100%.

at the phosphate of the phosphate-protonated nucleotide NTP·H gives rise to three complexes: open and closed phosphate-protonated species and a nucleic base-protonated complex. This last complex will form a macrochelate to a much smaller extent than phosphate-protonated complexes due to the acidification of a base proton by a base-coordinating metal ion; e.g., for Cu^{2+} [31] and Pd^{2+} [50] the acidification corresponds to about 2 log units. Therefore, we do not include an equilibrium with the macrochelated and base-protonated species H·N·M·TP but consider only the open H·NTP·M isomer. Hence, the situation is described by eq 11, where $K^M_{M(H\cdot NTP)}$ (eq 3) is the observed stability constant.

$$K^M_{M(H\cdot NTP)} = \frac{[NTP\cdot H\cdot M] + [N\cdot M\cdot TP\cdot H] + [H\cdot NTP\cdot M]}{[NTP\cdot H][M]} = K^M_{NTP\cdot H\cdot M}(1 + K_{I/P} + K_T) \quad (11)$$

In the calculations and reasoning described in the next part, $K^M_{NTP\cdot H\cdot M}$ is taken as the stability constant of the $H(UTP)^{3-}$ complexes, $K^M_{M(H\cdot UTP)}$. Some assumptions will have to be made about $K_{I/P}$ and K_T (Figure 2).

7. Quantification of the Isomers Formed with $M(H\cdot ATP)^-$ and $M(H\cdot CTP)^-$ for Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, and Cd^{2+}. Table V contains the values of $\log (K^M_{M(H\cdot NTP)}/K^M_{NTP\cdot H\cdot M})$ (see eq 11) for the complexes of $H(ATP)^{3-}$ and $H(CTP)^{3-}$ with the 3d ions, including Zn^{2+} and Cd^{2+}. These values were calculated from the constants in Table II, where $\log K^M_{NTP\cdot H\cdot M}$ is taken from $\log K^M_{M(H\cdot NTP)}$ for UTP, because $H(UTP)^{3-}$ cannot form a macrochelate or become protonated at the nucleic base residue. The corresponding ratios for the Mg^{2+} and Ca^{2+} complexes of $H(CTP)^{3-}$ and $H(ATP)^{3-}$ are not included in Table V because negative values are obtained. These negative values are due to the apparently rather high stability of $Mg(H\cdot UTP)^-$ and $Ca(H\cdot UTP)^-$ (see Table II). As these values are difficult to determine and as the errors are rather large, we are at present not certain whether this effect is real and what its origins are.

In column 4 of Table V is given the sum of the equilibrium constants $K_{I/P} + K_T$ as derived from eq 11. To estimate the individual contributions of $K_{I/P}$ and K_T (Figure 2) to this sum, we compare it with K_I; i.e., the macrochelate equilibrium constant (eq 7 and 8) for the unprotonated complex in Table IV. For $M(ATP)^{2-}$, except for Cu^{2+} (see part 8), the K_I values are either possibly greater than (Cd^{2+}) or within the experimental uncertainties of the $K_{I/P} + K_T$ sum. Thus for $M(H\cdot ATP)^-$, except for Cu^{2+}, we assign $K_T = 0$ (no base protonation) and set $K_{I/P}$ equal to the values in the fourth column of Table V; these values quantify the equilibrium between the two phosphate-protonated complexes ATP·H·M (open) and A·M·TP·H (closed) as indicated in Figure 2.

For the protonated complexes the ratio of closed to all open complexes is given by eq 12. Only when there is no nucleic base

$$K_{I/P/tot.} = \frac{[N\cdot M\cdot TP\cdot H]}{[NTP\cdot H\cdot M] + [H\cdot NTP\cdot M]} = \frac{K_{I/P}}{1 + K_T} \quad (12)$$

protonation, i.e., $K_T = 0$, does $K_{I/P/tot.} = K_{I/P}$ (as defined in Figure 2). The fraction (f_{cl}) of protonated complexes closed in a macrochelate is given by $f_{cl} = K_{I/P}/(1 + K_{I/P})$. The last column of Table V lists for the $M(H\cdot ATP)^-$ complexes (except for Cu^{2+}; part 8) the percentage of the closed species, $100f_{cl}$, as calculated by setting $K_T = 0$. Hence, we suggest for $M(H\cdot ATP)^-$ that base protonation is negligible.

The ratio (R) of nucleic base to phosphate-protonated complexes is given by eq 13. Only when the phosphate-protonated complex

$$R = \frac{[H\cdot NTP\cdot M]}{[NTP\cdot H\cdot M] + [N\cdot M\cdot TP\cdot H]} = \frac{K_T}{1 + K_{I/P}} \quad (13)$$

forms no macrochelate and $K_{I/P} = 0$ does $R = K_T$. For $M(CTP)^{2-}$ only the Cu^{2+} complex exhibits any degree of macrochelate formation in the unprotonated complex (part 4). Therefore, for all cases of $M(H\cdot CTP)^-$, except for Cu^{2+}, we set $K_{I/P} = 0$ and take K_T equal to the values in the fourth column of Table V. The fraction (f_N) of complexes protonated at the nucleic base is now given by $f_N = R/(1 + R)$. The fifth column of Table V lists for $M(H\cdot CTP)^-$ (except for Cu^{2+}) the percentage of nucleic base protonated complexes, $100f_N$, as calculated by setting $K_{I/P} = 0$. Hence, we conclude that the base-protonated open isomer of $M(H\cdot CTP)^-$ is important while any macrochelated isomer is negligible.

8. Estimation of the Isomer Distribution in $Cu(H\cdot ATP)^-$ and $Cu(H\cdot CTP)^-$. The Cu^{2+} complexes of both $H(ATP)^{3-}$ and $H(CTP)^{3-}$ present special cases. With $K_I = 2.02 \pm 0.20$ (Table IV) for $Cu(ATP)^{2-}$ and 0.48 ± 0.12 (part 4) for $Cu(CTP)^{2-}$, these values are both significantly smaller than the $K_{I/P} + K_T$ sum in Table V and also greater than zero. As the best guide available, we assume the intramolecular equilibrium constant for macrochelate formation is the same in unprotonated and protonated complexes and set $K_{I/P} = K_I$.

On the basis of the fourth column of Table V, we thus obtain for $Cu(H\cdot ATP)^-$ $K_T = 3.15$ and for $Cu(H\cdot CTP)^-$ $K_T = 8.5$ (see Figure 2). With this information we can now use the full eq 12 and 13 and find for $Cu(H\cdot ATP)^-$ $K_{I/P/tot.} = 0.49$ and $R = 1.04$ and for $Cu(H\cdot CTP)^-$ $K_{I/P/tot.} = 0.05$ and $R = 5.8$. The respective mole fractions of NTP·H·Cu, N·Cu·TP·H, and H·NTP·Cu become 0.16, *0.33*, and *0.51* for $Cu(H\cdot ATP)^-$ and 0.10, *0.05*, and *0.85* for $Cu(H\cdot CTP)^-$. The values printed in italics appear as percentages in the last two columns of Table V. Of course, assumption of a different $K_{I/P}$ value would alter the mole fraction values for H·NTP·M and N·M·TP·H, but the percentage for the open phosphate-protonated NTP·H·M species is independent of this assumption.

Conclusions

Inner-Sphere vs. Outer-Sphere Complexation: N-7 Back-Binding in $M(ATP)^{2-}$ Complexes. Up to this point no attempt has been made to distinguish between inner- and outer-sphere coordination of back-bonded N-7 in $M(ATP)^{2-}$ complexes. The phosphate-bound metal ion may coordinate directly to N-7 or contain a coordinated water molecule that hydrogen bonds to N-7; these closed isomers are designated as $M(ATP)^{2-}_{cl/i}$ and $M(ATP)^{2-}_{cl/o}$, respectively. Ultraviolet absorption and nuclear magnetic resonance spectroscopy techniques that detect perturbations on the adenine ring are sensitive mainly to inner-sphere coordination of the ring by a metal ion.

Table VI lists the percentage of back-bonded closed forms in $M(ATP)^{2-}$ complexes obtained with a variety of techniques. The second column tabulates the values from part 5, together with some

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data from the literature,[12,15,21,37] which were obtained by careful analysis of stability constants determined by potentiometric pH titration. This technique yields the total of closed forms over the sum of inner- and outer-sphere contributions; i.e., $M(ATP)^{2-}_{cl/tot.}$, which corresponds to the designation $M(ATP)^{2-}_{cl}$ used in part 5 and Table IV: $M(ATP)^{2-}_{cl/tot.} = M(ATP)^{2-}_{cl/i} + M(ATP)^{2-}_{cl/o}$.

The third column shows the inner-sphere percentage estimated by ultraviolet spectroscopy. The set of higher percentages from ref 17 are probably to be preferred over the lower values from ref 14, because a metal ion/tripolyphosphate complex, $M(H\cdot P_3O_{10})^{2-}$, was used as a perturbant in the former study and only the aqueous metal ions were used in the latter investigation. The $Ni(H\cdot P_3O_{10})^{2-}$ complex binds 11 times more strongly than aqueous Ni^{2+} to adenosine with a marked enhancement of binding to N-7 over N-1.[17] Aqueous Ni^{2+} binds preferentially to N-1 over N-7 of adenosine by 3:1.[9,24] It is the spectral perturbation due to binding at N-7 that is relevant to the back-bonded isomers of the $M(ATP)^{2-}$ species.

It is also possible to obtain some approximate estimates of inner-sphere back-bonded complexation in $M(ATP)^{2-}$ from ^1H NMR spectra of ATP in the presence of diamagnetic metal ions. Estimates[11] of 20% and 54% made for $Zn(ATP)^{2-}$ and $Cd(ATP)^{2-}$ are reduced by *half* to 10% and 27% to allow for the recently described[9,24] *even* distribution of Zn^{2+} between N-1 and N-7 of adenosine, which was used[11] as a reference in the percentage evaluation. In another approach, when it is bound to N-7 of AMP^{2-}, $Pd(dien)^{2+}$ induces a downfield shift at pH >8 of 0.70 ppm in the position of the H-8 peak.[50] We use this shift as a prototype for other diamagnetic metal ions and compare it with the downfield shifts of 0.08 ppm for $Zn(ATP)^{2-}$ and 0.21 ppm for $Cd(ATP)^{2-}$ given in ref 11: on the basis of the shift of 0.70 ppm for full complexation one obtains 11% for $Zn(ATP)^{2-}_{cl/i}$ and 30% for $Cd(ATP)^{2-}_{cl/i}$. This pair of values agrees closely with the 10% and 27% calculated at the beginning of this paragraph. We list as representative NMR inner-sphere back-bound percentages 10% for $Zn(ATP)^{2-}_{cl/i}$ and 30% for $Cd(ATP)^{2-}_{cl/i}$ in Table VI. For $Mn(ATP)^{2-}$ it was also shown[16] that some inner-sphere coordination occurs.

The percentage values for inner-sphere back-bonded coordination from UV spectra and NMR are combined to yield a "best" value that appears in the fifth column of Table VI. The difference between the total percentage in the second column and the "best" percentage for the inner-sphere isomer gives the outer-sphere percentage in the sixth column. The last column contains the difference of the sum of the two closed isomers to 100%, which is the percentage of the open isomer, $M(ATP)^{2-}_{op}$.

For $Ni(ATP)^{2-}$ the situation is certainly most clear-cut: the total amount of closed species obtained from the potentiometric pH titration data can never be explained alone by an inner-sphere back-bound isomer. In this case there exists about 30% as inner-sphere and about 25% as outer-sphere isomers; these estimates for $Ni(ATP)^{2-}$ agree closely with the original analysis.[17] In fact, it was concluded[17] for ATP → ADP → AMP that with the decreasing number of phosphate groups the Ni^{2+} complexes show an increasing mole fraction of the inner-sphere closed isomer at the expense of the mole fractions of the outer-sphere and open forms. This order may be compared with evidence[13,51] showing that the total extent of macrochelate formation apparently depends for all studied metal ions also on the number of phosphate groups, but in a different way: i.e., $\%\ M(AMP)_{cl/tot.} < \%\ M(ADP)^{-}_{cl/tot.} > \%\ M(ATP)^{2-}_{cl/tot.}$.

A similar clear-cut conclusion appears possible for $Mg(ATP)^{2-}$: UV spectrophotometric measurements show that no[17] or only a tracelike[14] direct N-7 interaction occurs; no evidence for a direct Mg^{2+}–N-7 interaction is observed by NMR.[11,52] Hence, according to the data summarized in Table VI, about 10% of $Mg(ATP)^{2-}$ exists as an outer-sphere macrochelate.

Except for $Cu(ATP)^{2-}$ and the mentioned case of $Mg(ATP)^{2-}$ the inner- and outer-sphere percentages occur in comparable amounts.[53] The percentages given in Table VI for $M(ATP)^{2-}_{cl/tot.}$ reflect the relative affinity of the corresponding metal ions for N-donor ligands.[48] In fact, the sequence of total and inner-sphere closed forms not only follows the usual stability series for dipositive first-row transition-metal ions but also follows closely the stability constants for imidazole binding[54,55] even to the relative placements of Zn^{2+} and Cd^{2+} as indicated in the stability ruler.[56]

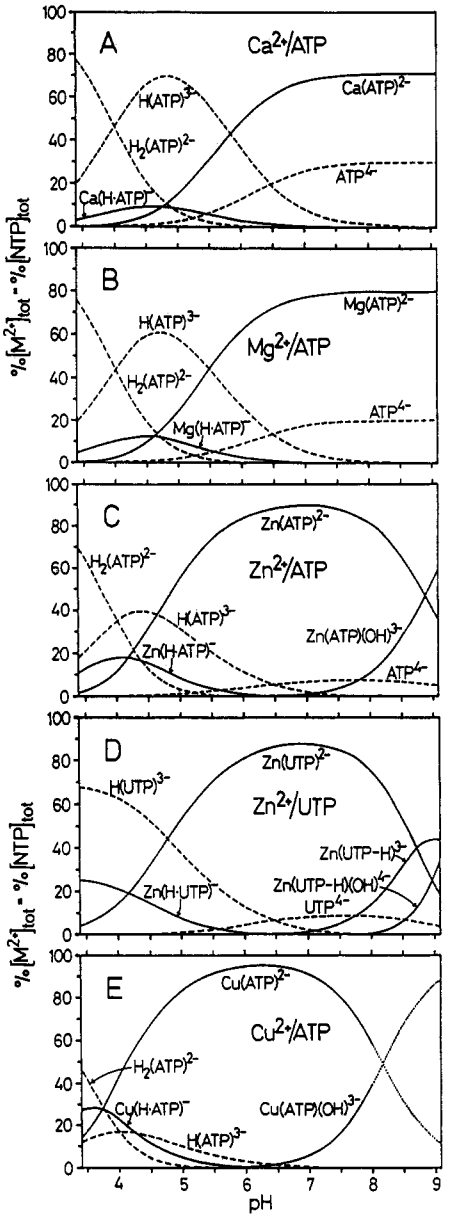

Figure 3. Comparison of the effect of pH on the concentration of the species present in an aqueous solution of (A) Ca^{2+}/ATP, (B) Mg^{2+}/ATP, (C) Zn^{2+}/ATP, (D) Zn^{2+}/UTP ($[H_2(UTP)^{2-}] \leq 3.4\%$ and $[(UTP\text{-}H)^{5-}] < 1\%$), and (E) Cu^{2+}/ATP ($[ATP^{4-}] < 1.8\%$; the dotted-line portions at pH >8 indicate uncertainty; see below). The results are given as the percentage of the total M^{2+} present (=total NTP). The broken lines indicate the free NTP species and the solid lines the complexes. The distribution curves were computed with the potentiometrically determined constants listed in Tables I and II, plus those given below, for concentrations of 10^{-3} M for each reactant at $I = 0.1$ and 25 °C: $pK^{H}_{Zn(ATP)(H_2O)} = 8.87$;[31] $pK^{H}_{UTP} = 9.70$,[31] $pK^{H}_{Zn(UTP)} = 8.71$,[31] $pK^{H}_{Zn(UTP\text{-}H)(H_2O)} = 9.24$;[31] $pK^{H}_{Cu(ATP)(H_2O)} = 8.17$ (this constant describes the situation well only in the pH range up to 8.0 as is indicated in ref 58; see also ref 59).

General Considerations. The results and conclusions show unequivocally that macrochelate formation is an important feature

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Table VI. Percentages of the Macrochelated Isomers of Several $M(ATP)^{2-}$ Systems As Determined by Stability Data from Potentiometric pH Titrations (stab) and by UV Spectrophotometric (spect) or NMR Measurements: Evidence for the Formation of Inner-Sphere (i) and Outer-Sphere (o) Macrochelated Isomers[a]

M^{2+}	% $M(ATP)^{2-}_{cl/tot}$ stab[b]	% $M(ATP)^{2-}_{cl/i}$ spect[g]	% $M(ATP)^{2-}_{cl/i}$ NMR[h]	estimates[a] % $M(ATP)^{2-}_{cl/i}$[j]	% $M(ATP)^{2-}_{cl/o}$	% $M(ATP)^{2-}_{op}$
Mg^{2+}	11 ± 6/13 ± 6[c]	1–3/0	0	0	10	90
Ca^{2+}	2 ± 6	1–3		0	~0	~100
Mn^{2+}	17 ± 10	3/10		~10	~10	80
Co^{2+}	38 ± 9	12/35		~25	~15	60
Ni^{2+}	56 ± 4/58[d]	20/29		30	25	45
Cu^{2+}	67 ± 2/68 ± 4[e]	80		67	~0	33
Zn^{2+}	28 ± 7	15	10[i]	15	15	70
Cd^{2+}	46 ± 4/52[f]		30[i]	30	20	50

[a] The inner-sphere macrochelate is designated by "i" and the outer-sphere by "o". The percentage of the macrochelates totally formed, $M(ATP)^{2-}_{cl/tot}$, is given by the results obtained from the potentiometric pH titrations, while the other methods give % $M(ATP)^{2-}_{cl/i}$; hence, % $M(ATP)^{2-}_{cl/o}$ = % $M(ATP)^{2-}_{cl/tot}$ − % $M(ATP)^{2-}_{cl/i}$ and % $M(ATP)^{2-}_{cl/i}$ + % $M(ATP)^{2-}_{cl/o}$ + % $M(ATP)^{2-}_{op}$ = 100%. [b] From Table IV (I = 0.1 ($NaClO_4$ or $NaNO_3$); 25 °C; error limits 2σ) except where another source is given. [c] Calculated from the averages of the constants (three values each) listed in Table III of ref 15 (I = 0.1; 15–25 °C): log $K^{Mg}_{Mg(ATP)}$ = 4.07 ± 0.03 (1σ); log $K^{Mg}_{Mg(PNTP)}$ = 4.01 ± 0.01, based on $Mg(CTP)^{2-}$ and $Mg(H{\cdot}P_3O_{10})^{2-}$. [d] Calculated from the following constants given in ref 15 (I = 0.1 (KNO_3); 15 °C): log $K^{Ni}_{Ni(ATP)}$ = 4.79 and log $K^{Ni}_{Ni(PNTP)}$ = 4.41 (based on $Ni(CTP)^{2-}$ and $Ni(H{\cdot}P_3O_{10})^{2-}$) give K_I = 1.4, which is in good agreement with K_I = 1.5 given in ref 37. [e] From ref 21 (I = 0.1 ($NaNO_3$); 25 °C). [f] From ref 12 (I = 0.1 ($NaNO_3$); 25 °C). [g] First value from ref 14, probably natural ionic strength and ambient temperature; second value from ref 17, I = 0.2 ($NaClO_4$), 23 °C. [h] By ^1H NMR from ref 11: D_2O, I = 0.1 ($NaNO_3$), 27 °C. [i] The values given in ref 11 are upper limits because Zn^{2+} and Cd^{2+} may coordinate at adenosine (which was used as a basis in the evaluations) not only to N-7 but also to N-1.[9] Regarding the above values, see text in the first Conclusion part. [j] "Best" value based on the two columns to the left.

of $M(ATP)^{2-}$ complexes that may be reversed by mixed-ligand-complex formation as recent studies show.[12,57] This process of base back-binding also occurs in those complexes common in biological systems as substrates: $Mn(ATP)^{2-}$ and $Zn(ATP)^{2-}$ certainly form in equilibrium macrochelated isomers. As indicated in Table VI, $Mg(ATP)^{2-}$ also occurs to about 10% as a macrochelate but only as an outer-sphere isomer in which a water molecule links the phosphate-bound Mg^{2+} and N-7.

Structural alterations of the indicated kind may be involved with the selectivity observed in nature. This statement is supported by the results summarized in Figure 3.[58,59] Despite the nearly 2.5 log unit difference in complex stability (see Table II), the overall formation degree of $M(ATP)^{2-}$ is very similar in the physiological pH range. However, for $Ca(ATP)^{2-}$ only a phosphate-coordinated species occurs, while for $Zn(ATP)^{2-}$ nearly 30% exists as base-back-bound isomers. With $Cu(ATP)^{2-}$ the situation is even more extreme: two-thirds of this complex is macrochelated, and there are indications[60] that $Cu(ATP)^{2-}$ might be a natural active form of Cu^{2+}.

Obviously, not only are there structural differences between $M(ATP)^{2-}$ complexes depending on the kind of metal ion involved but there are also differences between NTP complexes formed with the same metal ion; e.g., the formation degrees of $Zn(UTP)^{2-}$ and $Zn(ATP)^{2-}$ around pH 7 differ very little (Figure 3), but in the first case no metal ion–base interaction occurs while in the second one an isomer with such an interaction is important. Such subtle structural alterations between $M(NTP)^{2-}$ complexes, which differ little in their free energies (ΔG), appear to be ideal to promote enzymic selectivity.

There is one further aspect to be considered: from Figure 3 it is evident that the complexes $M(H{\cdot}NTP)^-$ in aqueous solution barely extend into the physiological pH range. However, in the active-site cavities of enzymes the equivalent solution or effective dielectric constants are lower than in water.[61] It is also known[21] that ionization of a proton from the γ-phosphate group is inhibited by addition of an organic solvent to an aqueous solution; i.e., a lower polarity solvent increases the pK_a values for R–OH ionization. The consequence is that those isomers of $M(H{\cdot}NTP)^-$ (see parts 6–8 and Figure 2) carrying the proton at the phosphate group may become favored, and it may therefore be that in active-site cavities of enzymes these species can exist and are playing a role in reactions. It is enough that the reactive conformation of a substrate occurs to some few percentages in equilibrium, especially if the energy barrier between several isomers is low, a condition clearly fulfilled in the present cases.

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