Nucleoside 5′-triphosphates: self-association, acid–base, and metal ion-binding properties in solution†

Helmut Sigel* and Rolf Griesser

Received 5th May 2005
First published as an Advance Article on the web 1st September 2005
DOI: 10.1039/b505986k

Adenosine 5′-triphosphate (ATP^{4-}) and related nucleoside 5′-triphosphates (NTP^{4-}) serve as substrates in the form of metal ion complexes in enzymic reactions taking part thus in central metabolic processes. With this in mind, the coordination chemistry of NTPs is critically reviewed and the conditions are defined for studies aiming to describe the properties of monomeric complexes because at higher concentrations (>1 mM) self-stacking may take place. The metal ion (M^{2+}) complexes of purine–NTPs are more stable than those of pyrimidine–NTPs; this stability enhancement is attributed, in accord with NMR studies, to macrochelate formation of the phosphate-coordinated M^{2+} with N7 of the purine residue and the formation degrees of the resulting isomeric complexes are listed. Furthermore, the formation of mixed-ligand complexes (including also those with buffer molecules), the effect of a reduced solvent polarity on complex stability and structure (giving rise to selectivity), the use of nucleotide analogues as antiviral agents, and the effect of metal ions on group transfer reactions are summarized.

Department of Chemistry, Inorganic Chemistry, University of Basel, Spitalstrasse 51, CH-4056, Basel, Switzerland.
E-mail: Helmut.Sigel@unibas.ch; Fax: +41-61-267-1017
† Dedicated in friendship to Professor Dr. Henryk Kozlowski, University of Wroclaw, on the occasion of his 60th birthday with best wishes for all his future endeavors.

1 Introduction

1.1 Role of metal ions and nucleoside 5′-triphosphates

"If you think that biochemistry is the organic chemistry of living systems, then you are misled, *biochemistry is the*

Helmut Sigel (left in the picture) is Emeritus Professor (since 2003) at the Department of Chemistry, University of Basel, Switzerland, where he has been Dozent since 1967 and Professor (Extraordinarius) from 1978 on. He is a member of several chemical societies and serves on various editorial and advisory boards, including the 'Research Center for Materials Science' at Nagoya University (Japan). Together with Astrid Sigel and Roland K. O. Sigel he is an editor of the series 'Metal Ions in Biological Systems', which he founded in 1973 (over 45 volumes have appeared), and also co-editor of three handbooks (Toxicity of Inorganic Compounds / Metals in Clinical and Analytical Chemistry / Metalloproteins). Dr. Sigel participated in various European programmes and research networks; he is one of the initiators of the 'International Conferences on Bioinorganic Chemistry' (ICBICs); he has lectured worldwide and published over 300 articles on metal ion complexes of nucleotides, nucleotide analogues, coenzymes, and other ligands of biological relevance. He was named Protagonist in Chemistry (2002) by Inorganica Chimica Acta (issue 339); among further honors are the P. Ray Award (Indian Chemical Society, of which he is also an Honorary Fellow), the Werner Award (Swiss Chemical Society), a Doctor of Science honoris causa degree (Kalyani University, India), appointments as Visiting Professor (e.g., Austria, China, UK) and Endowed Lectureships.

Rolf Griesser (right in the picture) studied chemistry at the University of Basel, Switzerland, where he received the Diploma in 1964 and completed the PhD studies in 1967 under the supervision of the late Professor Dr. Silvio Fallab. From 1967 to 1970 he worked in the Institute of Inorganic Chemistry of the same University, first as an Assistant to the late Professor Dr. Hans Erlenmeyer and then on bioinorganic topics with Professor Dr. Helmut Sigel. During the academic year 1970/1971 he was a Postdoctoral Research Associate with Professor Donald B. McCormick at Cornell University in Ithaca (N.Y., USA), where he studied the metal ion-binding properties of biotin and derivatives. After his return to Basel he joined Sandoz Ltd., where his research first focused on metal ion-containing pigments, then he moved to pharmaceutical development and finally he headed the Radiation Protection Office. In 1998, after the merger of Sandoz Ltd. and Ciba-Geigy Ltd. to Novartis Ltd., he retired from industry and returned to his old love Bioinorganic Chemistry as Research Associate to Professor Sigel at the University of Basel. His research interests focus on the stability and structure of simple and mixed ligand metal ion complexes of amino acids, peptides, nucleotides and nucleotide analogues; he has several patents and has published about 50 papers.

coordination chemistry of living systems". This statement by John Wood[1] made in 1975 is, of course, in some respect a deliberate exaggeration, but over the past 30 years it became evident that it contains much truth and that metal ions are playing a key role in biological systems.[2,3] Indeed, one is inclined to conclude that whenever nature has a difficult task to perform, a metal ion, or sometimes a cluster of such ions, is invariably employed.[4]

Similarly, nucleoside 5′-triphosphates (NTPs)‡ play a key role in all aspects of metabolism;[2,5] two representative examples are adenosine 5′-triphosphate (ATP^{4-})[6,7] and guanosine 5′-triphosphate.[7] ATP is an ubiquitous substrate for many biological reactions and generally regarded as an intracellular energy donor.[8] However, ATP is also recognized as an important neurotransmitter,[9] a property which is in part possibly interlinked with its ability to form stacks;[10] it can mediate fast ligand-gated synaptic transmission at nerve-nerve synapses,[9,11] and it appears that Zn^{2+} has a physiological role in the regulation of the excitatory action of ATP on mammalian neurons.[12] Indeed, from known metabolic pathways and the extent of the world's biomass, Boyer[8] calculated that ATP, and ADP and inorganic phosphate, from which it is formed, participate in more chemical reactions than any other compound on the earth's surface except water. Moreover, other calculations show,[13] again in accord with the central metabolic role of ATP, that an average person will daily synthesize and use the amount of ATP equivalent to the body weight. – The other prominent example is GTP which is utilized by so-called G-proteins in such diverse processes[14] as cellular signaling,[15] protein synthesis,[16] vesicular trafficking,[17] ion channel regulation,[18] nerve growth[19] or exocytosis.[20]

In agreement with the above given opening statement of Wood,[1] virtually all reactions of NTPs also involve metal ions,

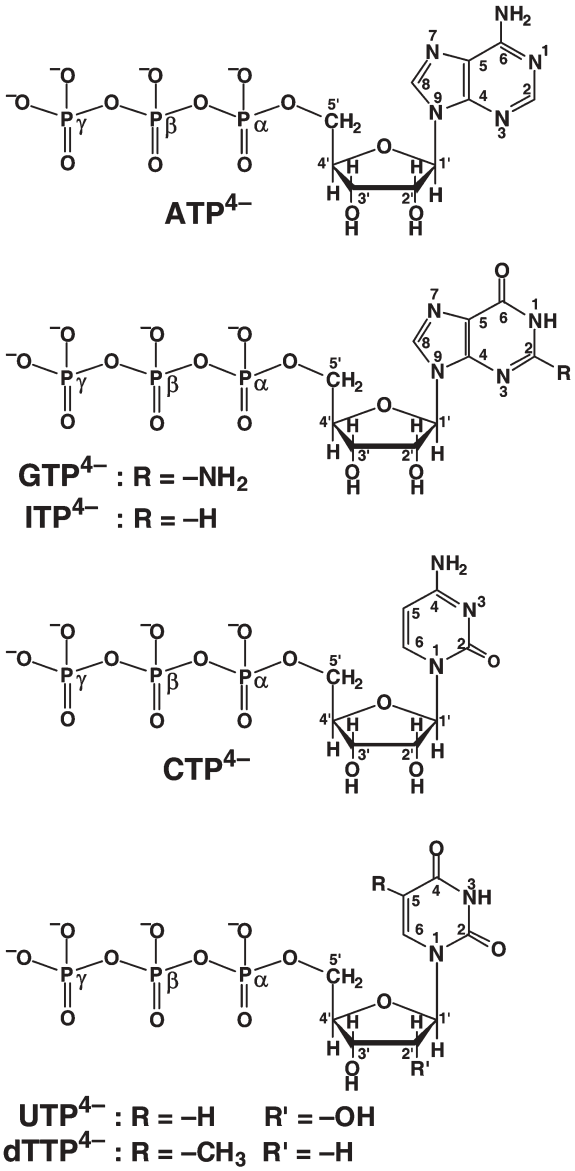


Fig. 1 Chemical structures of adenosine 5′-triphosphate (ATP^{4-}), guanosine 5′-triphosphate (GTP^{4-}), inosine 5′-triphosphate (ITP^{4-}), cytidine 5′-triphosphate (CTP^{4-}), uridine 5′-triphosphate (UTP^{4-}), and thymidine [= 1-(2′-deoxy-β-D-ribofuranosyl)thymine] 5′-triphosphate ($dTTP^{4-}$); all are shown in their dominating *anti* conformation.[42–46] Note, the phosphate groups in the nucleoside 5′-triphosphates (NTPs) are labeled as α, β, and γ, where γ refers to the terminal phosphate group. The analogous nucleoside 5′-monophosphates (NMPs) and nucleoside 5′-diphosphates (NDPs) have the corresponding structures with one or two phosphate groups, respectively; the terminal phosphate group in the NDPs is labeled as β. The nucleobase residues shown above are (from top to bottom) adenine, guanine (R = $–NH_2$), hypoxanthine (R = –H), cytosine, uracil (R = –H), and thymine (R = $–CH_3$); the corresponding nucleosides (Ns) are named as adenosine (Ado), guanosine (Guo), inosine (Ino), cytidine (Cyd), uridine (Urd), and thymidine (dThd).

since the NTPs serve as substrates only in the form of their metal ion complexes, *e.g.*, in the biosynthesis of nucleic acids.[21,22] In addition, many of the enzymes involved in these turnovers also contain metal ions as integral components,[23]

‡ Abbreviations and definitions: Only those abbreviations are listed which are not given or do not logically follow from the definitions provided in the legends of Figs. 1, 7 and 8. AA^-, amino-acid anion; ADP^{3-}, adenosine 5′-diphosphate; Ala^-, alaninate (anion of alanine); AMP^{2-}, adenosine 5′-monophosphate; AP, adenosine phosphate (= AMP^{2-}, ADP^{3-}, ATP^{4-}); Arm, heteroaromatic nitrogen base (like Bpy or Phen); Bpy, 2,2′-bipyridine; $dATP^{4-}$, 2′-deoxyadenosine 5′-triphosphate; $dCTP^{4-}$, 2′-deoxycytidine 5′-triphosphate; $dNTP^{4-}$, 2′-deoxynucleoside 5′-triphosphate; ε-ATP^{4-}, 1,N^6-ethenoadenosine 5′-triphosphate; FMN^{2-}, flavin mononucleotide; GMP^{2-}, guanosine 5′-monophosphate; *I*, ionic strength; Im, imidazole; IMP^{2-}, inosine 5′-monophosphate; K_a, acidity constant; L, general ligand; Lys, L-lysine; M^{2+}, general divalent metal ion; N, nucleoside and/or nucleotide; NMR, nuclear magnetic resonance; NP, nucleoside phosphate; Ns, nucleoside; Phen, 1,10-phenanthroline; p-Lys, poly-α,L-lysine; $PMEA^{2-}$, dianion of 9-[2-(phosphonomethoxy)ethyl]-adenine; $PMEApp^{4-}$, diphosphorylated $PMEA^{2-}$; $PyNTP^{4-}$, pyrimidine-nucleoside 5′-triphosphate; $R\text{-}DP^{3-}$, diphosphate monoester with a residue R that does not affect metal ion binding; $RibMP^{2-}$, D-ribose 5-monophosphate; $R\text{-}MP^{2-}$, monophosphate monoester (for R see $R\text{-}DP^{3-}$); $R\text{-}TP^{4-}$, triphosphate monoester (for R see $R\text{-}DP^{3-}$); UMP^{2-}, uridine 5′-monophosphate. – Species written in the text without a charge do not carry one or represent the species in general (*i.e.*, independent from their protonation degree); which of the two possibilities applies is always clear from the context. In formulas such as $M(H;NTP)^-$, the H^+ and NTP^{4-} are separated by a semicolon to facilitate reading; yet, they appear within the same parenthesis to indicate that the proton is at the ligand without defining its location. A formula like $(NTP - H)^{5-}$ means that the ligand has lost a proton and it is to be read as NTP^{4-} *minus* H^+. The term (aq) is used to indicate that water is acting as a ligand.

like, *e.g.*, nucleic acid polymerases.[21,24] Hence, metal ions, mostly Mg^{2+} (*e.g.*[25]) but also Mn^{2+} (*e.g.*[26]) or Zn^{2+} (*e.g.*[27]), *in combination* with the NTPs, are at the crossroads in biology. Already Szent-Györgyi[28] had recognized this nearly 50 years ago and he has postulated a structure for the "quadridentate Mg^{2+} chelate" of $H_2(ATP)^{2-}$ (!) in which the metal ion was coordinated to the twofold protonated triphosphate chain and also to the adenine moiety giving such rise to, what we call today, a macrochelate. Though Szent-Györgyi's picture needed significant revisions,[29] ever since coordination chemists have been fascinated by the interactions between metal ions and nucleotides, *i.e.* the stabilities,[7,30–34] structures,[7,29,33,35–37] and reactivities[38–41] of these complexes.

1.2 Structures and conformations of NTPs

Nucleotides are composed of three units, a nucleobase, a sugar, and a phosphate residue,[42] which gives in the case of a single phosphate group a nucleoside monophosphate, and with two or three phosphate groups a nucleoside di- or triphosphate, respectively (Fig. 1).[42–46] The sugar is commonly a cyclic, furanoside-type five-membered ring, *i.e.* β-D-ribose is part of ATP, CTP, *etc.* (see Fig. 1) and β-D-2′-deoxyribose of dATP, dCTP, *etc*. In the form of metal ion complexes the former ones are substrates for RNA polymerases and the latter ones for DNA polymerases.[21,24,27] The nucleobase residues which mainly occur in nucleic acids are the purine bases adenine and guanine, and the pyrimidine bases cytosine and uracil for ribonucleic acids (RNAs); in 2′-deoxyribonucleic acids (DNAs) the latter base is replaced by thymine (= 5-methyluracil).

It is important to note that in the solid state[42,46] and in solution as well[43–45,47,48] nucleosides and nucleotides exist predominantly in the so-called *anti* conformation (Fig. 1). This means, in the case of purines the N9–C8 bond and in the case of pyrimidines the N1–C6 bond project onto or near the sugar ring,[43] whereas in the *syn* conformation the N9–C4 bond of purines and the N1–C2 bond of pyrimidines project onto or near the sugar ring. The *syn*/*anti* barrier around the glycosyl bond C1′–N1 for cytidines has been estimated as being in the order of 6–7 kJ mol^{-1}.[49,50]

The mentioned dominating conformations have consequences for the metal ion-binding properties of nucleotides: A metal ion coordinated at the phosphate residue can reach N7 of the purine moiety[7,49] and in this way macrochelates are formed (see Section 4.4). In contrast, in pyrimidine-nucleotides the N3(C2)O site is directed away from the ribose ring and consequently this binding site cannot be reached by a phosphate-coordinated metal ion (see Fig. 1);[29,49] this would only be possible in the *syn* conformation but to obtain this is energetically costly and therefore pyrimidine-nucleotides behave as far as metal ion binding is concerned mostly as simple phosphate ligands (see Section 4.2).[34]

Regarding metal ion binding, a further point warrants emphasis here. The phosphoric acid anhydride bonds in a triphosphate ester can either be broken between the β,γ-phosphate units or between the α,β ones (Fig. 1). In the first case a kinase-type reaction with the transfer of a phosphoryl group takes place,[40] whereas in the second case a nucleotidyl group is split off as it is catalyzed by nucleic acid polymerases.[24] The point now is that dependent on the coordinating pattern of metal ions to the triphosphate chain either the one or the other site is activated, meaning that metal ion-coordination decides which type of reaction takes place (see Section 7).[38,51]

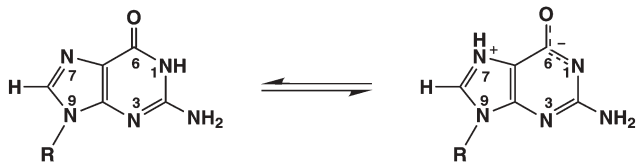


Fig. 2 Two tautomeric forms of guanosine (R = β-D-ribofuranosyl residue) which illustrate equilibrium (1).

A final point to be considered in this context refers to the structures of the nucleobase residues shown in Fig. 1. It was Martin[52] who emphasized in 1985 that to "the everlasting embarrassment of organic chemistry, by early 1953 it had not yet described definitely the dominant tautomeric structures that occur in the four nucleic bases of DNA. This deficiency caused base-pairing problems in formulations of the DNA double-helical structure".[53] In the meanwhile this problem can be considered as settled[52] and the predominant tautomeric forms (Fig. 1) and proton binding sites (see Section 3.1) of the common nucleobases have been confirmed by a variety of methods.[52] However, the formulation "predominant tautomeric forms" already indicates that minority species might exist. Indeed, recently[54,55] the micro acidity constants for the (N1)H and (N7)H sites of several purine derivatives have been determined and these values allow to define the intrinsic acid–base properties in aqueous solution of the individual N sites of nucleobases. For example, in guanosine the proton is overwhelmingly located at N1 leading to the tautomer seen in Fig. 1 but there is another, a zwitterionic tautomer present where the proton has moved from the (N1)H site to N7; this is expressed in equilibrium (1)

$$(\text{Guo})\text{N7–N1}\cdot\text{H} \rightleftharpoons (\text{Guo})^{+}\text{H}\cdot\text{N7–N1}^{-} \qquad (1)$$

and depicted in Fig. 2. Roughly speaking the ratio $(\text{Guo})^{+}\text{H}\cdot\text{N7–N1}^{-}/(\text{Guo})\text{N7–N1}\cdot\text{H}$ is 1:80000, meaning that among 80000 guanosine molecules one is present in the zwitterionic form.[54,55] Similarly, for adenosine it has been estimated[54,55] that among 100000 common tautomers (as shown in Fig. 1) there is one (C6)=NH imino tautomer (with a H at N1) present. Though rare, such tautomers could lead to mis-matches in DNA base pairing and thus give rise to mutations.[56]

1.3 Focus of this review

In Section 1.1 the interplay between metal ions and nucleoside 5′-triphosphates was shortly indicated and in Section 1.2 several pertinent properties of nucleobase residues and of NTPs were described. This means, the scene is set to a large part for a summary and a discussion of the acid–base and metal ion-binding qualities of NTPs. Only one further addition is necessary; this is the fact that nucleobase residues and consequently also NTPs may undergo self-association *via*

aromatic-ring stacking. Not knowing or ignoring this property has historically led to much confusion since, *e.g.*, spectrophotometric measurements in 10^{-5} M nucleotide solutions furnished results which differed from those obtained by ^{1}H-NMR in 0.1 M solutions. These historical contradictions and difficulties have previously been summarized.[36,38] For the present it is only necessary to define the conditions needed which allow to measure the properties of monomeric species; this is attempted in Section 2.

In the sections to follow and after having considered the acid–base properties of the NTPs (Section 3) we shall deal with metal ion complexes but restrict ourselves to those which contain the biologically important divalent metal ions, *i.e.* Ca^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+} and Zn^{2+}. All of these are labile in character, *i.e.* the exchange rates in their coordination spheres are high and equilibrium is "instantly" reached upon mixing of the components. This high lability is one of the main differences between the metal ions of life and those metal ions which are employed in the form of complexes as drugs; the latter ones are rather inert, *i.e.* their exchange rate is slow like the one of platinum(II) or ruthenium(III).[57] Finally, the equilibrium data for other labile metal ions, like Sr^{2+}, Ba^{2+}, Co^{2+}, Ni^{2+} and Cd^{2+}, are not listed here but the corresponding constants may usually be found in the same literature citations given for the essential metal ions.

2 Self-association of NTPs and related species

Considering that in the double helix of DNA the involved nucleobase residues are stacked,[42] it is not surprising that the self-association of purines and pyrimidines as well as of their nucleosides has long been recognized. Indeed, already more than 40 years ago it was shown that purines associate much better than pyrimidines;[58] regarding nucleotides and their self-association tendency the situation was less clear for many years.[59] Today it is generally accepted that self-association of all of these species occurs *via* aromatic-ring stacking of the nucleobases,[59–61] that it proceeds beyond the dimer stage, and that oligomers are formed.[59,61–65]

^{1}H-NMR shift measurements proved ideal to characterize the self-association of nucleosides and nucleotides (= N) (Fig. 1) in aqueous solution (D_2O).[59,63–65] The upfield shifts of the resonances, especially of the nucleobase protons, observed with increasing concentration of N confirm that the association occurs *via* stacking of the aromatic moieties. In most instances the association process is best quantified by application of the isodesmic model for an indefinite non-cooperative self-association;[47,59,61,63,66] *i.e.*, all association constants are considered as equal:

$$(N)_n + N \rightleftharpoons (N)_{n+1} \quad (2a)$$

$$K_{SA} = [(N)_{n+1}]/([(N)_n][N]) \quad (2b)$$

The relationship between the observed chemical shift (δ_{obsd}) and a solution with the total concentration N is given in eqn (3),

$$\delta_{obsd} = \delta_\infty + \{(\delta_\infty - \delta_0)[1 - (4K_{SA}[N] + 1)^{0.5}]/2K_{SA}[N]\} \quad (3)$$

where δ_0 represents the shift at infinite dilution (monomeric N), δ_∞ the shift of a molecule in an infinitely long stack, and K_{SA} is the self-association constant (eqn (2)). By ignoring species larger than dimers, a relationship analogous to eqn (3) is obtained:[59] *i.e.*, δ_∞ is replaced by δ_D, the upfield shift in a dimer, and K_{SA} is replaced by 2 K_D (*i.e.*, $K_D = 0.5\ K_{SA}$), K_D being the equilibrium constant for dimerization.[59,63,67] Whether a system dimerizes or polymerizes can only be concluded from the extent of the upfield shifts.[59,63,68]

2.1 NTP self-stacking and the effect of metal ions

Some self-association constants of purine-nucleobase derivatives (Fig. 1) are listed in Table 1.[61,63,64,69,70] From these data, plus some for the pyrimidines,[63] it is evident that the self-stacking tendency decreases for nucleosides in the series, Ado > Guo > Ino > Cyd ($K_{SA} = 1.4 \pm 0.5$ M) > Urd (1.2 ± 0.5), reflecting the decreasing aromaticity and hydrophobic properties of their nucleobase residues. The analogous series are observed within the error limits for the various nucleotides.[63,64] A further comparison of the data given in Table 1 shows that the association tendency also decreases in the series, Ns > NMP^{2-} > NDP^{3-} > NTP^{4-}. This decrease is evidently governed by the effect of the increasing negative charge of the phosphate residue. Hence, this observation suggests that charge neutralization at the phosphate group of a nucleotide facilitates its self-association.

Indeed, Mg^{2+} coordination to ATP^{4-} or ITP^{4-} significantly reduces the repulsive effect of the negatively charged triphosphate chains on the self-association of these NTPs as follows from the results in Table 1. However, for ATP^{4-} K_{SA} increases upon Mg^{2+} coordination from 1.3 M^{-1} to 4.0 M^{-1}, *i.e.* by a factor of about 3, but $K_{SA} = 4.0\ M^{-1}$ for $Mg(ATP)^{2-}$ is nearly twice as large as $K_{SA} = 2.1\ M^{-1}$ for AMP^{2-}, both species being twofold negatively charged. Hence, Mg^{2+} affects the stability of the NTP^{4-} (and NDP^{3-})[64] stacks beyond a pure charge neutralization indicating an *inter*molecular bridging by

Table 1 Association constants for self-stacking of purine-nucleosides and some of their nucleotides as determined by ^{1}H-NMR shift measurements in D_2O, as well as the effect of metal ion coordination on the self-stacking tendency of NTP^{4-} species (27 °C; $I = 0.1$ or $0.1 - \sim 2$ M, $NaNO_3$)[a]

Systems defined *via* Ns:	K_{SA} (M^{-1}) (eqn (2))		
	Ado	Guo	Ino
Ns	15 ± 3[b]	8 ± 3	3.3 ± 0.3
NMP^{2-}	2.1 ± 0.3[b]/1.9[c]	1.3[c]	1.4[c]
NDP^{3-}	1.8 ± 0.5[d]	1.0 ± 0.5[d]	1.3 ± 0.6[d]
NTP^{4-}	1.3 ± 0.2	0.8 ± 0.6	0.4 ± 0.3
$Mg(NTP)^{2-}$	4.0 ± 0.5		2.0 ± 0.6
$Zn(NTP)^{2-}$	~11.1 ± 4.5[e]	1.9 ± 0.6[e]	2.8 ± 1.2[e]
$Cd(NTP)^{2-}$	~17[e]		

[a] Average of the results (with *twice* the standard error) obtained from the chemical shifts of H2, H8, and H1′ of the purines (see Fig. 1). The constants are from ref. 63 if not otherwise indicated. [b] From ref. 69. [c] From ref. 61. [d] From ref. 64. [e] These values are only estimates, as the experimental data cannot be solely explained by the simple isodesmic model (eqns (2) and (3)); in these systems dimers are especially favored due to the participation of N7 in metal ion coordination; for details see ref. 63 as well as 64 and especially 70.

Mg^{2+} (reviewed in ref. 70). Since the chemical shift values for δ_∞ give no indication[63,64] for a selective Mg^{2+}-nucleobase interaction in the stacks, bridging by Mg^{2+} must occur *via* the phosphate chains.

For the ATP^{4-} complexes with Zn^{2+} and Cd^{2+} the self-association tendency is much larger than for the mentioned corresponding Mg^{2+} complex (Table 1).[63] This observation is explained by the formation of an *inter*molecular metal ion bridge in *dimeric* stacks by coordination of Zn^{2+} or Cd^{2+} to the phosphate moiety of one ATP^{4-} and to N7 of the other.[70] Of course, these relatively stable dimeric stacks, like $[Zn(ATP)]_2^{4-}$, may further associate to larger aggregates in the usual manner. The chemical shifts of the various hydrogens for complete stacking (δ_∞)[63,64] agree with this interpretation. It should be noted that the "constants" given in Table 1 for the Zn^{2+} and Cd^{2+} systems are based on the isodesmic model (eqn (3)) to enable direct comparisons with the other constants given, but they do not describe the experimental results in the best way (for details see refs. 63,64 and the review 70). Here, the important point is that metal ions like Zn^{2+} or Cd^{2+} favor the formation of dimers by bridging the phosphate *and* N7 sites of the two nucleotides forming the dimer. Furthermore, the facilitated formation of such dimeric complexes is also important for the metal ion-promoted dephosphorylation of ATP.[38]

In the above context it is interesting to note that 'small' alterations at the nucleobase can have dramatic effects: For example, the addition of the 1,N^6-ethenobridge to the adenine residue changes the binding properties of 1,N^6-ethenoadenosine 5′-triphosphate (ε-ATP^{4-}) towards certain metal ions drastically, if compared to those of ATP^{4-}.[71,72] This has severe consequences for the self-association, not so much for the free ligands but for their metal ion complexes: *i.e.*, stacking of ε-ATP^{4-} is more promoted[73] by Mg^{2+} than by Zn^{2+} (in contrast to the results discussed above for ATP^{4-}) and these different effects of metal ions also give rise to different mechanisms in the metal ion-facilitated hydrolysis[74] of ε-ATP, ATP, and CTP (see also ref. 70).

2.2 Purines and the effect of protonation and ion-pair formation on self-association

How does protonation affect self-association? This question shall be first addressed for adenosine. The concentration dependence of the chemical shifts for H2, H8 and H1′ of adenosine was measured[69] in D_2O under several degrees of protonation (27 °C; $I \sim 0.1$ M, $NaNO_3$). Maybe it is helpful to mention here that the pD of a D_2O solution is obtained by adding 0.40 to the pH meter reading[69,75] (the meter being calibrated with H_2O buffers) and that acidity constants valid for deprotonations in H_2O can be transformed[76] with eqn (4)

$$pK_{a/D_2O} = 1.015 \cdot pK_{a/H_2O} + 0.45 \quad (4)$$

into the corresponding constants which refer now to D_2O as solvent. This equation proved to give excellent results for the acid–base reactions of ATP[68,77] and the adenosine monophosphates.[45]

All ^{1}H-NMR shift results[69] are consistent with the isodesmic model (eqn (2)). The association constants decrease with increasing protonation at N1: Ado (K_{SA} = 15 M^{-1}) > D(Ado)$^+$/Ado = 1:1 (6.0 M^{-1}) > D(Ado)$^+$ (0.9 M^{-1}),[69] this result is expected and an analogous one is observed[47] for the systems with guanosine and inosine, where protonation occurs at N7,[78] because the creation of a positive charge at the aromatic rings leads to repulsion and thus to reduced stacking.[47,69] This simple pattern (see also Fig. 3) becomes more complicated if the effect of protonation on the self-association of nucleotides is considered.[47,68,69] With AMP a maximum of self-association is observed[69] in dependence on protonation (Fig. 3): AMP^{2-} (K_{SA} = 2.1 M^{-1}) < D(AMP)$^-$ (*3.5* M^{-1}) < D(AMP)$^-$/D_2(AMP)$^\pm$ = 1:1 (5.6 M^{-1}) ≫ D_2(AMP)$^\pm$ (*1* M^{-1}) > D_3(AMP)$^+$ (K_{SA} < *0.7* M^{-1}) (the values for K_{SA} printed in *italics* are intra- or extrapolated from the association constant–pD profile in ref. 69). This means, neutralization of one of the two negative charges of the $-PO_3^{2-}$ group by protonation leads to a reduced repulsion and thus to a somewhat increased stacking tendency; however, self-stacking is clearly most pronounced if 50% of the adenine residues are protonated at N1, whereas complete nucleobase protonation greatly reduces the stacking tendency as is evident from K_{SA} = 1 M^{-1} for D_2(AMP)$^\pm$.

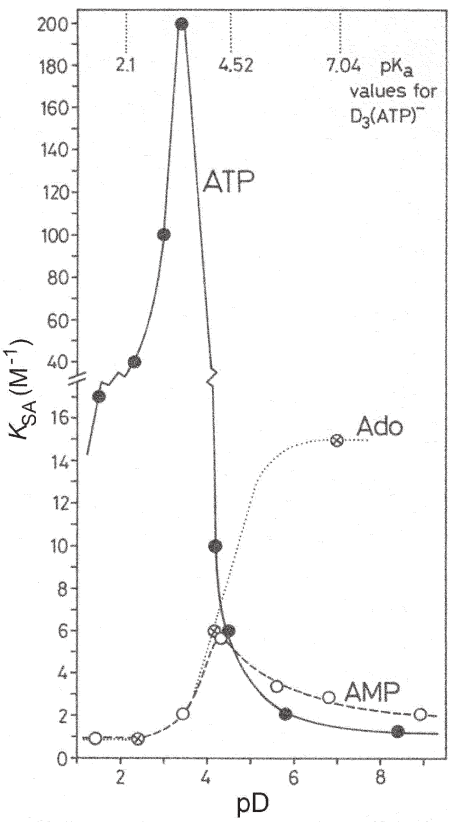


Fig. 3 Dependence of the self-association constant K_{SA} (M^{-1}) (eqns (2) and (3)) in D_2O on pD for ATP (●, full line),[68] AMP (○, broken line),[69] and adenosine (⊗, dotted line).[69] The course of the curves K_{SA} *versus* pD should be compared with the corresponding acidity constants $pK^D_{D_3(ATP)}$ = 2.1, $pK^D_{D_2(ATP)}$ = 4.52, $pK^D_{D(ATP)}$ = 7.04 (these values[68] are inserted at the top of the figure); $pK^D_{D_3(AMP)}$ = 0.9, $pK^D_{D_2(AMP)}$ = 4.33, $pK^D_{D(AMP)}$ = 6.83,[45] and $pK^D_{D(Ado)}$ = 4.14.[45] Reproduced in a slightly altered version by permission of the Federation of European Biochemical Societies (FEBS) from *Eur. J. Biochem.*, ref. 68.

Though apparently in part somewhat complicated, the mentioned self-association patterns in dependence on pH for adenosine and AMP (Fig. 3) are easily rationalized and possibly even predictable. This is different for the self-association of ATP in dependence on its protonation degree:[68] ATP^{4-} (K_{SA} = 1.3 M^{-1}) < $D(ATP)^{3-}$ (2.1 M^{-1}) < $D(ATP)^{3-}/D_2(ATP)^{2-}$ = 1:1 (6.0 M^{-1}) ≪ $D_2(ATP)^{2-}$ (*ca.* 200 M^{-1}) ≫ $D_3(ATP)^{-}$ (K_{SA} < *10* M^{-1}; extrapolated value; see Fig. 3). This result is surprising because the self-association tendency is most pronounced for $D_2(ATP)^{2-}$ which carries a proton each at the terminal γ-phosphate group and at N1. Furthermore, this most pronounced stability is due to a dimeric $[H_2(ATP)]_2^{4-}$ stack (for a structure see ref. 48 or 79) as is evident from the size of the observed upfield shifts, *i.e.* $\Delta\delta = \delta_0 - \delta_\infty$,[68] which is compatible with a dimer but not a polymer. This dimer is stabilized by intermolecular ion pair formation between $(N1)H^+$ and the phosphate group and hydrogen bonds between γ-$P(O)_2(OH)^-$ and N7.[68]

This interpretation agrees with the properties observed for $H_2(GTP)^{2-}$ and $H_2(ITP)^{2-}$;[47] both species show a low stacking tendency because their *neutral* (N1)H site is not able to form the *inter*molecular ion pair with the (also present) γ-$P(O)_2(OH)^-$ group, and H^+ – now located at N7 – leads to repulsion of the purine moieties. The above interpretation is further confirmed by a study[48] of the acid–base properties of the purine residues in ITP, GTP, and ATP in dependence on [NTP] (see Fig. 4):[80] The acidity of the $(N7)H^+$ site in $H_2(ITP)^{2-}$ and $H_2(GTP)^{2-}$ is enhanced upon stack formation (due to repulsion of the positive charges). Indeed, the $H(nucleoside)^+$ systems show the corresponding properties, whereas the acidity of the $(N1)H^+$ site in $H_2(ATP)^{2-}$ is *reduced* because this site is also involved in ion pair formation and hydrogen bonding. These observations generally demonstrate how aggregation can change the acid–base properties of certain sites of nucleobase residues in an unexpected way due to "fine tuning" by the kind of interactions that occur, and this may be of relevance for ribozyme catalysis.[81]

The described results show that the extent of aggregation is much affected by external conditions, such as the presence of metal ions or the pH of the solution. Apparently neutralization of negative charges at the phosphate groups facilitates stack formation of nucleotides but it is evident from the special properties of the dimeric $[H_2(ATP)]_2^{4-}$ species that hydrogen bonds and ionic interactions also play a role. This observation prompted a study of the effect of poly-α,L-lysine (= p-Lys) on the self-stacking properties of ATP in D_2O at pD 8.4.[10,82] Under these conditions all of the ε-amino groups of the side chains of p-Lys carry a positive charge (= $p(H{\cdot}Lys)_n^{n+}$) and thus ATP^{4-} with its negative phosphate groups is expected to be "lined up" along this $p(H{\cdot}Lys)_n^{n+}$ matrix. Indeed, under the mentioned conditions with $[p\text{-}Lys]_{side\ chains} \cong 0.4$ M and $[ATP^{4-}] \leqslant 0.25$ M, K_{SA} = 11.5 ± 2.1 M^{-1} based on the isodesmic model (eqns (2), (3)).[10] This value should be compared with K_{SA} = 1.3 ± 0.2 M^{-1} (Table 1) measured for ATP^{4-} in the absence of promoters. That actually the positively charged side chains of $p(H{\cdot}Lys)_n^{n+}$ are responsible for the increased stacking tendency of ATP^{4-} can be proven: at pD 12 the side chains of p-Lys are largely deprotonated and mainly present as uncharged amino groups; hence, the stack-promoting effect of p-Lys should be diminishing and this is observed[10] indeed: K_{SA} < 3.5 M^{-1}. This matrix-promoted self-association of ATP^{4-} is meaningful regarding the occurrence of certain cell organelles, such as chromaffin granules or the dense vesicles of blood platelets,[83] which contain high concentrations of nucleotides and of other solutes as well (for details see ref. 10). Such vesicles should be osmotically unstable but they are not; based on the indicated results one may conclude that the high nucleotide concentrations observed in such vesicles may be handled by nature *via* self-association and aggregate formation using suitable proteins as a matrix. Of course, opening of such a vesicle and dilution of its content leads instantly to a break-down of the aggregates and to monomeric species. Furthermore, due to the indicated properties ATP has recently been used as building blocks for the self-assembly of excitonic nanowires.[84]

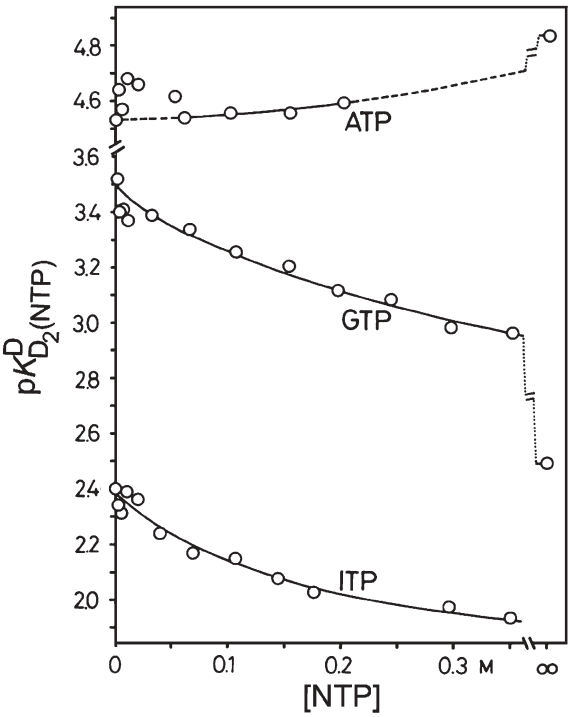


Fig. 4 Dependence of the negative logarithm of the acidity constant, $pK^D_{D_2(NTP)}$, for the $(N1)D^+$ deprotonation of $D_2(ATP)^{2-}$ and for the $(N7)D^+$ deprotonation of $D_2(GTP)^{2-}$ or $D_2(ITP)^{2-}$ on the NTP concentration. The plotted data are from Tables 3–5 of ref. 48; it should be noted that for ITP the micro acidity constant for the $(N7)D^+$ deprotonation of the $[D(N7){\cdot}ITP{\cdot}D]^{2-}$ species is plotted.[48,80] Reproduced in a slightly altered version by permission of the Federation of European Biochemical Societies (FEBS) from *Eur J. Biochem.*, ref. 48.

2.3 Conditions for studies on monomeric NTP species

To provide some information on the interrelations between the size of an association constant and the amounts of a stacked species formed, Fig. 5 is presented. In the figure the extent of stacking is given as a function of the concentration of the nucleoside or derivative, N, and the size of the association constant, K_{SA}. The two association constants used in these calculations were selected with the aim of being representative for the values mentioned and those listed in Table 1.

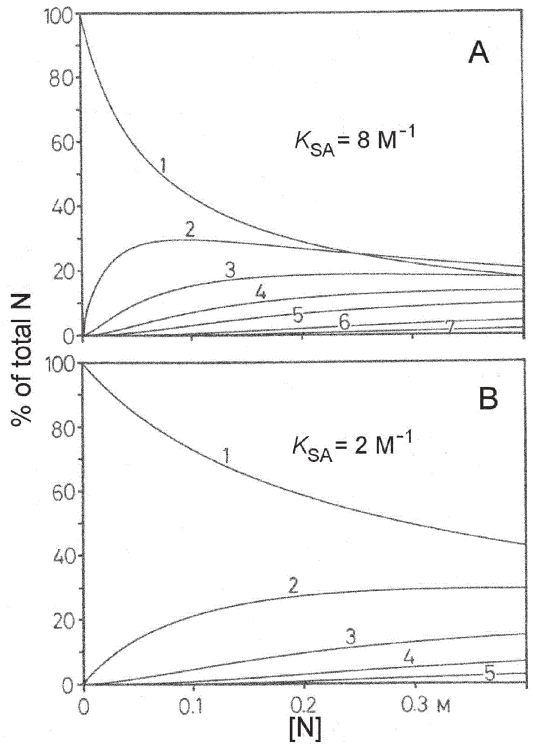


Fig. 5 Variation of the proportions of a nucleoside or derivative (=N) present in the monomer (1), dimer (2), trimer (3), *etc.*, in D_2O solutions as a function of the total concentration of N. The association constants used in the calculations are inserted in the figure, these values are representative examples from the constants mentioned in the text and those listed in Table 1. Reproduced in an altered version by permission of the Federation of European Biochemical Societies (FEBS) from *Eur. J. Biochem.*, ref. 47.

Corresponding distribution plots based on various other association constants, K_{SA}, are available in the literature[10,45,47,48,63,64,68–70,73,85–87] for many kinds of systems. For NDPs and corresponding metal ion complexes a tabulation exists in ref. 64.

In aiming for high concentrations of monomeric species, it may be helpful to know that in a 10^{-2} M UTP^{4-} solution 99% of UTP^{4-} are present in the monomeric form (K_{SA} = 0.4 M^{-1});[63] under the same conditions with ATP^{4-} only 97% of monomer are left (K_{SA} = 1.3 M^{-1}, Table 1). Metal ions affect self-association strongly, as discussed; consequently, one has to work in more dilute solutions to obtain high concentrations of monomers: *e.g.*, in 10^{-3} M solutions about 96% and 99% of $Zn(ATP)^{2-}$ and $Mg(ATP)^{2-}$, respectively, are monomers. One of the more extreme situations occurs[64] with $Zn(ADP)^{-}$ where one has to work at a $2{\cdot}10^{-4}$ M concentration to achieve an approximately 97% limit for the monomer.

To conclude, adenosine with its relatively high association constant (K_{SA} = 15 M^{-1}; Table 1) seems a good candidate for setting limiting concentrations. In this case, in a 10^{-3} M solution the monomers dominate with 97%. Hence, for studies aiming to quantify properties of monomeric species including metal ion complexes, we recommend working with 10^{-3} M (or more diluted) solutions; in fact, our potentiometric pH titrations used to determine the stability constants of nucleotide-metal ion complexes were in general carried out with $5{\cdot}10^{-4}$ or $3{\cdot}10^{-4}$ M nucleotide solutions.

3 Acid–base properties of NTPs and of some related derivatives

3.1 Definition of the acidity constants and site attributions

Adenosine, guanosine and inosine (Fig. 1) are used below for various comparisons. All three nucleotides (Ns) may accept a proton at the purine moiety: Ado at N1 and Guo as well as Ino at N7,[7,43,78,88] and the latter two can in addition release a proton from their (N1)H site. Hence, the following two equilibria are of relevance for $H(Ns)^+$ species:

$$H(Ns)^+ \rightleftharpoons Ns + H^+ \quad (4a)$$

$$K^{H}_{H(Ns)} = [Ns][H^+]/[H(Ns)^+] \quad (4b)$$

$$Ns \rightleftharpoons (Ns - H)^- + H^+ \quad (5a)$$

$$K^{H}_{Ns} = [(Ns - H)^-][H^+]/[Ns] \quad (5b)$$

For the release of a proton from the ribose moiety of nucleosides $pK_a > 12$ holds;[78,89] this reaction is thus not of relevance for the physiological pH range. Similarly, the release of the first proton from twofold protonated D-ribose 5-monophosphate, H_2(RibMP), occurs with $pK^{H}_{H_2(RibMP)} \cong pK^{H}_{H_2(UMP)} = 0.7 \pm 0.3$;[90] hence, for the present only equilibrium (6) is of relevance:

$$H(RibMP)^- \rightleftharpoons RibMP^{2-} + H^+ \quad (6a)$$

$$K^{H}_{H(RibMP)} = [RibMP^{2-}][H^+]/[H(RibMP)^-] \quad (6b)$$

Purine-nucleoside 5′-monophosphates are tribasic species; they accept two protons at their phosphate group and a further one at the purine moiety. In a first approximation[78,80] (see also Section 3.2) one may conclude that $H_3(IMP)^+$ and $H_3(GMP)^+$ release their first proton from the $-P(O)(OH)_2$ group, the second one from the $(N7)H^+$ site and the third one again from the phosphate group; a fourth proton is released in the alkaline pH range from the (N1)H site. Exactly the same reactions occur with the corresponding $H_3(ITP)^-$ and $H_3(GTP)^-$ species (see Fig. 1), the third proton being released from the terminal γ-phosphate group. These four steps are expressed by the following equilibria and equations, where charges are partly omitted and NP represents a nucleoside phosphate, *i.e.* IMP^{2-} and GMP^{2-} or ITP^{4-} and GTP^{4-}:

$$H_3(NP)^{+\text{ or }-} \rightleftharpoons H_2(NP)^{\pm\text{ or }2-} + H^+ \quad (7a)$$

$$K^{H}_{H_3(NP)} = [H_2(NP)][H^+]/[H_3(NP)] \quad (7b)$$

$$H_2(NP)^{\pm\text{ or }2-} \rightleftharpoons H(NP)^{-\text{ or }3-} + H^+ \quad (8a)$$

$$K^{H}_{H_2(NP)} = [H(NP)][H^+]/[H_2(NP)] \quad (8b)$$

$$H(NP)^{-\text{ or }3-} \rightleftharpoons NP^{2-\text{ or }4-} + H^+ \quad (9a)$$

$$K^{H}_{H(NP)} = [NP][H^+]/[H(NP)] \quad (9b)$$

$$NP^{2-\text{ or }4-} \rightleftharpoons (NP - H)^{3-\text{ or }5-} + H^+ \quad (10a)$$

$$K^{H}_{NP} = [(NP - H)][H^+]/[NP] \quad (10b)$$

For completeness one needs to add that equilibria/equations (7) through (9) also apply to $H_3(AMP)^+$ or $H_3(ATP)^-$, but the second proton (eqn (8)) is released in these cases from the $(N1)H^+$ site (cf. Fig. 1).

As far as the pyrimidine-nucleoside 5′-triphosphates are concerned, the situation is as follows: For $H_3(CTP)^-$ equilibria (7a) to (9a) are valid, the second proton (eqn (8)) being released from the $(N3)H^+$ site of the cytosine moiety. Since $H(UTP)^{3-}$ and $H(dTTP)^{3-}$ do not accept a proton at their pyrimidine ring, only $H_2(UTP)^{2-}$ and $H_2(dTTP)^{2-}$ species are of relevance; in these cases the first proton is released from one of the three primary sites of the twofold protonated triphosphate chain (Fig. 1); of course, the $-P_3O_{10}H_2^{2-}$ residue contains two more such primary sites, but these are protonated only at pH about 1 or below[80] (naturally this holds analogously for all NTPs) and are therefore not considered here. The second constant is due to proton loss from the monoprotonated terminal γ-phosphate group. Finally, in the alkaline pH range UTP^{4-} and $dTTP^{4-}$ can lose a proton from their (N3)H site to yield a species with an overall charge of −5, *i.e.* $(NTP - H)^{5-}$ (eqn (10)).

3.2 Comparison of the acidity constants of $H_2(NTP)^{2-}$ species with some related values

As indicated in Section 3.1 the first proton from $H_2(UTP)^{2-}$ is mainly released from one of the three primary sites (which may be in equilibrium with each other) of the twofold protonated triphosphate chain; this release occurs[85] with $pK^H_{H_2(UTP)} = 2.0 \pm 0.1$ and the one for $H_2(dTTP)^{2-}$ is expected to be very similar.[80] Since all of the other NTPs carry a further proton at the nucleobase, *e.g.* ATP at N1 and GTP at N7, some charge repulsion is expected and the proton from the primary phosphate sites should be released at a lower pH; indeed, for the two mentioned cases the values are $pK^H_{H_3(ATP)} = 1.7 \pm 0.1$ (ref. 68) and $pK^H_{H_3(GTP)} = 1.3 \pm 0.2$ (ref. 80). Of course, in the nucleoside 5′-monophosphates there is only a single primary phosphate site and correspondingly the acidity should be higher since there are no other negative charges close by; indeed, this is the case, but the overall pattern remains the same as is seen from the following constants: $pK^H_{H_2(UMP)} = 0.7 \pm 0.3$,[90] $pK^H_{H_3(AMP)} = 0.4 \pm 0.2$,[45] and $pK^H_{H_3(GMP)} = 0.3 \pm 0.2$.[78]

The acidity constants for equilibria (8a) through (10a) are listed in Table 2.[90–92] The second column contains the pK_a values for the release of the proton from the protonated nucleobase residues. In the context with the discussion in the preceding paragraph it is evident that deprotonation of the $(N7)H^+$ site in $H_3(ITP)^-$, and $H_3(IMP)^+$ as well, is expected to overlap somewhat with the release of the primary phosphate proton. Indeed, the interrelation between the measured macro acidity constants and the corresponding micro acidity constants, which quantify the properties of a given site, has been resolved.[78,80] For $H_2(ITP)^{2-}$ it was shown that the ratio between the species $(H{\cdot}ITP{\cdot}H)^{2-}$, with one proton each at N7 and the γ phosphate, and $(ITP{\cdot}H_2)^{2-}$, with both protons at the phosphate chain, is close to 1,[80] whereas for $H_2(IMP)^{\pm}$ the $(H{\cdot}IMP{\cdot}H)^{\pm}$ species dominates with a formation degree of about 80%.[78] These tautomeric equilibria are of no relevance in the physiological pH range but the basicity properties, which they define for a given site, may well be (see, *e.g.*, Section 4.4).

Table 2 Negative logarithms of the acidity constants (see eqns (8) to (10)) of several $H_2(NTP)^{2-}$ species as determined by potentiometric pH titrations in aqueous solution at 25 °C and $I = 0.1$ M ($NaNO_3$ or $NaClO_4$), together with some related data that refer to the same conditions[a]

Acid	$pK^H_{H_2(NP)}$ or $pK^H_{H(Ns)}$	$pK^H_{H(NP)}$	pK^H_{NP} or pK^H_{Ns}
$H(Ado)^+$	3.61 ± 0.03[bc]		
$H(Guo)^+$	2.11 ± 0.04[c]		9.22 ± 0.01[c]
$H(Ino)^+$	1.06 ± 0.06[c]		8.76 ± 0.03[c]
$H(RibMP)^-$		6.24 ± 0.01[d]	
$H_2(AMP)^{\pm}$	3.84 ± 0.02[be]	6.21 ± 0.01[e]	
$H_2(GMP)^{\pm}$	2.48 ± 0.04[c]	6.25 ± 0.02[c]	9.49 ± 0.02[c]
$H_2(IMP)^{\pm}$	1.30 ± 0.10[c]	6.22 ± 0.01[c]	9.02 ± 0.02[c]
$H_2(ATP)^{2-}$	4.00 ± 0.01[bf]	6.47 ± 0.01[f]	
$H_2(GTP)^{2-}$	2.94 ± 0.02	6.50 ± 0.02	9.57 ± 0.02
$H_2(ITP)^{2-}$	2.19 ± 0.05	6.47 ± 0.02	9.11 ± 0.03
$H_2(CTP)^{\pm}$	4.55 ± 0.03[b]	6.55 ± 0.02	
$H(UTP)^{3-}$		6.48 ± 0.02	9.57 ± 0.02[g]
$H(dTTP)^{3-}$		6.52 ± 0.02	10.08 ± 0.05[g]

[a] NP = nucleoside phosphate = NMP^{2-} or NTP^{4-}. So-called practical (or mixed) constants are listed.[91] The error limits given are *three times* the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. Those values for which no source is given are from ref. 80. For the sites at which the protons are located see also the text in Sections 3.1 and 3.2. There is a further aspect to be noted; the given equilibrium constants 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 the data for the complex formation between Na^+ and NTP to prevent any ambiguity. However, if desired, the competition between Na^+ and H^+ can easily be taken into account: for details see footnote (33) of ref. 49. [b] This value refers to the deprotonation of the $(N1)H^+$ site of the adenine residue; all the other values in this column refer (largely; see text) to the deprotonation of the $(N7)H^+$ unit of the purines, except in the case of $H_2(CTP)^{2-}$ where the proton is at N3 (*cf.* Fig. 1). [c] From ref. 78. [d] From ref. 90. [e] From refs. 33 and 92. [f] From ref. 49. [g] This value refers to the deprotonation of the (N3)H site of a pyrimidine residue; all the other values in this column refer to the deprotonation of a purine-(N1)H site.

Comparisons of the other values in the second column of Table 2 confirm the site attributions given in Section 3.1. The increase in the pK_a values for the release of the proton from the $(N1)H^+$ site of an adenine residue within the series $H(Ado)^+ < H_2(AMP)^{\pm} < H_2(ATP)^{2-}$ reflects the influence of the negatively charged phosphate group(s) and is as expected. This observation is corroborated for the $(N7)H^+$ site, where the series for increasing pK_a values is $H(Guo)^+ < H_2(GMP)^{\pm} < H_2(GTP)^{2-}$ (Table 2). The property of the $(N3)H^+$ unit in $H(cytidine)^+$ and $H_2(CTP)^{2-}$ with the acidity constants[50,93] $pK^H_{H(Cyt)} = 4.14 \pm 0.02$ and $pK^H_{H_2(CTP)} = 4.55$ (Table 2) also fits excellently in this picture.

The values in column 3 of Table 2 refer to the loss of the final proton from the (terminating) phosphate group(s). The value for $H(RibMP)^-$ confirms the site attributions of Section 3.1. It is further evident from these data that the nucleobase residue has only little influence on the release of this proton within the series of the 5′-phosphates: All of the $pK^H_{H(NMP)}$ values are close to 6.2 and those for $pK^H_{H(NTP)}$ are close to 6.5. If the position of the phosphate group at the ribose moiety is

altered, the pK_a values change as is evident from the series of the three AMP isomers:[94] H(3′-AMP)$^-$ (p$K^H_{H(3'\text{-AMP})}$ = 5.77 ± 0.02) < H(2′-AMP)$^-$ (p$K^H_{H(2'\text{-AMP})}$ = 5.95 ± 0.01) < H(5′-AMP)$^-$ (p$K^H_{H(5'\text{-AMP})}$ = 6.21 ± 0.01).

The highest values in Table 2 (column 4) refer to the ionization of the (N1)H and (N3)H sites (eqn (10)) of the purines and the pyrimidines, respectively, which yields in the case of the NTPs, species with an overall charge of −5, *i.e.* (NTP − H)$^{5-}$. All of these values are consistent with each other as the comparisons below demonstrate. For example, comparison (and there are many others possible) of the acidity constants of the nucleosides with those of their corresponding nucleotides (Table 2) reveals the effect of the 4-fold negatively charged triphosphate chain on the deprotonation of the (N1)H site:

$$\begin{aligned} pK^H_{GTP} - pK^H_{Guo} &= (9.57 \pm 0.02) - (9.22 \pm 0.01) \\ &= 0.35 \pm 0.02 \end{aligned}$$

$$\begin{aligned} pK^H_{ITP} - pK^H_{Ino} &= (9.11 \pm 0.03) - (8.76 \pm 0.03) \\ &= 0.35 \pm 0.04 \end{aligned}$$

As one might expect, the release of the proton from (N1)H in GTP^{4-} and ITP^{4-} is inhibited by the triphosphate chain and the effect is very similar to that observed with pyrimidines and their (N3)H site:

$$\begin{aligned} pK^H_{UTP} - pK^H_{Urd} &= (9.57 \pm 0.02) - (9.19 \pm 0.02; \text{ ref. 95}) \\ &= 0.38 \pm 0.03 \end{aligned}$$

$$\begin{aligned} pK^H_{dTTP} - pK^H_{Thy} &= (10.08 \pm 0.05) - (9.69 \pm 0.03; \text{ ref. 95}) \\ &= 0.39 \pm 0.06 \end{aligned}$$

Of course, the corresponding comparisons can also be made between the listed pK_a values of the NMP^{2-} and Ns species. In addition, the effect described above also operates when a positively charged (N1)H$^+$ site is considered as in H$_2$(ATP)$^{2-}$ and H(adenosine)$^-$:

$$\begin{aligned} pK^H_{H_2(ATP)} - pK^H_{H(Ado)} &= (4.00 \pm 0.01) - (3.61 \pm 0.03) \\ &= 0.39 \pm 0.03 \end{aligned}$$

For further details ref. 80 should be consulted. Related comparisons have recently also been made for the release of the two protons from a –P(O)(OH)$_2$ group in NMPs.[96]

4 Stability and structure of binary NTP–metal ion complexes

In this section the focus is on the biologically more important divalent metal ions, *i.e.* Ca^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+} and Zn^{2+}, but equilibrium data for the complexes of Sr^{2+} and Ba^{2+}, as well as for those of Co^{2+}, Ni^{2+} and Cd^{2+} can usually also be found in the cited references (see also Section 1.3).

4.1 Definition of the stability constants of the complexes

The equilibrium constants summarized below were determined by potentiometric pH titrations. The experimental data for the various M^{2+}/NTP systems are completely described by the acid–base equilibria (8) and (9), and by the complex-forming equilibria (11) and (12):

$$M^{2+} + H(NTP)^{3-} \rightleftharpoons M(H;NTP)^- \quad (11a)$$

$$K^M_{M(H;NTP)} = [M(H;NTP)^-]/([M^{2+}][H(NTP)^{3-}]) \quad (11b)$$

$$M^{2+} + NTP^{4-} \rightleftharpoons M(NTP)^{2-} \quad (12a)$$

$$K^M_{M(NTP)} = [M(NTP)^{2-}]/([M^{2+}][NTP^{4-}]) \quad (12b)$$

Of course, care has to be taken that the evaluation of the data is not carried into the pH range where hydroxo complexes form or where (N1)H of ITP or GTP as well as (N3)H of UTP or dTTP are deprotonated.[7,49]

The two equilibria (11) and (12) are evidently connected by the deprotonation equilibrium (13):

$$M(H;NTP)^- \rightleftharpoons M(NTP)^{2-} + H^+ \quad (13a)$$

$$K^H_{M(H;NTP)} = [M(NTP)^{2-}][H^+]/[M(H;NTP)^-] \quad (13b)$$

The corresponding acidity constant is calculated with eqn (14):

$$pK^H_{M(H;NTP)} = pK^H_{H(NTP)} + \log K^M_{M(H;NTP)} - \log K^M_{M(NTP)} \quad (14)$$

4.2 Complexes of pyrimidine–NTPs

The collected stability constants for the complexes of pyrimidine–nucleoside 5′-triphosphates (PyNTPs) are listed in Table 3. The values for the monoprotonated complexes, M(H;NTP)$^-$, are given in column 3 and those for the M(NTP)$^{2-}$ species in column 4.

It is interesting to consider the deprotonation of the M(H;UTP)$^-$ complexes according to equilibrium (13) and to compare the corresponding acidity constants (Table 3, column 5) with the value due to the deprotonation of H(UTP)$^{3-}$ (eqn (9); p$K^H_{H(UTP)}$ = 6.48 ± 0.02, Table 2). Depending on the kind of metal ion, an acidification of about 1.6 (Ca^{2+}) to 3.1 (Cu^{2+}) pK units is observed. This acidified proton must be located at the terminal γ-phosphate group as there is no other basic site available in UTP^{4-}; hence, the metal ion and the proton are at the triphosphate chain. The same reasoning applies for the M(H;dTTP)$^-$ complexes (see also footnote "e" of Table 3).

With the M(H;CTP)$^-$ complexes the situation is more complicated because of the presence of a further basic site, *i.e.* N3. In this case the values of column 5 in Table 3 need to be compared with p$K^H_{H_2(CTP)}$ = 4.55 (eqn (8)) and p$K^H_{H(CTP)}$ = 6.55 (eqn (9)) (Table 2). Evidently, all of the values for p$K^H_{M(H;CTP)}$ are below p$K^H_{H(CTP)}$, as one expects, but most of them are also close or even below p$K^H_{H_2(CTP)}$. Hence, the question arises: Where is the proton located? At N3 of the cytosine residue or at the γ-phosphate group? Detailed evaluations[49] have led to the conclusion that, *e.g.*, in Mn(H;CTP)$^-$ and Zn(H;CTP)$^-$ the ratio is about 1:1 between complexes with the proton at N3 and the metal ion at the phosphate chain, (H·CTP·M)$^-$, and the species with both the proton and M^{2+} at the phosphate

Table 3 Logarithms of the stability constants of $M(H;NTP)^-$ and $M(NTP)^{2-}$ complexes (eqns (11), (12)) for NTP^{4-} = UTP^{4-}, $dTTP^{4-}$ and CTP^{4-} as determined by potentiometric pH titrations in aqueous solution, together with the negative logarithms of the acidity constants (eqns (13), (14)) of the corresponding $M(H;NTP)^-$ complexes at 25 °C and I = 0.1 M ($NaNO_3$ or $NaClO_4$). In the bottom part of the table the averaged values generally valid for complexes of pyrimidine-nucleoside 5′-triphosphates ($PyNTP^{4-}$) are given[abc]

NTP^{4-}	M^{2+}	log $K^{M}_{M(H;NTP)}$	log $K^{M}_{M(NTP)}$	p$K^{H}_{M(H;NTP)}$
UTP^{4-}	Ca^{2+}	2.2 ± 0.25[d]	3.82 ± 0.05[d]	4.86 ± 0.25
	Mg^{2+}	2.3 ± 0.25[d]	4.21 ± 0.05[d]	4.57 ± 0.25
	Mn^{2+}	2.70 ± 0.12	4.91 ± 0.08	4.27 ± 0.14
	Cu^{2+}	2.80 ± 0.08	5.87 ± 0.03	3.41 ± 0.08
	Zn^{2+}	2.73 ± 0.09	5.01 ± 0.03	4.20 ± 0.09
$dTTP^{4-}$	Ca^{2+}	—[e]	3.85 ± 0.02	
	Mg^{2+}	—[e]	4.23 ± 0.06	
	Mn^{2+}	—[e]	5.01 ± 0.17	
	Cu^{2+}	—[e]	5.83 ± 0.17	
	Zn^{2+}	—[e]	5.03 ± 0.09	
CTP^{4-}	Ca^{2+}	2.2 ± 0.4	3.85 ± 0.06	4.9 ± 0.4
	Mg^{2+}	2.27 ± 0.27	4.20 ± 0.08	4.62 ± 0.28
	Mn^{2+}	3.1 ± 0.45	4.90 ± 0.02	4.75 ± 0.45
	Cu^{2+}	3.80 ± 0.09	6.03 ± 0.05	4.32 ± 0.10
	Zn^{2+}	3.05 ± 0.11	5.03 ± 0.08	4.57 ± 0.14
$PyNTP^{4-}$	Ca^{2+}	2.2 ± 0.2	3.84 ± 0.05	4.85 ± 0.2[f]
	Mg^{2+}	2.3 ± 0.2	4.21 ± 0.04	4.6 ± 0.2[f]
	Mn^{2+}	2.70 ± 0.12	4.93 ± 0.03	4.27 ± 0.13[f]
	Cu^{2+}	2.80 ± 0.08	5.86 ± 0.03	3.44 ± 0.10[f]
	Zn^{2+}	2.73 ± 0.09	5.02 ± 0.02	4.21 ± 0.10[f]

[a] The error limits given are *three times* the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. The error limits of the derived data, in the above case of p$K^{H}_{M(H;NTP)}$ were calculated according to the error propagation after Gauss. [b] Those values for which no source is given are from Table II of ref. 49. The values for the M^{2+}/PyNTP systems are from Table 2 of ref. 7 (see also ref. 49); they were obtained, with the exception of the Cu^{2+} system (see text in Section 4.2), by averaging the data measured for the UTP, dTTP and CTP systems by using the number of independent titrations as weighting factors.[7,49] [c] The listed constants are conditional constants, which apply to solutions in which the Na^+ concentration is about 0.1 M (see also footnote "a" in Table 1). However, if desired, the competition between Na^+ and M^{2+} can be taken into account; see footnote (33) of ref. 49. [d] From ref. 7. [e] At the time[49] not enough compound was available to determine these constants reliably but the values for $M(H;UTP)^-$ hold in a first approximation certainly also here. [f] Calculated with eqn (14) and the average value[29,49,80] p$K^{H}_{H(NTP)}$ = 6.50 ± 0.05.

residue, $(CTP{\cdot}H{\cdot}M)^-$. For $Cu(H;CTP)^-$ it is estimated[49] that about 85% occur as the $(H{\cdot}CTP{\cdot}Cu)^-$ isomer, the remaining 15% being present as $(CTP{\cdot}H{\cdot}Cu)^-$ and a macrochelated isomer formed by the phosphate-coordinated Cu^{2+} and N3 (see also the next paragraph and equilibrium (15), *vide infra*).

Comparison of the three sets of data in the upper part of column 4 of Table 3 shows that the stability constants of the $M(NTP)^{2-}$ complexes with Ca^{2+}, Mg^{2+}, Mn^{2+} and Zn^{2+} are within their error limits identical for the ligands UTP^{4-}, $dTTP^{4-}$ and CTP^{4-}. Considering that the acidity constants for the release of the proton from the terminal γ-phosphate group in the corresponding $H(NTP)^-$ species are also the same within ±0.05 pK units, *i.e.* p$K^{H}_{H(NTP)}$ = 6.50 ± 0.05 (*cf.* Table 2), this result is no surprise. The only exception is the stability of the $Cu(CTP)^{2-}$ species which is by about 0.17 log units more stable than $Cu(UTP)^{2-}$ or $Cu(dTTP)^{2-}$; this means that Cu^{2+}, in contrast to all the other metal ions studied,[49] is able to force part of CTP^{4-} in $Cu(CTP)^{2-}$ into the *syn* conformation (see Section 1.2). This then allows the phosphate-coordinated Cu^{2+} to form a macrochelate with N3 of the cytosine residue; about 30% of $Cu(CTP)^{2-}$ exist in this macrochelated form (equilibrium (15), *vide infra*).[49]

With the above considerations in mind one can now define stability constants for M^{2+} complexes formed with pyrimidine 5′-triphosphates ($PyNTP^{2-}$). This means, for each metal ion the stability constants of the $M(UTP)^{2-}$, $M(dTTP)^{2-}$ and $M(CTP)^{2-}$ complexes were averaged by using the number of titrations as weighting factors.[7,49] The resulting stability constants, $K^{M}_{M(PyNTP)}$ (eqn (12)), are listed in the lower part of column 4 in Table 3; in the case of $Cu(PyNTP)^{2-}$ only the values for $Cu(UTP)^{2-}$ and $Cu(dTTP)^{2-}$ were averaged. The values for $M(H;PyNTP)^-$ (Table 3, column 3, lower part) are mainly based on those for $M(H;UTP)$.[7] Note, in all these $M(PyNTP)^{2-}$ complexes M^{2+} is coordinated only to the phosphate chain and in the $M(H;PyNTP)^-$ species both M^{2+} and H^+ are also located at the phosphate moiety only. In accord herewith is the stability order of all of these complexes[7] which corresponds to those for phosphate monoesters[90] and diphosphate monoesters:[34] $Ba^{2+} < Sr^{2+} < Ca^{2+} < Mg^{2+} < Ni^{2+} < Co^{2+} < Mn^{2+} < Cu^{2+} > Zn^{2+} < Cd^{2+}$. The fact that the stabilities of phosphate-metal ion complexes do not strictly follow the Irving–Williams sequence[97] is a long standing experience[98] (see also Section 8).

It may be added here that values for Fe^{2+}–nucleotide complexes have hardly been measured[30–32] and a recent tabulation[30] of stability constants does not contain a single "recommended" value for a Fe^{2+} complex of a nucleotide. The reason is that it is difficult to obtain Fe^{2+} solutions completely free of Fe^{3+} and especially to prevent oxidation of traces of Fe^{2+} to Fe^{3+}(by traces of dioxygen from air), a reaction that is facilitated by phosphate coordination. In other words, there is a high danger for measuring artefacts. Therefore, based on interpolations the stability constant for the Fe^{2+} complex formed with a 'simple' triphosphate monoester, $R\text{-}TP^{4-}$, *i.e.* a triphosphate in which the residue R does not affect metal ion binding at the triphosphate chain, has recently been estimated:[34] log $K^{Fe}_{Fe(R\text{-}TP)}$ = log $K^{Fe}_{Fe(PyNTP)}$ = 4.85 ± 0.1.

Similarly, since again no values appear to be available,[30–32] a stability constant for Pb^{2+} complexes of triphosphate monoesters of the mentioned kind, has also been estimated: log $K^{Pb}_{Pb(R\text{-}TP)}$ = log $K^{Pb}_{Pb(PyNTP)}$ = 6.3 ± 0.25.[99] However, this estimation[99] is based on the so-called *Stability Ruler*.[100]

4.3 Complexes of purine–NTPs

The stability constants of the $M(H;NTP)^-$ and $M(NTP)^{2-}$ complexes formed according to equilibria (11a) and (12a) are listed in columns 3 and 4 of Table 4, respectively; column 5 contains the acidity constants determined for the release of a proton from the $M(H;NTP)^-$ species (equilibrium (13a)). Comparison of the corresponding p$K^{H}_{M(H;NTP)}$ values with p$K^{H}_{H(NTP)}$ = 6.50 (Table 2) reveals for the $M(H;ITP)^-$ and $M(H;GTP)^-$ species acidifications between about 1.3 to 2.8 pK units. Since the acidity constants for the release of a proton from $H_2(NTP)^{2-}$ are p$K^{H}_{H_2(GTP)}$ = 2.94 and p$K^{H}_{H_2(ITP)}$ = 2.19 (Table 2) (to be more exact, p$k^{ITP{\cdot}H}_{H{\cdot}ITP{\cdot}H}$ = 1.89 ± 0.07)[80] due to

Table 4 Logarithms of the stability constants of $M(H;NTP)^-$ and $M(NTP)^{2-}$ complexes (eqns (11), (12)) for NTP^{4-} = ATP^{4-}, ITP^{4-} and GTP^{4-} as determined by potentiometric pH titrations in aqueous solution, together with the negative logarithms of the acidity constants (eqns (13), (14)) of the corresponding $M(H;NTP)^-$ complexes at 25 °C and I = 0.1 M ($NaNO_3$ or $NaClO_4$)[ab]

NTP^{4-}	M^{2+}	log $K^{M}_{M(H;NTP)}$	log $K^{M}_{M(NTP)}$	p$K^{H}_{M(H;NTP)}$
ATP^{4-}	Ca^{2+}	2.20 ± 0.08	3.91 ± 0.03	4.76 ± 0.08
	Mg^{2+}	2.42 ± 0.08	4.29 ± 0.03	4.60 ± 0.08
	Mn^{2+}	2.74 ± 0.09	5.01 ± 0.08	4.20 ± 0.12
	Cu^{2+}	3.59 ± 0.08	6.34 ± 0.03	3.72 ± 0.08
	Zn^{2+}	2.86 ± 0.11	5.16 ± 0.06	4.17 ± 0.13
ITP^{4-}	Ca^{2+}	2.4 ± 0.25	3.93 ± 0.05	4.95 ± 0.25
	Mg^{2+}	2.4 ± 0.25	4.29 ± 0.04	4.6 ± 0.25
	Mn^{2+}	3.1 ± 0.3	5.21 ± 0.06	4.35 ± 0.3
	Cu^{2+}	3.9 ± 0.4	6.71 ± 0.10	3.65 ± 0.4
	Zn^{2+}	3.1 ± 0.3	5.32 ± 0.06	4.25 ± 0.3
GTP^{4-}	Ca^{2+}	2.6 ± 0.3	3.96 ± 0.03	5.15 ± 0.3
	Mg^{2+}	2.6 ± 0.3	4.31 ± 0.04	4.8 ± 0.3
	Mn^{2+}	3.36 ± 0.16	5.36 ± 0.03	4.50 ± 0.16
	Cu^{2+}	4.6 ± 0.2	7.38 ± 0.08	3.7 ± 0.2
	Zn^{2+}	3.45 ± 0.25	5.52 ± 0.05	4.45 ± 0.25

[a] For the error limits (3σ) see footnote "a" of Table 3. [b] The values for the ATP systems are from Table II in ref. 49 and those for the ITP and GTP systems from Table 1 in ref. 7.

$(N7)H^+$ deprotonation, it is evident that the proton in $M(H;ITP)^-$ and $M(H;GTP)^-$ must be located at the more basic terminal γ-phosphate group; of course, M^{2+} is also at the triphosphate chain (for the detailed structure see Section 4.4).

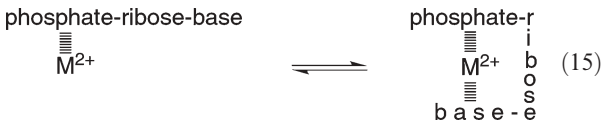
For the $M(H;ATP)^-$ complexes the situation is more complicated: Of course, all p$K^{H}_{M(H;ATP)}$ values (Table 4, column 5) are well below p$K^{H}_{H(ATP)}$ = 6.47 (Table 2). Indeed, detailed evaluations have shown[49] that because of p$K^{H}_{Cu(H;ATP)}$ = 3.72, which is also below p$K^{H}_{H_2(ATP)}$ = 4.00 (Table 2), in about 51% of the $Cu(H;ATP)^-$ species the proton is at the N1 site of the adenine residue; in the remaining part both the proton and the metal ion are at the phosphate chain. This latter part can be further subdivided: about 33% form a macrochelate and about 16% exist in a simple phosphate-bound 'open' form (see equilibrium (15); *vide infra*). In all the other $M(H;ATP)^-$ complexes the proton is at the γ-phosphate group[49] and to some extent macrochelates form also in these instances; *e.g.*, for $Zn(H;ATP)^-$ they amount to about 25%.

There is however, another interesting detail: comparison of the stability constants of the $M(H;ITP)^-$ and $M(H;GTP)^-$ complexes (Table 4, column 3) with those of the $M(H;PyNTP)^-$ species given in the lower part of Table 3 reveals (more clearly than for the $M(H;ATP)^-$ species) that the stabilities of the $M(H;ITP)^-$ and $M(H;GTP)^-$ complexes are higher. This is clear evidence for the participation of a further binding site,[101] *i.e.* again for macrochelate formation. Indeed, such an increased stability is also clearly observed for most of the $M(NTP)^{2-}$ complexes listed in Table 4, including the $M(ATP)^{2-}$ species, if their stability constants (column 4) are compared with those of the $M(PyNTP)^{2-}$ complexes in Table 3 (column 4). This means that in the complexes of the purine–NTPs the nucleobase residue must be involved at least to some extent in metal ion binding. This point will be considered in more detail in Section 4.4.

For reasons of completeness one may add the following stability constants for Fe^{2+} and Pb^{2+} complexes, since values for their nucleotide complexes are hardly found in the literature. Based on the stability constant given in Section 4.2 for the $Fe(PyNTP)^{2-}$ complex one may estimate, by taking into account the average of the stability increase observed for the $Mn(ATP)^{2-}$ and $Co(ATP)^{2-}$ species,[7] a value for $Fe(ATP)^{2-}$: log $K^{Fe}_{Fe(ATP)}$ = 5.00 ± 0.15. For the corresponding Pb^{2+} complex the same value must be anticipated as for the $Pb(PyNTP)^{2-}$ species,[99] *i.e.* log $K^{Pb}_{Pb(ATP)}$ = 6.3 ± 0.25. The estimated stability constants for the $Pb(ITP)^{2-}$ and $Pb(GTP)^{2-}$ complexes are log $K^{Pb}_{Pb(ITP)}$ = 6.42 ± 0.27 and log $K^{Pb}_{Pb(GTP)}$ = 6.57 ± 0.27, respectively.[99]

4.4 Intramolecular equilibria in metal ion complexes of purine–NTPs

The increased stability indicated shortly in Section 4.3 is to be attributed to macrochelate formation which occurs in metal ion complexes of purine–nucleotides by an interaction of the phosphate-coordinated metal ion with N7. That it is the N7 site of the purine moiety which is responsible for this interaction has been repeatedly proven, *e.g.*, by ^{1}H-NMR shift experiments[63,64] or by replacing N7 by a CH unit which gives then a 7-deazapurine derivative and complexes which show the properties of simple phosphate monoesters.[92] Hence, purine–nucleotide complexes may adopt in solution two families of conformations as described previously:[7,35–37,49,78] an 'open' form in which the metal ion is only phosphate-coordinated, designated as $M(NTP)^{2-}_{op}$ for nucleoside 5′-triphosphates, and a 'closed' or chelated form, designated as $M(NTP)^{2-}_{cl}$, in which the triphosphate-bound metal ion forms a bridge to N7 of the purine-nucleobase giving thus rise to the intramolecular equilibrium (15).



(15)

The corresponding dimensionless equilibrium constant K_I is defined in eqn (16):

$$K_I = [M(NTP)^{2-}_{cl}]/[M(NTP)^{2-}_{op}] \quad (16)$$

The observed overall stability of purine–nucleotide complexes is thus the sum of the individual stability constants for the open and the closed or macrochelated forms; in other words, eqn (12) may be redefined as follows:

$$K^{M}_{M(NTP)} = \frac{\left[M(NTP)^{2-}_{op}\right] + \left[M(NTP)^{2-}_{cl}\right]}{\left[M^{2+}\right]\left[NTP^{4-}\right]} \quad (17a)$$

$$= K^{M}_{M(NTP)op} + K^{M}_{M(NTP)cl} \quad (17b)$$

The stability constant for the open form is well defined by the stability of $M(PyNTP)^{2-}$ complexes (Table 3, lower part) that do not form macrochelates (Section 4.2). Hence, eqn (18) holds:

$$K^{M}_{M(NTP)op} = K^{M}_{M(PyNTP)} = \frac{\left[M(NTP)^{2-}_{op}\right]}{\left[M^{2+}\right]\left[NTP^{4-}\right]} \quad (18)$$

Combining the last two equations with the definition of K_I (eqn (16)) yields[36,78,92,101] eqn (19):

$$K^{M}_{M(NTP)} = K^{M}_{M(NTP)op} (1 + K_I) \quad (19)$$

This equation shows that the observed overall stability is equal to the stability constant for the open form augmented by the factor $(1 + K_I)$, which includes the contribution from the closed form.

If we define now the difference in the logarithms of the observed overall stability constants of the $M(NTP)^{2-}$ complexes, where NTP^{4-} = purine-NTP^{4-}, and those of the $M(PyNTP)^{2-}$ species, we have defined the previously mentioned stability increase by eqn (20):

$$\log \Delta_{M/NTP} = \log K^{M}_{M(NTP)} - \log K^{M}_{M(PyNTP)} \quad (20a)$$

$$= \log K^{M}_{M(NTP)} - \log K^{M}_{M(NTP)op} \quad (20b)$$

$$= \log \Delta \quad (20c)$$

The equality of the various terms in eqns (20a) and (20b) does not need any explanation. However, the combination between eqns (19) and (20b) defines now the relation between the observed stability increase, log Δ, and the dimensionless constant K_I, *i.e.*, the position of equilibrium (15). This relation[92,101] is given in eqn (21):

$$K_I = \frac{K^{M}_{M(NTP)}}{K^{M}_{M(NTP)op}} - 1 \quad (21a)$$

$$= 10^{\log \Delta} - 1 \quad (21b)$$

Knowledge of K_I allows the percentage of the closed or macrochelated form to be calculated as given by eqn (22):

$$\%\ M(NTP)^{2-}_{cl} = 100 \cdot K_I/(1 + K_I) \quad (22)$$

In column 3 of Table 5 the results for log $\Delta_{M/NTP}$ are listed; these values were obtained by forming according to eqn (20) the differences between the data in columns 4 of Tables 4 and 3 (lower part). To provide a more comprehensive overview also the results for the corresponding Co^{2+} and Ni^{2+} complexes[7] are included. It is evident that the differences log $\Delta_{M/NTP}$ are positive throughout proving the existence of the intramolecular equilibrium (15). Of course, the corresponding differences may also be formed[7] for the monoprotonated complexes (eqn (11)) $M(H;NTP)^{-}$ of Table 4 and the $M(H;PyNTP)^{-}$ species of Table 3 (see also the second to the last paragraph in Section 4.3). Indeed, it is remarkable to find that the values[7] for log $\Delta_{M/H;NTP}$ are quite similar, in fact, mostly identical within the error limits, to those for log $\Delta_{M/NTP}$. However, because of the large error limits of the log $\Delta_{M/H;NTP}$ values, we shall concentrate the further evaluation on the $M(NTP)^{2-}$ systems for the purine–NTPs.

The results for K_I (eqns (16), (21)) and % $M(NTP)^{2-}_{cl}$ (eqn (22)) for the ATP^{4-}, ITP^{4-} and GTP^{4-} complexes with each of the seven metal ions mentioned are provided in columns 4 and 5 of Table 5, respectively. The situation in which log $\Delta_{M/NTP}$ (eqn (20)) and K_I (eqn (21)) both equal zero, which indicates that no macrochelate forms, does not occur in Table 5. The largest amount of macrochelate occurs with Cu^{2+}, followed by Ni^{2+}. Indeed, the log $\Delta_{M/NTP}$ values follow the Irving–Williams series[97] which is in accord with the fact that these values reflect the interaction with a nitrogen site, namely N7. As one would expect, the closed or macrochelated forms are lower in percentage for the two alkaline earth metal ions.

Table 5 Increased complex stabilities, log $\Delta_{M/NTP}$ (eqn (20)), and extent of chelate formation (equilibrium (15)) in the $M(ATP)^{2-}$, $M(ITP)^{2-}$ and $M(GTP)^{2-}$ complexes, as quantified by the dimensionless equilibrium constants K_I (eqns (16), (21)) and the percentages of $M(NTP)^{2-}_{cl}$ (eqn (22)) for aqueous solutions at 25 °C and I = 0.1 M ($NaNO_3$ or $NaClO_4$)[a]

NTP^{4-}	M^{2+}	log $\Delta_{M/NTP}$	K_I	% $M(NTP)^{2-}_{cl}$
ATP^{4-}	Ca^{2+}	0.07 ± 0.06	0.17 ± 0.16	15 ± 12
	Mg^{2+}	0.08 ± 0.05	0.20 ± 0.14	17 ± 10
	Mn^{2+}	0.08 ± 0.08	0.20 ± 0.22	17 ± 15
	Co^{2+}	0.21 ± 0.09	0.62 ± 0.34	38 ± 13
	Ni^{2+}	0.36 ± 0.06	1.29 ± 0.32	56 ± 6
	Cu^{2+}	0.48 ± 0.04	2.02 ± 0.28	67 ± 3
	Zn^{2+}	0.14 ± 0.06	0.38 ± 0.19	28 ± 10
ITP^{4-}	Ca^{2+}	0.09 ± 0.07	0.23 ± 0.20	19 ± 13
	Mg^{2+}	0.08 ± 0.06	0.20 ± 0.17	17 ± 11
	Mn^{2+}	0.28 ± 0.07	0.91 ± 0.31	48 ± 8
	Co^{2+}	0.32 ± 0.08	1.09 ± 0.38	52 ± 9
	Ni^{2+}	0.51 ± 0.10	2.24 ± 0.75	69 ± 7
	Cu^{2+}	0.85 ± 0.10	6.08 ± 1.63	86 ± 3
	Zn^{2+}	0.30 ± 0.06	1.00 ± 0.28	50 ± 7
GTP^{4-}	Ca^{2+}	0.12 ± 0.06	0.32 ± 0.18	24 ± 10
	Mg^{2+}	0.10 ± 0.06	0.26 ± 0.17	21 ± 11
	Mn^{2+}	0.43 ± 0.04	1.69 ± 0.25	63 ± 3
	Co^{2+}	0.58 ± 0.06	2.80 ± 0.53	74 ± 4
	Ni^{2+}	0.92 ± 0.05	7.32 ± 0.96	88 ± 1
	Cu^{2+}	1.52 ± 0.08	32.11 ± 6.10	97 ± 1
	Zn^{2+}	0.50 ± 0.05	2.16 ± 0.36	68 ± 4

[a] For the error limits see footnote "a" of Table 3. The above values are abstracted from Table 4 in ref. 7 but most of them can also be calculated by forming the adequate differences between the data in Tables 4 and 3; see also text in Section 4.4.

For the five transition metal ions in Table 5, substantial amounts of macrochelates form; for example, 97% macrochelated and only 3% open species are observed for $Cu(GTP)^{2-}$. Generally, for a given metal ion the percentage of macrochelate falls off in the order $M(GTP)^{2-}_{cl} > M(ITP)^{2-}_{cl} > M(ATP)^{2-}_{cl}$. This order corresponds to the decreasing N7 basicity as far as GTP^{4-} and ITP^{4-} are concerned (Table 2 and text in Section 3.2).[54] For the adenosine residue the matter is more complicated because the most basic site is N1; however, recently the micro acidity constant has been determined for the adenosine tautomer in which N1 is free and the proton resides at N7. The corresponding micro acidity constant, $pk^{N7\text{-}N1}_{H\cdot N7\text{-}N1}$ = 2.20 ± 0.17,[54] is very similar to the acidity constant of N7-protonated guanosine: $pK^{H}_{H(Guo)}$ = 2.11 ± 0.04 (Table 2). Hence, the reason for the lower formation degree of the $M(ATP)^{2-}_{cl}$ species is the steric inhibition which the $(C6)NH_2$ group exercises (not only at the N1 site[102] but also) at N7 as far as metal ion binding is concerned.[103] Of course, metal ion binding to one site in a nucleobase will affect the acid–base properties at another nearby site, at present we are only at the brink of

understanding and quantifying such effects.[55,104] Though the presently available data[105] are only of a preliminary nature it is clear that binding of metal ions at N7, especially of Cu^{2+}, *via* macrochelate formation acidifies the (N1)H site[105] of GTP^{4-} and ITP^{4-} and after this site is deprotonated the formation degree of the macrochelates in the $M(NTP - H)^{3-}$ species increases further.[63]

There is another most fascinating aspect: If one compares the formation degrees of $M(AMP)_{cl}$, $M(ADP)^{-}_{cl}$ and $M(ATP)^{2-}_{cl}$ as assembled in Table 6, one makes the remarkable observation that for the biologically most important metal ions Ca^{2+}, Mg^{2+}, Mn^{2+} and Zn^{2+}, the formation degrees of the macrochelated species of a given metal ion are identical within the error limits, this means, independent of the number of phosphate groups present in the adenosine phosphates (AP) and thus in the coordination spheres of the metal ions. There are three, but not dramatic, exceptions; for Co^{2+} and Ni^{2+} one observes the series $M(AMP)_{cl} > M(ADP)^{-}_{cl} \cong M(ATP)^{2-}_{cl}$ and for Cu^{2+}, $Cu(AMP)_{cl} \cong Cu(ADP)^{-}_{cl} < Cu(ATP)^{2-}_{cl}$ (Table 6). This indicates that mainly the properties of the N7 site are responsible for the extent of macrochelate formation.

The above observation is even more surprising when one considers the overall stabilities of the M(AP) complexes (Table 4),[7,33,49] which are determined to the very largest part by the coordination of the metal ions to the phosphate residues: The stability differences between the M(AMP) and $M(ADP)^{-}$ complexes amount to about 1.2 to 2.4 log units,[33] whereas those between the $M(ADP)^{-}$ (ref. 33) and $M(ATP)^{2-}$(ref. 49) complexes are in the order of about 1 log unit. To give an example, the log stability constants for Mn(AMP), $Mn(ADP)^{-}$ and $Mn(ATP)^{2-}$ are about 2.2, 4.2 and 5.0; those for the corresponding Mg^{2+} and Zn^{2+} complexes are about 1.6, 3.4 and 4.3 as well as 2.4, 4.3 and 5.2, respectively. The given stability constants demonstrate nicely that, for example, upon hydrolysis of the terminal γ-phosphate group of the ATP substrate the resulting product can relatively easily be replaced in the coordination sphere of the metal ion because its binding affinity is drastically reduced.

Finally it needs to be emphasized that from potentiometric pH titrations only overall (global) stability constants can be obtained, and hence, different types of macrochelates cannot be distinguished. Measured is the concentration of all complexes, including the sum of all possible macrochelated isomers. However, from the studies on the $M(ATP)^{2-}$ complexes it is well known that (at least) two types of macrochelates can form:[29,49] one in which the phosphate-coordinated metal ion binds innersphere to N7 of the adenine residue and one in which this interaction is of an outersphere type, that is, with a water molecule between N7 and M^{2+} (for details about the data see ref. 29). These two isomeric forms are depicted in Fig. 6.[106–108] For example, for $Cu(ATP)^{2-}_{cl}$ it was concluded[29] that all N7 binding is innersphere, whereas for $Mg(ATP)^{2-}_{cl}$ only outersphere species form; for $Ni(ATP)^{2-}_{cl}$ evidence exists[29] which led to the suggestion that about 30% are N7 innersphere, 25% N7 outersphere, and 45% exist as $Ni(ATP)^{2-}_{op}$ (see also equilibrium (15)).

Similar situations occur for $M(ITP)^{2-}$ and $M(GTP)^{2-}$ species. ^{1}H-NMR shift experiments of the corresponding Mg^{2+} systems[63] gave no indication for macrochelate formation involving N7, which is clearly proven to occur by the results based on potentiometric pH titrations which are presented in Table 5; hence, it must be concluded[7] that $Mg(ITP)^{2-}_{cl}$ and $Mg(GTP)^{2-}_{cl}$ are of an N7 outersphere type, which is in accord with the conclusions reached for $Mg(ATP)^{2-}$. The same structure is most probably also relevant for the complexes of

Table 6 Comparison of the extent of intramolecular macrochelate formation (equilibrium (15)) in the M(AMP), $M(ADP)^{-}$, and $M(ATP)^{2-}$ complexes for aqueous solutions at 25 °C and I = 0.1 M ($NaNO_3$)[a]

M^{2+}	% $M(AMP)_{cl}$	% $M(ADP)^{-}_{cl}$	% $M(ATP)^{2-}_{cl}$
Ca^{2+}	7 ± 13	9 ± 8	15 ± 12
Mg^{2+}	13 ± 10	13 ± 9	17 ± 10
Mn^{2+}	15 ± 11	21 ± 7	17 ± 15
Co^{2+}	56 ± 7	37 ± 8	38 ± 13
Ni^{2+}	75 ± 4	59 ± 6	56 ± 6
Cu^{2+}	50 ± 7	54 ± 5	67 ± 3
Zn^{2+}	44 ± 12	31 ± 9	28 ± 10

[a] For the error limits see footnote "*a*" of Table 3. The values in columns 2 and 3 are abstracted from Table 4 in ref. 33; those of column 4 are from Table 5 (upper part, column 5).

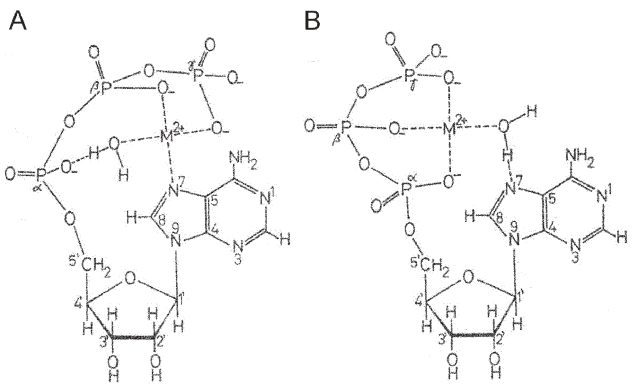


Fig. 6 Tentative and simplified structure for the macrochelated innersphere (A) and outersphere (B) $M(ATP)^{2-}$ isomers (reproduced by permission of the Federation of European Biochemical Societies (FEBS) from *Eur. J. Biochem.*, ref. 29). It should be noted that the terms innersphere and outersphere are used above solely with regard to the M^{2+}-N7 coordination (see also below). The depicted M^{2+}-triphosphate coordination follows an early suggestion of Martin and Mariam:[43] if an intramolecular direct M^{2+}-N7 coordination occurs, then it is sterically more favorable to have a water molecule between M^{2+} and the α-phosphate group as shown in A, though it should be noted that space-filling molecular models indicate[106] that a simultaneous innersphere binding of both N7 and the α-phosphate group is also possible.[78,92] With an outersphere N7 binding, as shown in B, innersphere coordination of all three phosphate groups is suggested,[43] but this will certainly also depend on the kind of metal ion involved. For example, there is evidence[107] that in $Mg(ATP)^{2-}$ phosphate binding occurs as a mixture of β,γ-bidentate and α,β,γ-tridentate complexation. Hence, further isomers differing in the phosphate coordination are possible; *e.g.*, direct β,γ-phosphate and N7 coordination leaving the α group free. One may also mention here a very early conclusion regarding metal ion-phosphate interactions in general,[108] namely, "the lower the charge, the more predominant are outersphere complexes". Finally, it should be noted that for the sake of clarity in the above structures the equatorial positions of an octahedral coordination sphere are used, but binding to other positions is of course also possible and this gives then rise to further isomers.

the other alkaline earth ions. From an early line-broadening study[109] of the Mn^{2+}/ITP system, it follows that at least some innersphere binding occurs with N7. Comparisons of the results given in Table 5 for the Zn^{2+} and Cd^{2+} complexes of ITP^{4-} and GTP^{4-} with those of a ^{1}H-NMR shift study[63] indicate that macrochelate formation for the $M(ITP)^{2-}_{cl}$ and $M(GTP)^{2-}_{cl}$ species of these two metal ions is largely innersphere with N7 (analogous to Fig. 6A). It is evident that further detailed studies, either by NMR and/or spectrophotometry, are desirable to reveal the ratios of the macrochelated isomers for other metal ions as well. In addition, it should be noted that the (C6)O carbonyl group present in inosine and guanosine (Fig. 1) may also participate in outersphere metal ion binding as discussed in detail[78] for M(GMP) complexes.

5 Stabilities and solution structures of mixed ligand complexes containing NTPs

5.1 Some general comments and definitions regarding equilibria of ternary complexes

As one might guess based on the results discussed in Section 4.4, the 'weak' point in macrochelate formation of $M(ATP)^{2-}$ complexes is the coordination of N7 of the adenine residue. Indeed, the release of N7 from $M(ATP)^{2-}_{cl}$ upon the formation of mixed ligand complexes in solution has been demonstrated with ligands as different as OH^-,[77,110] NH_3,[110] imidazole,[110,111] 2,2′-bipyridine,[77,112,113] 1,10-phenanthroline,[112] and tryptophanate.[112,114] This release of N7 from the metal ion-coordination sphere has also been confirmed for the solid state.[115]

Not all of the indicated ternary complexes can be discussed here; we shall concentrate in this section on two aspects: (*i*) Considering that many experiments in biochemistry are carried out in buffers to stabilize the pH of a solution, we feel that it is important to indicate possible drawbacks of this procedure and therefore in Section 5.2 mixed ligand complexes containing a buffer molecule and an NTP will be discussed. (*ii*) Equally important are recognition reactions and here nucleobase stacking is an important tool in nature; therefore some examples with intramolecular stacking in mixed ligand complexes will be discussed in Section 5.3. However, at first it will be necessary to define the equilibria which allow a quantification of the properties of ternary complexes.

A ternary complex of the kind considered here is composed of a metal ion to which two different ligands are coordinated. There are various ways to quantify the stability of such ternary complexes.[116,117] We restrict ourselves to complexes which have a nucleoside 5′-triphosphate (NTP^{4-}) bound in the coordination sphere of M^{2+} and to which a second ligand L is binding, leading thus to the ternary $M(NTP)(L)^{2-}$ complex. For convenience possible charges of L are deleted; the relevant equilibrium may then be written as:

$$M(NTP)^{2-} + L \rightleftharpoons M(NTP)(L)^{2-} \quad (23a)$$

$$K^{M(NTP)}_{M(NTP)(L)} = [M(NTP)(L)^{2-}]/([M(NTP)^{2-}][L]) \quad (23b)$$

Evidently this equilibrium is best compared with the following one:

$$M^{2+} + L \rightleftharpoons M(L)^{2+} \quad (24a)$$

$$K^{M}_{M(L)} = [M(L)^{2+}]/([M^{2+}][L]) \quad (24b)$$

The difference $\Delta \log K_{M/NTP/L}$,

$$\Delta\log K_{M/NTP/L} = \log K^{M(NTP)}_{M(NTP)(L)} - \log K^{M}_{M(L)} \quad (25a)$$

$$= \log K^{M(L)}_{M(L)(NTP)} - \log K^{M}_{M(NTP)} \quad (25b)$$

characterizes the coordination tendency of L toward $M(NTP)^{2-}$ (eqn (23)) relative to that toward $M(aq)^{2+}$ (eqn (24)) and *vice versa* (eqn (25));[116,117] hence, factors that arise through direct[118–120] or indirect (*i.e.*, metal ion mediated)[116,117,121] ligand-ligand interactions in a ternary complex should show up in this description.

It is important to note that $\Delta \log K_{M/NTP/L}$ is the difference between two log stability constants and thus it has to be a constant itself; indeed, it quantifies the position of the following equilibrium (26a).

$$M(NTP)^{2-} + M(L)^{2+} \rightleftharpoons M(NTP)(L)^{2-} + M^{2+} \quad (26a)$$

$$10^{\Delta \log K_{M/NTP/L}} = \frac{[M(NTP)(L)^{2-}][M^{2+}]}{[M(NTP)^{2-}][M(L)^{2+}]} \quad (26b)$$

Since more coordination positions are available for binding of L to M^{2+} than to $M(NTP)^{2-}$, one expects on statistical grounds[116,117] and in accord with the general rule[31,32] $K_1 > K_2$ *negative* values for $\Delta \log K_{M/NTP/L}$. For example, to be precise, for two different bidentate ligands A and B $\Delta \log K_{M/A/B}$ is between -0.38 for an octahedral coordination sphere and about -0.9 for the distorted coordination sphere of Cu^{2+};[116] hence, smaller values are expected for ternary Cu^{2+} systems than for those with Mn^{2+} or Co^{2+}; Zn^{2+} with its 'Chameleon'-like coordination sphere[122] is a special case.

5.2 Ternary metal ion complexes containing ATP^{4-} and a buffer molecule as ligand

Tris is one of the buffers most often used in biochemical studies, as its buffer region is in the neutral to slightly alkaline pH range;[123] the same is true for Bistris which buffers in the pH range 6 to 7.5.[124] Bicine,[125] also known as one of "Good's buffers",[126] is widely employed as well, especially in the pH range 7.5 to 9. The structures of the three mentioned buffers are shown in Fig. 7.

Bicine is evidently derived from the amino acid glycine. Consequently, already in 1966 Bicinate was expected to form chelates with metal ions just like its parent compound, glycinate.[126] For Tris and Bistris the awareness that in the presence of metal ions the interactions between the buffers and these ions need to be considered is much lower and the fact that also mixed ligand complexes can be formed has hardly been realized. Therefore, the stabilities of ternary complexes formed between these buffers (Fig. 7) and ATP is briefly summarized below.

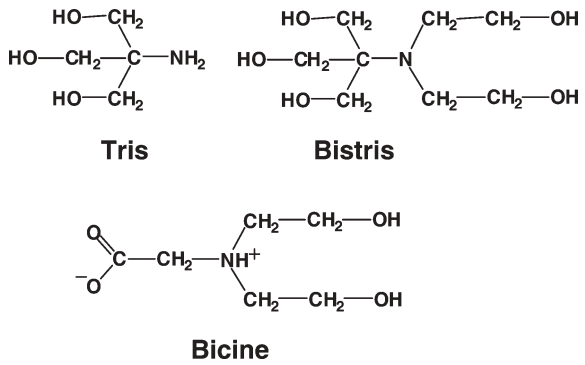


Fig. 7 Chemical structures of 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris), 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)-1,3-propanediol (Bistris), and *N*,*N*-bis(2-hydroxyethyl)glycine (Bicine).

The stability constants according to equilibria (23) and (24) are listed in Table 7. The stabilities of the binary $M(L)^{2+}$ complexes of Tris and Bistris are quite large and it has been recognized[123,124] that this is due to chelate formation with hydroxy groups; substitution of a hydrogen by a methyl or ethyl group at NH_3 alone leads to a steric inhibition and a reduction of complex stabilities.[123,124] That the hydroxy groups of these buffers play a significant role is also confirmed by a comparison of the stability constants of the $M(Tris)^{2+}$ complexes of Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} with those of the corresponding $M(Bistris)^{2+}$ species (Table 7, column 4); the stability increase is between about 0.1 to 1.2 log units and this despite the fact that the basicity of the nitrogen in Bistris is about 1.5 pK units lower (see footnotes "a" and "b" in Table 7). This proves the earlier conclusion[124] that most of the hydroxy groups of Bistris participate in metal ion binding.

An especially interesting case is $Ca(Bistris)^{2+}$ which is significantly more stable than the complexes of its neighbouring elements. This may be explained[124] by a 'cage-like' orientation of several hydroxy groups and the nitrogen, a structural arrangement into which Ca^{2+} fits well (log $K^{Ca}_{Ca(Bistris)}$ = 2.25; Table 7), while the ionic radius of Mg^{2+} is too small (log $K^{Mg}_{Mg(Bistris)}$ = 0.34; Table 7) and the radii of Sr^{2+} (log $K^{Sr}_{Sr(Bistris)}$ = 1.44 ± 0.02)[124] and Ba^{2+} (log $K^{Ba}_{Ba(Bistris)}$ = 0.85 ± 0.03)[124] are too large to allow an optimal interaction with the hydroxy groups. This selectivity of Bistris to complex preferably with Ca^{2+} within the alkali earth ion series is of biological relevance and corresponds to observations made with 'crown' ethers and other macrocyclic ligands.[124]

That the hydroxy groups also play a significant role in the ternary complexes is evident from the small negative values observed for Δ log $K_{M/ATP/L}$ (Table 7, column 5). The role of the hydroxy groups for the stability of the $M(ATP)(Tris)^{2+}$ complexes follows from comparisons with the stability constants of the $M(ATP)(NH_3)^{2-}$ complexes for Ni^{2+} (log $K^{Ni(ATP)}_{Ni(ATP)(NH_3)} \leqslant 2.3$)[127] and Cu^{2+} (log $K^{Cu(ATP)}_{Cu(ATP)(NH_3)}$ = 3.4);[127] these constants are somewhat smaller than those of the corresponding $M(ATP)(Tris)^{2-}$ complexes (Table 7) and this despite the much lower basicity of Tris (p$K^{H}_{H(Tris)}$ = 8.13; Table 7, footnote "a") compared to that of NH_3 (p$K^{H}_{NH_4}$ = 9.38).[127] To which extent the participation of the hydroxy groups in the formation of the ternary complexes occurs *via* direct metal ion binding or *via* hydrogen bonding to the phosphate oxygens of the coordinated ATP^{4-} is open. However, the *positive* Δ log $K_{Mg/ATP/Bistris}$ value observed for the Mg^{2+}/ATP^{4-}/Bistris system and the relatively small negative values for Δ log $K_{M/ATP/Bistris}$ observed for the Ca^{2+},

Table 7 Logarithms of the stability constants of some ternary $M(ATP)(L)^{2-}$ (eqn (23)) and binary $M(L)^{2+}$ (eqn (24)) complexes, where L = Tris,[123,a] Bistris[124,b] or Bicinate,[124,c] as determined by potentiometric pH titrations[123,124] or spectrophotometric measurements[125] in aqueous solutions at 25 °C, together with the stability differences according to eqn (25)[d]

L	M^{2+}	log $K^{M(ATP)}_{M(ATP)(L)}$	log $K^{M}_{M(L)}$	Δ log $K_{M/ATP/L}$
Tris	Mg^{2+}	$\leqslant$0.7	$\leqslant$0.7	
	Co^{2+}	1.57 ± 0.05	1.73 ± 0.02	−0.16 ± 0.05
	Ni^{2+}	2.35 ± 0.05	2.74 ± 0.02	−0.39 ± 0.05
	Cu^{2+}	3.50 ± 0.05	4.05 ± 0.02	−0.55 ± 0.05
	Zn^{2+}	$\leqslant$1.8[e]	1.94 ± 0.03	$\leqslant$−0.14
	Cd^{2+}	1.17 ± 0.05	1.94 ± 0.02	−0.77 ± 0.05
Bistris	Mg^{2+}	0.59 ± 0.10	0.34 ± 0.05	0.25 ± 0.11
	Ca^{2+}	1.85 ± 0.09	2.25 ± 0.02	−0.40 ± 0.09
	Mn^{2+}	0.6 ± 0.2	0.70 ± 0.05	−0.1 ± 0.2
	Co^{2+}	1.33 ± 0.03	1.78 ± 0.03	−0.45 ± 0.04
	Ni^{2+}	2.77 ± 0.04	3.59 ± 0.02	−0.82 ± 0.04
	Cu^{2+}	3.62 ± 0.03	5.27 ± 0.01	−1.65 ± 0.03
	Zn^{2+}	$\leqslant$2.0[f]	2.38 ± 0.03	$\leqslant$−0.38
Bicinate	Co^{2+}	4.53 ± 0.22	5.08 ± 0.13	−0.55 ± 0.26
	Ni^{2+}	5.44 ± 0.19	6.02 ± 0.09	−0.58 ± 0.21
	Cu^{2+}	6.57 ± 0.32	8.24 ± 0.09	−1.67 ± 0.33

[a] p$K^{H}_{H(Tris)}$ = 8.13 ± 0.01; I = 0.1 M (KNO_3).[123] [b] p$K^{H}_{H(Bistris)}$ = 6.72 ± 0.01; I = 1.0 M (KNO_3).[124] For the acidity constant of $H(Bistris)^+$ at 25 °C and I = 0.1 M (KNO_3) it holds p$K^{H}_{H(Bistris)}$ = 6.56 ± 0.04.[124] [c] The acidity constants of $H_2(Bicinate)^+$ as determined by potentiometric pH titrations at 25 °C and I = 1.0 M (KNO_3) are p$K^{H}_{H_2(Bicinate)}$ = 2.13 ± 0.06 and p$K^{H}_{H(Bicinate)}$ = 8.33 ± 0.03.[125] The stability constants given above were determined by spectrophotometry also at I = 1.0 M (KNO_3).[125] [d] For the error limits (3σ) see footnote "*a*" of Table 3. [e] This value is an upper limit of the stability constant but the actual value is expected to be close to this limit.[123] [f] Same comment as in footnote "e".[124]

Mn^{2+} and Co^{2+} complexes suggest, because of the "saturation" of the coordination spheres of these metal ions by, *e.g.*, three sites each from ATP^{4-} and Bistris, as the most plausible explanation the formation of hydrogen bonds between some OH groups of the coordinated Bistris and phosphate-oxygens of the also coordinated ATP^{4-}.[124] It should be noted here that the expected statistical value for a regular octahedral coordination sphere of M^{2+} and the coordination of two different but simple and symmetrical tridentate ligands amounts already to $\Delta \log K_{oh/3/3} = -1.03$.[124]

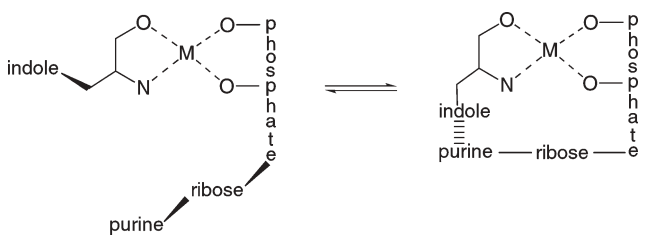
The stability constants of the binary $M(Bicinate)^+$ complexes (Table 7, lower part) speak for themselves; these values are so large that Bicine, if used as pH buffer in the presence of metal ions, will certainly complex a very significant amount of the metal ions – and the hydroxy groups participate in this reaction.[128] However, the mixed ligand complexes formed with ATP^{4-} are very stable as well!

Finally it may be mentioned that there is a study[129] which focuses on the effect of mixed aqueous solvents (1,4-dioxane, dimethyl sulfoxide, methanol) on the stability of several binary complexes of Tris, Bistris and triethanolamine (Tea), a compound also often employed as pH buffer.

5.3 Intramolecular stacking interactions in ternary NTP^{4-} complexes

Considering that purine residues self-stack so well (see Section 2) it is to be expected that stacking interactions also occur with other aromatic-ring systems. Indeed, this is the case (*e.g.*[77,79,85,111–114,130]) and it is now well recognized[131] that such noncovalent interactions, to which also electrostatic and hydrogen bonding[132] as well as hydrophobic interactions belong, are important for specificity, selectivity and recognition reactions as they occur in enzyme–substrate, nucleobase–nucleobase, nucleic acid–protein and neurotransmitter–receptor adducts. We shall concentrate here on aromatic-ring stacking and on interactions between an aliphatic residue, *e.g.* of an amino acid, and an aromatic ring, previously classified as hydrophobic interactions but recently addressed as CH/π interactions.[133] To give an example each, a typical amino acid that may provide an aromatic ring in its side chain is tryptophan and one that can offer an aliphatic side chain is leucine (Fig. 8).

The first mixed ligand complex studied containing ATP^{4-} and an amino acid anion (AA^-) was the one with tryptophanate (Trp^-), *i.e.* $Zn(ATP)(Trp)^{3-}$. By ^{1}H-NMR shift experiments it was shown that an indole–adenine interaction takes place[134] which may be promoted by Zn^{2+}. Later, the position of the intramolecular equilibrium (27)



(27)

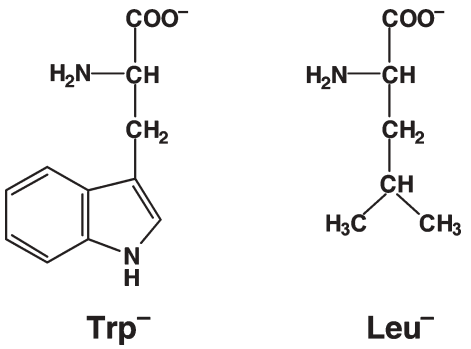


Fig. 8 Chemical structures of the anions of the amino acids (AA^-) tryptophanate (Trp^-) and leucinate (Leu^-).

was determined with Zn^{2+} as metal ion and it was concluded[114] that the stacked species occurs with a formation degree of approximately 75%. Other metal ions serving as bridges were studied as well[71,114,135,136] and the occurrence of intramolecular stacks in $M(ATP)(Trp)^{3-}$ complexes was confirmed by several groups.[137] Related complexes containing phenyl and imidazole residues have also been investigated.[111,138,139]

Intramolecular ligand–ligand interactions of the mentioned kind have to be reflected in an increased complex stability[101] and therefore stability constant comparisons allow to quantify the position of, *e.g.*, equilibrium (27). A comparison of stability constants as defined in eqns (23) to (25), where for the present L = anion of an amino acid (AA^-), is the basis of such an evaluation.[101] To make this more clear we rewrite equilibrium (26a) with the ligands now considered,

$$M(ATP)^{2-} + M(AA)^+ \rightleftharpoons M(ATP)(AA)^{3-} + M^{2+} \quad (28)$$

and remember that the position of equilibrium (28) is defined by $\Delta \log K_{M/ATP/AA}$ (eqn (25)). It is immediately evident that if we select for AA^- once tryptophanate (Trp^-) and once alaninate (Ala^-) then the coordination spheres of the metal ions involved in equilibrium (28) remain identical but in the ternary $M(ATP)(Trp)^{3-}$ complex an adenine–indole stack may form giving rise to an increased stability and thus to a shift of this equilibrium to its right-hand side, compared with the position of the corresponding equilibrium involving $M(ATP)(Ala)^{3-}$. Hence, the difference

$$\log \Delta_{M/ATP/Trp} = \Delta \log K_{M/ATP/Trp} - \Delta \log K_{M/ATP/Ala} \quad (29)$$

is a reflection of the intramolecular stack in $M(ATP)(Trp)^{3-}$. Of course, $10^{\log \Delta_{M/ATP/Trp}}$, as it follows from eqn (29), quantifies the position of equilibrium (30):

$$M(Trp)^+ + M(ATP)(Ala)^{3-} \rightleftharpoons M(ATP)(Trp)^{3-} + M(Ala)^+ \quad (30)$$

Note, if there is no ligand–ligand interaction in $M(ATP)(Trp)^{3-}$ then equilibrium (30) is in the middle and $\log \Delta_{M/ATP/Trp} = 0$ and $10^{\log \Delta_{M/ATP/Trp}} = 1$.

The position of the intramolecular equilibrium (27) is now defined by eqn (31) (see also ref. 118):

$$K_{I/st} = \frac{10^{\Delta \log K_{M/ATP/Trp}}}{10^{\Delta \log K_{M/ATP/Ala}}} - 1 \quad (31a)$$

$$= \frac{10^{\Delta \log K_{M/ATP/Trp(st)}}}{10^{\Delta \log K_{M/ATP/Trp(op)}}} - 1 \quad (31b)$$

$$= 10^{\log \Delta_{M/ATP/Trp}} - 1 \quad (31c)$$

Of course, the percentage of the stacked isomer, $M(ATP)(Trp)^{3-}_{st}$, is calculated in analogy to eqn (22); the difference from 100% represents the formation degree of the 'open' (op) isomer, $M(ATP)(Trp)^{3-}_{op}$, seen at the left in equilibrium (27).

Exactly the same type of evaluation can be carried out for the $M(ATP)(Leu)^{3-}$ system in which a hydrophobic or CH/π interaction is expected to occur. For convenience we use also here the term 'stack'. The results for the $M(ATP)(Trp)^{3-}$ and $M(ATP)(Leu)^{3-}$ systems are summarized in Table 8 together with some results obtained from ^{1}H-NMR shift measurements, a method by which the intramolecular ligand–ligand interactions could be proven in an independent way.

The results of Table 8 show that equilibrium (27) actually exists. From the first four entries in the table it follows that the formation degree of the intramolecular stack in the $M(ATP)(Trp)^{3-}$ complexes amounts on average to about 50% and is thus quite pronounced. As expected, the formation degree of the hydrophobic or CH/π adduct in the $M(ATP)(Leu)^{3-}$ complexes is lower but with, on average, approximately 25% still remarkable. Of course, the stability of the binary, unbridged adducts between AMP^{2-} or ATP^{4-} and Trp^- or Leu^- is rather low: *e.g.*, for AMP^{2-}/Trp^- it holds $K = 2.24 \pm 0.58\ M^{-1}$ (2σ)[136] and for ATP^{4-}/Leu^- $K = 0.4 \pm 0.2\ M^{-1}$,[114] yet, already an ionic bridge, as it occurs in $AMP^{2-}/H(Trp)^{\pm}$ or in $ATP^{4-}/H(Trp)^{\pm}$ as well as in $ATP^{4-}/H(Leu)^{\pm}$, helps as is evident from $K = 6.83 \pm 1.62\ M^{-1}$,[136] $6.2 \pm 1.2\ M^{-1}$,[112] and $0.6 \pm 0.3\ M^{-1}$,[114] respectively (for a compilation of such data see Table 5 in ref. 79).

One may further mention in the present context that the evaluation of ^{1}H-NMR shift experiments[111] led to the conclusion that in aqueous solution the $Zn(ATP)(Im)^{2-}$ and $Cd(ATP)(Im)^{2-}$ complexes form an intramolecular stack between the adenine residue and imidazole (Im) with a formation degree between about 15–50%.[111] This result agrees with a suggestion,[138] based on thermodynamic parameters, that in $Zn(ATP)(histamine)^{2-}$ stacking occurs. This result, together with the one obtained for $M(ATP)(Ala)^{3-}$ species that by ^{1}H-NMR measurements no indications for an interaction between the methyl group of alaninate (Ala^-) and the purine residue of ATP^{4-} could be found,[114] allows the following conclusion: The recognition between the adenine residue and amino acid side chains in mixed ligand complexes of the type $M(ATP)(AA)^{3-}$ decreases in the order: indole residue (tryptophan) > phenyl residue (phenylalanine; tentative) ⩾ isopropyl residue (leucine) ⩾ imidazole residue (histidine) > methyl residue (alanine). We are convinced that this series of selectivity also holds for other instances in which amino acids/proteins interact with nucleotides/nucleic acids.

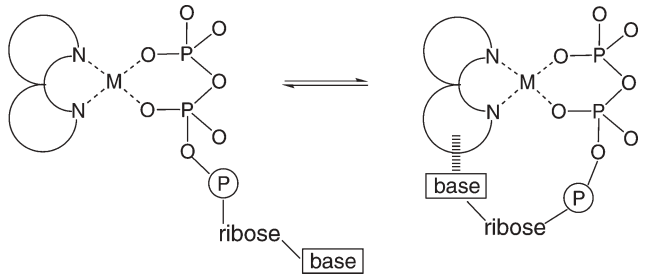
Of course, stacking interactions of purine moieties are also possible with other aromatic-ring systems (Arm) like 2,2′-bipyridine (Bpy) or 1,10-phenanthroline (Phen). Indeed, the stability of several binary adducts measured either by UV spectrophotometry or ^{1}H-NMR is known: *e.g.*, for the stability of the Phen/Ado adducts it holds $\log K = 1.33 \pm 0.05\ M^{-1}$ (UV)[119] and for that of $Phen/ATP^{4-}$ $\log K = 1.19 \pm 0.02\ M^{-1}$ (UV)[119] and $1.58 \pm 0.09\ M^{-1}$ (NMR);[85] for Bpy/ATP^{4-} $\log K = 0.91 \pm 0.22\ M^{-1}$ (UV)[140] and $1.20 \pm 0.11\ M^{-1}$ (NMR)[85] was measured (for a listing see Table 3 of ref. 79 and also ref. 141). As one might expect, the different size of the aromatic system in Bpy and Phen is reflected in the stability of their adducts: the Bpy/ATP^{4-} adducts are somewhat less stable than the $Phen/ATP^{4-}$ adducts (note, values obtained by the same method should be compared). However, overall these binary Arm/ATP^{4-} adducts are relatively unstable, yet again by linking the parts together *via* a metal ion bridge the formation degree of the stacks increases dramatically at a given total concentration of the reactants because it is now linked to the stability of the metal ion complexes.

Table 8 Extent of intramolecular aromatic-ring stacking or hydrophobic adduct formation in ternary $M(ATP)(AA)^{3-}$ complexes, where AA^- = tryptophanate (Trp^-) or leucinate (Leu^-), according (or analogous) to equilibrium (27), calculated from stability constants (analogous to eqns (12b) and (17a)) determined *via* potentiometric pH titrations (Pot.): Given is the stability enhancement $\log \Delta_{M/ATP/AA}$ (eqn (29)), the intramolecular and dimensionless equilibrium constant $K_{I/st}$ (eqn (31)), and the percentage (analogous to eqn (22)) of the 'closed' or stacked $M(ATP)(AA)^{3-}_{st}$ species in aqueous solution at 25 °C and $I = 0.1$ M ($NaClO_4$).[a] For comparison some results[a] obtained from ^{1}H-NMR shift experiments are also given

			% $M(ATP)(AA)^{3-}_{st}$		
$M(ATP)(AA)^{3-}$	$\log \Delta_{M/ATP/AA}$	$K_{I/st}$	Pot.	NMR	Ref.
$Mg(ATP)(Trp)^{3-}$				44 ± 19[b]	71
$Mn(ATP)(Trp)^{3-}$	0.32 ± 0.11	1.09 ± 0.52	52 ± 12		114
$Cu(ATP)(Trp)^{3-}$	0.19 ± 0.09	0.55 ± 0.33	35 ± 14		114
$Zn(ATP)(Trp)^{3-}$	0.59 ± 0.06	2.89 ± 0.51	74 ± 3	40 ± 15[b]	114,71
$Mn(ATP)(Leu)^{3-}$	0.23 ± 0.22	0.70 ± 0.87	41 ± 30		114
$Cu(ATP)(Leu)^{3-}$	0.10 ± 0.08	0.26 ± 0.23	21 ± 14		114
$Zn(ATP)(Leu)^{3-}$	0.02 ± 0.09	0.05 ± 0.22	5 ± 20	~30(20/75)[c]	114

[a] For the error limits (3σ) see footnote "*a*" of Table 3. The above information is abstracted from Table 6 of ref. 79. [b] Based on ^{1}H-NMR shift experiments in D_2O at 27 °C and $I = 0.1$ M ($NaNO_3$).[71,112] [c] Based on ^{1}H-NMR shift experiments in H_2O at 34 °C and $I = 0.1 - 1.8$ M (KNO_3); the values in parentheses give the lower and upper limits, respectively.[114]

Furthermore, since Bpy and Phen are considerably less flexible, if coordinated to a metal ion, than the ligands discussed above, *e.g.* in equilibrium (27), the formation degrees of the stacks in M(Arm)(NTP)$^{2-}$ complexes as indicated in the intramolecular equilibrium (32)



(32)

are expected to be larger, since the aromatic moieties in the mixed ligand complexes "find" each other more easily.

For this type of M(Arm)(NTP)$^{2-}$ complexes the same evaluation procedure as described above for M(ATP)(AA)$^{3-}$ complexes can be used. The increased formation degree of the stacks in the M(Arm)(NTP)$^{2-}$ species, compared with those in the M(ATP)(AA)$^{3-}$ ones (Table 8), is nicely seen from the results in Table 9, where values from potentiometric pH titrations as well as from ^{1}H-NMR shift experiments are assembled.

Entries 1–4 of Table 9 show that the formation degree of the intramolecular stacks in the M(Phen)(ATP)$^{2-}$ complexes is large and amounts to about 90% independent of the metal ion involved, *i.e.* Mg^{2+}, Ca^{2+}, Cu^{2+} or Zn^{2+}. This result is interesting since the overall stability constant, $\beta^{M}_{M(Phen)(ATP)}$, of these complexes is very different,[85,119] but evidently once the complex is formed, the flexibility of ATP^{4-} in these ternary M(Phen)(ATP)$^{2-}$ complexes is high enough to overcome steric restrictions possibly imposed due to the different coordination geometries of these metal ions. The formation degree of the stacks in M(Bpy)(ATP)$^{2-}$ systems is somewhat smaller, indicating again that the size of the aromatic moiety has an effect. The same trend is borne out if the data for Cu(Phen)(ATP)$^{2-}$ are compared with those for Cu(Phen)(UTP)$^{2-}$ (entries 3 and 5 of Table 9) or those of Zn(Bpy)(ATP)$^{2-}$ with the ones of Zn(Bpy)(UTP)$^{2-}$ (entries 7 and 9).

Maybe it should again be emphasized that the stability of the binary adducts is low, *e.g.* for Bpy/UTP^{4-} it holds $K \cong 1\ M^{-1}$,[142] a value that could hardly be determined. However, the formation degree of about 60% for the intramolecular stacks in Cu(Bpy)(UTP)$^{2-}$ and Zn(Bpy)(UTP)$^{2-}$ is quite considerable (Table 9, entries 8 and 9). Hence, the message is: a metal ion may facilitate the "recognition" between two moieties by linking them together into the same species (see also the terminating paragraph in Section 6)!

In the above discussion we have concentrated our attention on mixed ligand complexes formed with NTPs; however, there are also data available[79] about stacking interactions in ternary complexes of NDPs[130,143] and also of NMPs.[144,145] For example, the extent of intramolecular stack formation in ternary complexes formed between Cu(Arm)$^{2+}$ and 2′-AMP^{2-}, 3′-AMP^{2-} or 5′-AMP^{2-} differs considerably and depends on the position of the phosphate group at the ribose residue.[145] FMN^{2-} does not only self-stack;[87] it also forms heterostacks in Cu(Arm)(FMN) complexes.[146] 9-[2-(Phosphonomethoxy)-ethyl]adenine (PMEA), an analogue of AMP^{2-} with antiviral properties,[51] is also able to form intramolecular stacks with Cu(Arm)$^{2+}$,[141,147] as are several of its derivatives;[120,148] these stacks are remarkably similar in their stability to those of Cu(Arm)(AMP).

Table 9 Extent of intramolecular stack formation according to equilibrium (32) in ternary M(Arm)(NTP)$^{2-}$ complexes as calculated from stability constants (analogous to eqns (12b) and (17a)) determined *via* potentiometric pH titrations (Pot.): Given is the stability enhancement log $\Delta_{M/Arm/NTP}$ (analogous to eqn (29)), the intramolecular and dimensionless equilibrium constant $K_{I/st}$ (analogous to eqn (31)), and the percentage (analogous to eqn (22)) of the stacked M(Arm)(NTP)$^{2-}_{st}$ species in aqueous solution at 25 °C and I = 0.1 M ($NaNO_3$).[a] For comparison some results obtained from ^{1}H-NMR shift experiments are also given

No.	M(Arm)(NTP)$^{2-}$	log $\Delta_{M/Arm/NTP}$	$K_{I/st}$	M(Arm)(NTP)$^{2-}_{st}$ Pot.	NMR[b]	Ref.
1	Mg(Phen)(ATP)$^{2-}$	~1.0	~9	~90	~100	117[c],112
2	Ca(Phen)(ATP)$^{2-}$	~1.0	~9	~90		117[c]
3	Cu(Phen)(ATP)$^{2-}$	1.07	11	92		85
4	Zn(Phen)(ATP)$^{2-}$				~95	112
5	Cu(Phen)(UTP)$^{2-}$			~57		85
6	Cu(Bpy)(ATP)$^{2-}$	0.84	5.9	86		85
7	Zn(Bpy)(ATP)$^{2-}$	~0.54	~2.5	~70	~55	77,112
8	Cu(Bpy)(UTP)$^{2-}$			~55		85
9	Zn(Bpy)(UTP)$^{2-}$	~0.45	~1.8	~65	~40	77,112
10	Zn(Bpy)(CTP)$^{2-}$	~0.57	~2.7	~75		77

[a] The above information is abstracted from Table 4 of ref. 79. Regarding entries 3, 6 and 8 see also Table 12 in Section 6. [b] Based on ^{1}H-NMR shift experiments in D_2O at 27 °C and I = 0.1 M ($NaNO_3$).[112] [c] The data given in ref. 117 are based on experiments described in ref. 119 which apply to 25 °C and I = 0.1 M ($NaClO_4$).

6 Effect of a reduced solvent polarity on complex stability

It is now well established that the so-called 'effective' or 'equivalent solution' dielectric constants in proteins[149] or in active-site cavities of enzymes[150,151] are reduced compared to the situation in bulk water, and it is agreed that different types of water exist in cells.[152] In other words, the activity of water is decreased[153] due to the presence of aliphatic and aromatic amino acid side chains at the protein–water interface.[139] To what extent are metal ion complex equilibria affected by these effects?

Estimates for the effective dielectric constants (permittivities) in such locations range[149–151] from about 30 to 70 compared with the approximately 80 of bulk water; hence, by employing aqueous solutions that contain a certain amount of organic solvents, *e.g.*, about 20–50% 1,4-dioxane, one may expect to simulate to some degree the situation in active site cavities.[139] The dielectric constants of the two indicated mixed solvents are about 60 and 35, respectively.[154] It may further be mentioned that with regard to biological systems it has been pointed out[155] that metal ions like to be coordinated to a hydrophilic shell which then may be embedded into a larger hydrophobic shell.

Table 10 Negative logarithms of the acidity constants of $H(RibMP)^-$ and $H(UTP)^{3-}$ (eqn (9)) as well as logarithms of the stability constants of the corresponding Cu(RibMP) and $Cu(UTP)^{2-}$ complexes (analogous to eqns (12) and (24)) in dependence on the amount of 1,4-dioxane added to water (25 °C; I = 0.1 M, $NaNO_3$)[a]

% (v/v) Dioxane[b]	$RibMP^{2-}$		UTP^{4-}	
	$pK^{H}_{H(RibMP)}$	$\log K^{Cu}_{Cu(RibMP)}$	$pK^{H}_{H(UTP)}$	$\log K^{Cu}_{Cu(UTP)}$
0	6.24 ± 0.01	2.96 ± 0.02	6.46 ± 0.01	5.81 ± 0.06
20	6.70 ± 0.01	3.45 ± 0.01		
30	6.93 ± 0.01	3.77 ± 0.02	6.84 ± 0.01	6.16 ± 0.05
40	7.19 ± 0.01	4.09 ± 0.03		
50	7.38 ± 0.01	4.38 ± 0.05	6.92 ± 0.01	6.24 ± 0.03

[a] For the error limits (3σ) see footnote "*a*" of Table 3. The values for RibMP are from Table I of ref. 156 and those for UTP of Table 4 of ref. 85. [b] For the mol fractions and the corresponding dielectric constants (ε) see Table 11.

In the preceding sections we have seen that the phosphate–metal ion interactions determine the overall stabilities of nucleotide complexes to a very large part;[7,29,34,36] therefore, it seems appropriate to consider first the effect of a reduced solvent polarity on the stability of simple phosphate–metal ion complexes. To this end we have collected the stability constants given in Table 10 for the Cu^{2+} complexes of D-ribose 5-monophosphate[156] and uridine 5′-triphosphate,[85] which despite being a nucleotide behaves like a simple triphosphate monoester (Section 4.2).

From the equilibrium constants given in Table 10 it is evident that both, ligand basicity as well as complex stability increase for both phosphate ligands with increasing amounts of 1,4-dioxane present in the aqueous solvent mixture. Plots of $\log K^{M}_{M(L)}$ *versus* $pK^{H}_{H(L)}$ for the two Cu^{2+}/phosphate systems lead to straight lines with slopes close to 1; in fact, this is a rather general observation which appears to hold for Zn^{2+} complexes as well.[156] This indicates that the solvent effects on proton and metal ion binding at a phosphate residue are approximately of the same size. The different spans in the $\log K^{M}_{M(L)}$ and $pK^{H}_{H(L)}$ values for $RibMP^{2-}$ and UTP^{4-} are most likely due to the different charges of the ligands; UTP^{4-} is expected to attract locally more water molecules than does $RibMP^{2-}$ and thus the effective dielectric constant around the triphosphate is expected to be less affected.[36]

However, it is evident that complex stability may be affected quite drastically by a change in the solvent; for example, the stability of Cu(RibMP) increases by a factor of about 25 by going from water ($\log K^{Cu}_{Cu(RibMP)}$ = 2.96; Table 10) to a water mixture containing 50% (v/v) 1,4-dioxane ($\log K^{Cu}_{Cu(RibMP)}$ = 4.38). This means, that the stability of a complex may change significantly indeed by a shift along the surface of a protein if the local effective dielectric constant changes.

With the results of Table 10 in mind it is fascinating to view the data in Table 11, where the percentages of the macrochelated species according to equilibrium (15) for Cu(AMP) and $Cu(ATP)^{2-}$ are assembled in dependence on the amount of 1,4-dioxane being present in the solvent.[157] First of all it needs to be emphasized that the stability of the complexes and the acid–base properties of the phosphate residues of AMP^{2-} and ATP^{4-} are rather closely represented by the corresponding values for $RibMP^{2-}$ and UTP^{4-} (Table 10), respectively. As far as the release of a proton from the $(N1)H^+$ site of the adenine residue is concerned $H_2(AMP)^{\pm}$ and $H_2(ATP)^{2-}$ behave identically; *i.e.*, in both instances the acidity increases by 0.42 pK units in going from water to water containing 50% (v/v) 1,4-dioxane.[157] This is important because any differences observed between $Cu(AMP)_{cl}$ and $Cu(ATP)^{2-}_{cl}$ can thus not be explained by different basicity properties of N7.

Clearly, the intriguing result of Table 11 is that the formation degree of $Cu(AMP)_{cl}$ passes through a minimum at about 30% (v/v) dioxane–water despite the fact that the overall complex stability[157] of Cu(AMP) increases rather "regularly" by about 1.5 log units in going from water to water containing 50% 1,4-dioxane (*cf.* also the data in Table 10 for $RibMP^{2-}$). It may be noted that the same observation has been made for $Cu(PMEA)_{cl}$.[158] In other words, the overall stability of these complexes behaves largely as predicted based on the results of Table 10 whereas the structural changes of these complexes involving equilibrium (15) appear as quite surprising. Furthermore, the overall stability of $Cu(ATP)^{2-}$ is only relatively little affected by the change in solvent composition[85] (see also the example of UTP^{4-} in Table 10) whereas the formation degree of its macrochelate decreases quite significantly (Table 11).

The results of Table 11 regarding $Cu(AMP)_{cl}$ and $Cu(ATP)^{2-}_{cl}$ are not easily explained;[36] they must be due to a combination of (opposing) solvent effects. Maybe the organic solvent molecules preferentially solvate the adenine residue at low concentration of dioxane and inhibit in this way the Cu^{2+}–adenine interaction in $Cu(AMP)_{cl}$ (as well as in $Cu(PMEA)_{cl}$), whereas at higher dioxane concentrations the solvation of those binding sites of the metal ion that are not yet occupied by

Table 11 Extent of chelate formation (equilibrium (15)) in the Cu(AMP) and $Cu(ATP)^{2-}$ complexes as quantified by the stability enhancement $\log \Delta_{Cu/AP}$ (eqn (20)) and the percentage of $Cu(AP)_{cl}$ (eqn (22)) in aqueous solution and in water containing various amounts of 1,4-dioxane at 25 °C and I = 0.1 M ($NaNO_3$),[a] together with some information about the solvents[b]

% (v/v) Dioxane	mol fract.	ε[b]	Cu(AMP)		$Cu(ATP)^{2-}$	
			$\log \Delta_{Cu/AMP}$	% $Cu(AMP)_{cl}$	$\log \Delta_{Cu/ATP}$	% $Cu(ATP)^{2-}_{cl}$
0	0	78.5	0.30 ± 0.06	50 ± 7	0.49 ± 0.05	68 ± 4
20	0.050	61.3	0.07 ± 0.04	15 ± 8		
30	0.083	52.7	0.04 ± 0.04	9 ± 8	0.26 ± 0.05	45 ± 6
40	0.124	44.1	0.11 ± 0.06	22 ± 11		
50	0.175	35.2	0.28 ± 0.04	48 ± 5	0.12 ± 0.05	24 ± 9

[a] For the error limits (3σ) see footnote "*a*" of Table 3. The entry regarding Cu(AMP) for aqueous solution is from ref. 33 and those for the dioxane–water mixtures are from ref. 157; the data for $Cu(ATP)^{2-}$ are from Table 6 of ref. 85. [b] The dielectric constants or permittivities (ε) for the dioxane–water mixtures are interpolated from the data given in ref. 154.

the ligand but by water is hindered and that this effect enhances the affinity of these copper(II) sites for ligand sites. Should this interpretation be correct then one should also be able to observe a minimum in the formation degree of $Cu(ATP)_{cl}^{2-}$ at larger dioxane concentrations; unfortunately, the corresponding experiments cannot be made as the reactants become insoluble at higher amounts of dioxane. Clearly, more work is needed here.

The described effects of a reduced solvent polarity on the properties of binary complexes are quite interesting and, at least in part, they were clearly not predictable. Hence, it is desirable to consider also the effect of a reduced solvent polarity on the stability of stacking adducts as they occur in mixed ligand complexes (see equilibrium (32)). Of course, at first it is appropriate to consider the stability of binary and unbridged stacks as they occur in the Phen/ATP^{4-} system: the stability of the corresponding stack in water is defined by $K_{st} = 38 \pm 8\ M^{-1}$, and those in water containing 30 and 50% (v/v) 1,4-dioxane by $K_{st} = 4.8 \pm 1.1\ M^{-1}$ and $1.8 \pm 0.7\ M^{-1}$, respectively.[85] In other words, a dramatic reduction in the stability of the stacks is observed.

The above results should be compared with those given in Table 12, where the effect of 1,4-dioxane on the stability of the intramolecular stacks according to equilibrium (32) in $Cu(Arm)(NTP)^{2-}$ complexes is seen. It is evident that even in 50% dioxane the formation degree of the intramolecular stacks is still remarkably high. Clearly, from the data in Table 12 it is apparent that equilibrium (32) involving the formation of intramolecular and metal ion-bridged stacks between the aromatic rings of 1,10-phenanthroline (entries 1–3) or 2,2′-bipyridine (entries 4–9) and the nucleobase moieties of ATP^{4-} (entries 1–6) and UTP^{4-} (entries 7–9) are affected by dioxane but by far not as much as the binary stacks formed between Phen and ATP^{4-} as mentioned above. By going from an aqueous solution to 50% (v/v) dioxane–water the stability of the unbridged Phen/ATP^{4-} adduct decreases by a factor of about 1/20, while the metal-ion-bridged stack in the ternary $Cu(Phen)(ATP)^{2-}$ complex (Table 12, entries 1–3) is disfavored only by a factor of about 1/2. In a first approximation the same observation is made for the $Cu(Bpy)(NTP)^{2-}$ complexes (entries 4–9)[85] though it is evident once again that the stability of the stacks depends on the size of the aromatic moieties forming the stacks. In this connection one should also mention that cases are known[139,159] where the addition of 1,4-dioxane or ethanol to an aqueous solution promotes the formation of intramolecular hydrophobic adducts or stacks in ternary Cu^{2+} and Zn^{2+} complexes. This contrasts with any experience regarding binary adducts.

To further illustrate the point that the formation of a metal ion bridge between the individual parts of a stacking adduct greatly favors the stability of this adduct, one may compare the percentage of the stacked adduct present in 10^{-3} M solutions of the reactants for Phen/ATP^{4-} and for the Cu^{2+}/Phen/ATP^{4-} systems by using for the calculations the appropriate equilibrium constants given in ref. 85. In an aqueous solution of the binary system the stacked adduct is present to about 3.5% (based on the total concentrations),[79] whereas in the ternary system (at pH $\sim$ 7) about 90% of the stacked isomer is formed; hence, we observe a promotion factor of approximately 25. Even more dramatic is the situation in 50% (v/v) dioxane–water. Here in the binary system only around 0.18% of the reactants exist in the stacked form, whereas in the ternary system the stacked isomers are still present with about 46%; *i.e.*, the promotion factor is now close to 250. Such effects certainly influence selectivity in biological systems.

7 A short appraisal of the metal ion-promoted group-transfer reactions involving NTPs

NTPs, and the same is true for the 2′-deoxy-NTPs (dNTPs) (see Fig. 1), are excellent ligands with significant ambivalent properties which give rise to varying structures of the complexes in solution as is evident from the results summarized in the preceding sections. Considering further that NTPs are substrates in enzymic reactions in the form of metal ion complexes, it is not surprising to find that the binding mode of a metal ion affects the type of reaction that occurs.

NTPs serve as substrates for group transfer reactions; the two most important types are: (i) The γ-phosphate group is transferred to another molecule, in the most simple case to

Table 12 Extent of intramolecular stack formation according to equilibrium (32) in ternary $Cu(Arm)(NTP)^{2-}$ complexes as calculated from stability constants (analogous to eqns (12b) and (17a)) determined *via* potentiometric pH titrations: Given is the stability enhancement log $\Delta_{Cu/Arm/NTP}$ (analogous to eqn (29)), the intramolecular and dimensionless equilibrium constant $K_{I/st}$ (analogous to eqn (31)), and the percentage (analogous to eqn (22)) of the stacked $Cu(Arm)(NTP)_{st}^{2-}$ species in aqueous solution as well as in water containing 30% (v/v) or 50% (v/v) 1,4-dioxane at 25 °C and $I = 0.1$ M ($NaNO_3$)[a]

No.	$Cu(Arm)(NTP)^{2-}$	Solvent[b]	log $\Delta_{Cu/Arm/NTP}$	$K_{I/st}$	%$Cu(Arm)(NTP)_{st}^{2-}$
1	$Cu(Phen)(ATP)^{2-}$	water	1.07 ± 0.15	10.7 ± 4.1	91 ± 3
2	$Cu(Phen)(ATP)^{2-}$	30% diox.	0.41 ± 0.15	1.57 ± 0.89	61 ± 13
3	$Cu(Phen)(ATP)^{2-}$	50% diox.	0.29 ± 0.15	0.95 ± 0.67	49 ± 18
4	$Cu(Bpy)(ATP)^{2-}$	water	0.84 ± 0.15	5.92 ± 2.39	86 ± 5
5	$Cu(Bpy)(ATP)^{2-}$	30% diox.	0.30 ± 0.15	1.00 ± 0.69	50 ± 17
6	$Cu(Bpy)(ATP)^{2-}$	50% diox.	0.21 ± 0.15	0.62 ± 0.56	38 ± 21
7	$Cu(Bpy)(UTP)^{2-}$	water	0.45 ± 0.22	1.82 ± 1.43	65 ± 18
8	$Cu(Bpy)(UTP)^{2-}$	30% diox.	0.12 ± 0.13	0.32 ± 0.39	24 ± 23
9	$Cu(Bpy)(UTP)^{2-}$	50% diox.	0.11 ± 0.11	0.29 ± 0.33	22 ± 20

[a] The above information is abstracted from Table 8 of ref. 79. The results are based on data published in ref. 85. The given values are in a systematic way rather lower limits and the given errors are in part (oversized) estimates.[79] [b] For the mol fraction and the corresponding dielectric constants (ε) see Table 11.

water, *i.e.* a hydrolysis reaction is then taking place. Such transphosphorylations are catalyzed, *e.g.*, by kinases, and they depend on the presence of metal ions. (ii) The other type is the transfer of a nucleotidyl unit, *i.e.* the break occurs now between the α- and β-phosphate groups; this nucleotidyl transfer is needed in the synthesis of nucleic acids and the connected polymerization reaction is catalyzed by DNA and RNA polymerases and again metal ions are involved.

Comprehensive kinetic experiments,[74,113,160] carried out mainly on the metal ion-facilitated hydrolysis of ATP and other NTPs, revealed that two metal ions need to be coordinated to the triphosphate chain[38,113] to obtain a significant promotion of the reaction. Coordination of a single Mg^{2+}, which occurs *via* the (α),β,γ-phosphate groups, rather prevents the hydrolysis reaction whereas synergism occurs in combination with Cu^{2+} or Zn^{2+} and the system becomes highly reactive.[39] Combination of all the results leads to the conclusion[38] that binding of two metal ions in a M(α,β)-M(γ)-type fashion, which occurs at a triphosphate chain (if the metal ion concentration is large enough) without any additional enforcement by a third partner (enzyme), gives rise to transphosphorylations and it is this metal ion pattern which is relevant for kinases and related enzymes; this coordination pattern is depicted in the upper part of Fig. 9.[161,162] Of course, one of the two metal ions could be replaced by an ionic interaction, *e.g.* with an arginyl group, and a reactive intermediate would still result,[38,163] especially under conditions of a low polarity, *i.e.* in a hydrophobic environment.[139] The M(α)-M(β,γ)-type coordination pattern[38,164] which is shown in the lower part of Fig. 9, needs to be enforced by the enzyme (see legend to Fig. 9); this pattern,

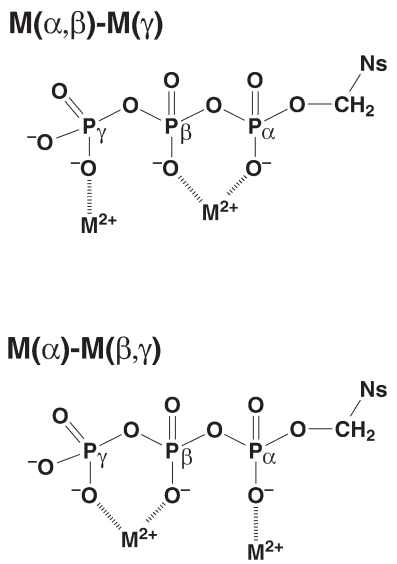


Fig. 9 Simplified structures of two M_2(NTP) complexes. Once with an M(α,β)-M(γ)-coordination mode (upper part) relevant for transphosphorylations (kinases, *etc.*), and once with an M(α)-M(β,γ)-type mode (lower part) relevant for the transfer of a nucleotidyl unit as catalyzed by polymerases (see also Fig. 10). This latter binding mode needs to be enforced by the enzyme, *i.e.* both metal ions are anchored[38,113] to amino acid side chains, often carboxylate groups of aspartate or glutamate units of the enzyme[24,161,162] (CH_2–Ns = nucleosidyl residue).

which facilitates the break between P_α and P_β, allows the transfer of a diphosphoryl or a nucleotidyl group, the latter being relevant for nucleic acid polymerases.

The described transphosphorylation mechanism was confirmed years later by an X-ray structural study[40] of *Escherichia coli* phosphoenolpyruvate carboxykinase. Similarly, X-ray structural studies of nucleic acid polymerases also verified the involvement of two metal ions and mechanisms similar to the one indicated in the lower part of Fig. 9 were proposed.[24,161,162] The crucial step in the polymerase reaction indicated above is to force a metal ion into the α-position of the triphosphate chain[38,51,164] of a (d)NTP (Fig. 9, lower part).

In the above context 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA), an analogue of (2′-deoxy)-AMP^{2-}, with remarkable antiviral properties, needs to be mentioned.[165] In fact, the oral prodrug of PMEA, that is its bis(pivaloyloxymethyl)ester, also named *Adefovir dipivoxil*, *Preveon*[166] or *Hepsera*,[167] was approved in 2002 by the US Food and Drug Administration (FDA) for use in hepatitis B therapy[168] and in March 2003 it was also granted *Community Marketing* by the European Agency for the Evaluation of Medicinal Products (EMEA) for the 'treatment of chronic hepatitis B in adults'.[167] The mentioned diester was synthesized to facilitate the transport of the drug into the cell. Inside the cell the ester is hydrolyzed and the resulting $PMEA^{2-}$ is converted by cellular nucleotide kinases into its diphosphate,[169] $PMEApp^{4-}$, and as such it inhibits viral and to a lesser extent cellular DNA synthesis of the host.[170,171] In other words, $PMEApp^{4-}$, which is an analogue of $dATP^{4-}$ and ATP^{4-}, is recognized by nucleic acid polymerases as a substrate[165] and incorporated into the growing nucleic acid chain which is terminated thereafter due to the lack of a 3′-hydroxy group.[172,173]

As indicated above, $PMEApp^{4-}$ is initially a substrate for several polymerases[170,173,174] and indeed, an excellent one. For example, even in the presence of a 20-fold excess of dATP, *in vitro* DNA synthesis by avian myeloblastosis-virus reverse-transcriptase is depressed to 50% within 5 minutes.[170,174] This observation is astonishing and the question arises why $PMEApp^{4-}$ is initially such an effective substrate. The reason is that M_2(PMEApp) is formed in a facilitated manner compared to M_2(dATP) or M_2(ATP) as is evident from a comparison of the structures shown in Fig. 10 and in the lower part of Fig. 9, respectively.[51,164,175] The ether oxygen present in PMEA facilitates metal ion binding to the α-phosph(on)ate

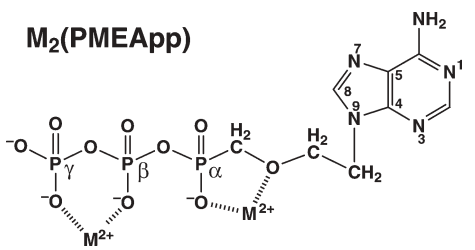


Fig. 10 Simplified structure of the M_2(PMEApp) intermediate. Note, the M(α)-M(β,γ)-binding mode, which is crucial for the polymerase reaction, is favored due to the formation of the 5-membered chelate with the ether oxygen of the aliphatic chain.[51,164] See also the situation in the parent complex, M_2[(d)NTP], shown in the lower part of Fig. 9.

group due to the formation of a 5-membered chelate ring.[164,175] This together with the increased basicity of the α group (phosphonates are more basic than phosphates)[176] leads to a higher formation degree of the needed M(α)-M(β,γ)-type binding mode of the metal ions making $PMEApp^{4-}$ a better substrate than $(d)ATP^{4-}$ for the polymerase reaction.[51,164] At the same time the formation of such a 5-membered chelate should make the transfer of a phosphoryl group more difficult. Indeed, there are indications in the literature, given unfortunately without experimental details,[177] that $PMEApp^{4-}$ is a somewhat poorer substrate than ATP for ATPases because for these, like for kinases,[40] a M(α,β)-M(γ) metal ion-coordination pattern is needed.[38]

8 Concluding remarks

At this point it is interesting to compare the metal ion-binding properties of mono-, di-, and triphosphate monoesters directly with each other in a quantitative way for a whole series of complexes. To this end we selected for the calculations, summarized in Table 13, pK_a values which are representative for pyrimidine-nucleoside phosphates; *i.e.*, $pK^{H}_{H(R\text{-}MP)} = 6.20$ for monophosphate monoesters,[36,90] $pK^{H}_{H(R\text{-}DP)} = 6.40$ for diphosphate monoesters,[34] and $pK^{H}_{H(R\text{-}TP)} = 6.50$ for triphosphate monoesters.[29,49,80] Application of the first mentioned pK_a value to the straight-line equations for M(R-MP) complexes[78,178] gives the results in column 2 of Table 13; column 3 lists the results based on the equations given in ref. 34 for the $M(R\text{-}DP)^{-}$ complexes, and column 4 provides the values for the $M(R\text{-}TP)^{2-}$ species (see also Table 3, lower part).[7,49]

To obtain a better overview, the results of Table 13 are plotted in an Irving–Williams sequence-type fashion in Fig. 11. The figure confirms the previous observation that phosphate complexes do not strictly follow the Irving–Williams series as already discussed in Section 4.2. It also shows that addition of a further phosphate unit to $R\text{-}MP^{2-}$, giving $R\text{-}DP^{3-}$, increases the stability of the complexes by approximately 1.1 to 2.4 log units, the effect being especially pronounced for Cu^{2+}. The addition of one more phosphate unit, giving $R\text{-}TP^{4-}$, has a somewhat smaller effect but the stability increase is still on the order of nearly 1 log unit throughout, with the exception of Cu^{2+} where it only reaches 0.6 log units; the latter observation is certainly connected with the Jahn–Teller distorted octahedral coordination sphere of Cu^{2+} which allows strong coordination only at the equatorial but not at the apical positions of the coordination sphere.

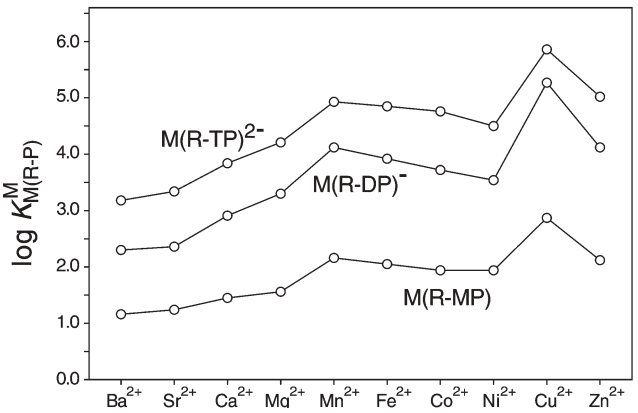


Fig. 11 Irving–Williams sequence-type plots for the 1:1 complexes of Ba^{2+} through Zn^{2+} formed with mono- ($R\text{-}MP^{2-}$), di- ($R\text{-}DP^{3-}$), and triphosphate monoesters ($R\text{-}TP^{4-}$) (= R-P). The plotted data are from Table 13; they also represent the stability constants for the M^{2+} complexes of the pyrimidine-nucleoside 5′-mono-, di-, or triphosphate [except for $Cu(CTP)^{2-}$; see Section 4.2] (25 °C; I = 0.1 M, $NaNO_3$).

The fact that the stability increase of the complexes varies significantly from metal ion to metal ion only by going from M(R-MP) to $M(R\text{-}DP)^{-}$, *i.e.*, within the large span from 1.1 to 2.4 log units, whereas it is quite constant from $M(R\text{-}DP)^{-}$ to $M(R\text{-}TP)^{2-}$ (if the mentioned special case of Cu^{2+} is ignored), *i.e.*, it stays within the narrow range of 0.8 to 1.0 log units, indicates in our view that outersphere species play a significant role in the M(R-MP) complexes[90] (see also the legend to Fig. 6), but hardly in the corresponding di- and triphosphate species.

Of course, for the complexes formed with the purine-nucleotides the stability constants are usually somewhat larger due to macrochelate formation (see equilibrium (15), the log $\Delta_{M/NTP}$ values in Table 5, and Section 4.4). In this context the IUPAC publication[30] on 'Stability Constants for Nucleotide Complexes with Protons and Metal Ions' needs to be mentioned. This compilation, as well as several others[31,32] are very helpful for finding access to the literature regarding equilibrium constants and (in part) their connected enthalpy changes. However, great care should be exercised with regard to the advice given in this publication[30] (see also ref. 32), *i.e.*, differentiating between the values which are *recommended* and those not *recommended*. To give just a single example: … ''the values of … (references) … are tentatively recommended for Cd(5′-dTMP). The value of … (reference) … for Cd(5′-GMP) *is much larger than the above values* and is not recommended''.

Table 13 Comparison of the stability constants (analogous to eqns (12) and (24)) of M^{2+} complexes formed with mono- ($R\text{-}MP^{2-}$), di- ($R\text{-}DP^{3-}$), and triphosphate monoesters ($R\text{-}TP^{4-}$) in aqueous solution at 25 °C and I = 0.1 M ($NaNO_3$)[a]

M^{2+}	log $K^{M}_{M(R\text{-}MP)}$[b]	log $K^{M}_{M(R\text{-}DP)}$[c]	log $K^{M}_{M(R\text{-}TP)}$[d]
Ba^{2+}	1.16 ± 0.04	2.30 ± 0.03	3.18 ± 0.04
Sr^{2+}	1.24 ± 0.04	2.36 ± 0.04	3.34 ± 0.05
Ca^{2+}	1.45 ± 0.05	2.91 ± 0.03	3.84 ± 0.05
Mg^{2+}	1.56 ± 0.03	3.30 ± 0.03	4.21 ± 0.04
Mn^{2+}	2.16 ± 0.05	4.12 ± 0.03	4.93 ± 0.03
Fe^{2+}	2.05 ± 0.10[e]	3.92 ± 0.10[e]	4.85 ± 0.10[e]
Co^{2+}	1.94 ± 0.06	3.72 ± 0.05	4.76 ± 0.03
Ni^{2+}	1.94 ± 0.05	3.54 ± 0.06	4.50 ± 0.03
Cu^{2+}	2.87 ± 0.06	5.27 ± 0.04	5.86 ± 0.03
Zn^{2+}	2.12 ± 0.06	4.12 ± 0.03	5.02 ± 0.02
$pK^{H}_{H(R\text{-}P)}$	6.20	6.40	6.50

[a] For the error limits see footnote ''*a*'' of Table 3. [b] Calculated with the $pK^{H}_{H(R\text{-}P)} = pK^{H}_{H(R\text{-}MP)}$ value given in the bottom row of the above table and the straight-line equations listed in Table 5 (and Table 6; error limits) of ref. 178 or in Table 3 of ref. 78. [c] From Table 7 of ref. 34. [d] The values for the alkaline earth ion complexes are from Table 2 of ref. 7 and all the others from Table IV of ref. 49. [e] The values for the Fe^{2+} complexes are estimates as are the error limits; they are taken from the terminating paragraph of ref. 34.

For the reader who recalls Sections 4.2 to 4.4 and the results discussed in connection with equilibrium (15) the apparent discrepancy is quite clear: The stability of the Cd^{2+} complexes with the three pyrimidine-nucleoside 5′-monophosphates is solely determined by the basicity of the corresponding phosphate groups, *i.e.*, there is *no* nucleobase–metal ion interaction, while the stability of Cd(5′-GMP) is significantly increased, *i.e.*, log $\Delta_{Cd/5'\text{-GMP}} = 0.79 \pm 0.06$,[36] owing to considerable nucleobase-backbinding to N7 of the phosphate-coordinated Cd^{2+}; indeed, $Cd(5'\text{-GMP})_{cl}$ is formed to 82 ± 2%.[36] It is evident that users of stability constant-compilations[30–32] have to make their own judgments in selecting stability constants to prevent being misguided! It is the hope that the values listed in this review are useful to facilitate such selections.

One may also recall in this context that 1 log unit of a stability constant corresponds approximately to a change in free energy (ΔG^0) of 5.7 kJ mol^{-1} at 25 °C.[179] Clearly, the high energy binding sites of the phosphate residue of purine-nucleotides are in contrast to the weak structuring interactions as they occur with N7 of the purine moieties: A stability difference log $\Delta_{M/NP}$ of 0.1 log unit gives rise to a formation degree of about 20% for the macrochelated $M(NP)_{cl}$ species (see equilibrium (15)), yet the change in free energy involved, which creates the special structure, corresponds only to about 0.6 kJ mol^{-1}.[179] On the other hand, it is evident that if 20% of a substrate are in the correct conformation/orientation needed by the enzyme for a reaction, this is more than enough, especially as equilibration is fast, with all the naturally important metal ions.

Finally, it needs to be emphasized that many questions are still open as far as the coordination chemistry of nucleotides is concerned. For example, it has already been indicated that there are hints that outersphere–innersphere equilibria occur for the binding of metal ions to (a) phosphate-group(s) (see also the legend to Fig. 6). Similarly, the outersphere–innersphere equilibria involving N7 are only rudimentarily understood and quantified (Section 4.4). Here evidently detailed studies are highly desirable.

Another point refers to the metal ion-binding properties of artificially altered nucleotides: For example, quite a bit of information is now available on antivirally active acyclic nucleotide analogues (see Section 7)[51,96,120,148,158,164,175,178] or on 1,N^6-ethenoadenosine phosphates (ε-APs),[70–74,106] whereas our knowledge is very scarce, *e.g.*, on thiophosphate derivatives: A few data of Mg^{2+} and Cd^{2+} complexes of thiophosphate derivatives of ADP and ATP are available[180] and the stability constants for a series of metal ion complexes formed with methyl thiophosphate, uridine 5′-*O*-thiomonophosphate ($UMPS^{2-}$)[181,182] and adenosine 5′-*O*-thiomonophosphate[183] have been measured. Also here more work is needed since again isomeric equilibria exist: *e.g.*, Mg^{2+} is nearly to 100% oxygen-coordinated in its Mg(UMPS) complex, whereas Zn^{2+} is to about 75% sulfur-bound in Zn(UMPS), the remaining 25% being oxygen-coordinated, and Cd^{2+} exists even to nearly 100% as the sulfur-bonded isomer in Cd(UMPS), and it needs to be mentioned that this Cd^{2+}-sulfur interaction gives rise to a stability increase of about 2.4 log units compared to the situation when only oxygen-binding occurs.[182] Such information is totally missing at present for nucleoside-triphosphate complexes but it is urgently needed considering the prominent role that these and related types of complexes play in the so-called antisense strategy.[184]

Acknowledgements

The competent technical assistance of Mrs. Rita Baumbusch and Mrs. Astrid Sigel in the preparation of this review and the support of our research on nucleotides over many years by the Swiss National Science Foundation as well as more recently on antivirally active nucleotide analogues (Section 7) within the COST D8 and D20 programmes by the State Secretariat for Education & Research are gratefully acknowledged.

References

1 J. M. Wood, *Naturwissenschaften*, 1975, **62**, 357–364.
2 J. J. R. Fraústo da Silva and R. J. P. Williams, *The Biological Chemistry of the Elements*; Clarendon Press; Oxford; 1991, pp. 1–561.
3 (*a*) A. Sigel and H. Sigel, (eds), *Metal Ions in Biological Systems*; **Volumes 1–42**. M. Dekker, Inc.; New York; 1973–2004; (*b*) A. Sigel, H. Sigel and R. K. O. Sigel, (eds), *Metal Ions in Biological Systems*; **Volumes 43** and **44**; Taylor & Francis; Boca Raton; 2005; (*c*) A. Sigel, H. Sigel and R. K. O. Sigel, (eds), *Neurodegenerative Diseases and Metal Ions*, **Vol. 45** of *Metal Ions in Biological Systems*; Wiley & Sons; Chichester, UK; to be published 2006.
4 H. Sigel, *Communications, The Journal of the European Science Foundation*, 1991, No 24 (April), 16–17.
5 A. Sigel and H. Sigel, (eds), *Interactions of Metal Ions with Nucleotides, Nucleic Acids and Their Constituents*; **Vol. 32** of *Metal Ions in Biological Systems*; M. Dekker, Inc.; New York; 1996, pp. 1–814.
6 H. Sigel, in *Metal–DNA Chemistry*, (T. D. Tullius, ed); *ACS Symposium Series*, 402; American Chemical Society; Washington, DC; 1989, 159–204.
7 H. Sigel, E. M. Bianchi, N. A. Corfù, Y. Kinjo, R. Tribolet and R. B. Martin, *Chem. Eur. J.*, 2001, **7**, 3729–3737.
8 P. D. Boyer, *Biochemistry*, 1987, **26**, 8503–8507.
9 A. Surprenant and R. J. Evans, *Nature*, 1993, **362**, 211–212.
10 H. Sigel and N. A. Corfù, *Eur. J. Biochem.*, 1996, **240**, 508–517.
11 R. J. Evans, V. Derkach and A. Surprenant, *Nature*, 1992, **357**, 503–505.
12 C. Y. Li, R. W. Peoples, Z. W. Li and F. F. Weight, *Proc. Nat. Acad. Sci. USA*, 1993, **90**, 8264–8267.
13 B. E. C. Bank, *Chem. Brit.*, 1996, **32**, May, 28.
14 (*a*) G. Schatz, *FEBS Lett.*, 2004, **572**, 1–2; (*b*) J. Garzon, I. De Antonio and P. Sanchez-Blazquez, *Methods Enzymol. B*, 2000, **314B**, 3–20; (*c*) L. Dryer and A. Berghard, *Trends Pharmacol. Sci.*, 1999, **20**, 413–417; (*d*) K. Kaibuchi, S. Kuroda and A. Amano, *Annu. Rev. Biochem.*, 1999, **68**, 459–486.
15 C. A. Parent and P. N. Devreotes, *Science*, 1999, **284**, 765–770.
16 E. Cabib, J. Drgonova and T. Drgon, *Annu. Rev. Biochem.*, 1998, **67**, 307–333.
17 F. Schimmoller, I. Simon and S. R. Pfeffer, *J. Biol. Chem.*, 1998, **273**, 22161–22164.
18 H. A. Lester and A. Karschin, *Annu. Rev. Neurosci.*, 2000, **23**, 89–125.
19 J. W. Gysbers, S. Guarnieri, M. A. Mariggio, T. Pietrangelo, G. Fano and M. P. Rathbone, *Neuroscience*, 2000, **96**, 817–824.
20 E. L. Watson, *Crit. Rev. Oral Biol. Med.*, 1999, **10**, 284–306.
21 J. W. Arndt, W. Gong, X. Zhong, A. K. Showalter, J. Liu, C. A. Dunlap, Z. Lin, C. Paxson, M.-D. Tsai and M. K. Chan, *Biochemistry*, 2001, **40**, 5368–5375.
22 P. H. Patel and L. A. Loeb, *Nature Struct. Biol.*, 2001, **8**, 656–659.
23 H. Sigel and A. Sigel, (eds), *Interrelations Among Metal Ions, Enzymes, and Gene Expression*, **Vol. 25** of *Metal Ions in Biological Systems*; M. Dekker, Inc., New York; 1989, pp. 1–557.
24 T. A. Steitz, *J. Biol. Chem.*, 1999, **274**, 17395–17398.

25 (*a*) A. Matte and L. T. J. Delbaere, in *Handbook on Metalloproteins*, (I. Bertini, A. Sigel and H. Sigel, eds); M. Dekker, Inc.; New York; 2001, pp. 59–91; (*b*) H. Sigel and A. Sigel, (eds), *Compendium on Magnesium and Its Role in Biology, Nutrition, and Physiology*, **Vol. 26** of *Metal Ions in Biological Systems*; M. Dekker, Inc.; New York; 1990, pp. 1–744; (*c*) A. E. Remmers and R. R. Neubig, *J. Biol. Chem.*, 1996, **271**, 4791–4797; (*d*) B. Flachner, Z. Kovari, A. Varga, Z. Gugolya, F. Vonderviszt, G. Naray-Szabo and M. Vas, *Biochemistry*, 2004, **43**, 3436–3449.

26 (*a*) J. D. Crowley, D. A. Traynor and D. C. Weatherburn, *Met. Ions Biol. Syst.*, 2000, **37**, 209–278; (*b*) T. Schweins, K. Scheffzek, R. Assheuer and A. Wittinghofer, *J. Mol. Biol.*, 1997, **266**, 847–856; (*c*) D. C. Weatherburn, in *Handbook on Metalloproteins*, (I. Bertini, A. Sigel and H. Sigel, eds); M. Dekker, Inc.; New York; 2001, pp. 193–268.

27 (*a*) F. Y.-H. Wu and C.-W. Wu, *Met. Ions Biol. Syst.*, 1983, **15**, 157–192; (*b*) G. Auerbach, A. Herrmann, A. Bracher, G. Bader, M. Gütlich, M. Fischer, M. Neukamm, M. Garrido-Franco, J. Richardson, H. Nar, R. Huber and A. Bacher, *Proc. Nat. Acad. Sci. USA*, 2000, **97**, 13567–13572.

28 A. Szent-Györgyi, in *Enzymes, Units of Biological Structure and Function*, (O. H. Gaebler, ed); Academic Press; New York; 1956, pp. 393–397.

29 H. Sigel, *Eur. J. Biochem.*, 1987, **165**, 65–72.

30 R. M. Smith, A. E. Martell and Y. Chen, *Pure Appl. Chem.*, 1991, **63**, 1015–1080.

31 (*a*) IUPAC Stability Constants Database; Release 5, Version 5.16; Compiled by L. D. Pettit and H. K. J. Powell, Academic Software; Timble, Otley, West Yorkshire, UK; 2001; (*b*) Joint Expert Speciation System (JESS); Version 6.4; Joint venture by K. Murray and P. M. May, Pretoria, South Africa, and Murdoch, Western Australia, Division of Water Technology, CSIR, and School of Mathematic and Physical Sciences, Murdoch University, 2001.

32 NIST Critically Selected Stability Constants of Metal Complexes; Reference Database 46, Version 6.0; Compiled by R. M. Smith and A. E. Martell, US Department of Commerce, National Institute of Standards and Technology; Gaithersburg, MD, USA; 2001.

33 E. M. Bianchi, S. A. A. Sajadi, B. Song and H. Sigel, *Chem. Eur. J.*, 2003, **9**, 881–892.

34 S. A. A. Sajadi, B. Song, F. Gregáň and H. Sigel, *Inorg. Chem.*, 1999, **38**, 439–448.

35 (*a*) H. Sigel, *Chem. Soc. Rev.*, 1993, **22**, 255–267; (*b*) H. Sigel, in *Handbook of Metal–Ligand Interactions in Biological Fluids. Bioinorganic Chemistry*, (G. Berthon, ed); **Vol. 1**; M. Dekker, Inc.; New York; 1995, pp. 451–465; (*c*) C. Klevickis and C. M. Grisham, *Met. Ions Biol. Syst.*, 1996, **32**, 1–26.

36 H. Sigel and B. Song, *Met. Ions Biol. Syst.*, 1996, **32**, 135–205.

37 H. Sigel, *Pure Appl. Chem.*, 2004, **76**, 375–388.

38 H. Sigel, *Coord. Chem. Rev.*, 1990, **100**, 453–539.

39 H. Sigel, *Inorg. Chim. Acta*, 1992, **198–200**, 1–11.

40 L. W. Tari, A. Matte, H. Goldie and L. T. J. Delbaere, *Nature Struct. Biol.*, 1997, **4**, 990–994.

41 H. Sigel, *Pure Appl. Chem.*, 1998, **70**, 969–976.

42 W. Saenger, *Principles of Nucleic Acid Structure*; Springer Verlag, New York, Berlin; 1984, pp. 1–556.

43 R. B. Martin and Y. H. Mariam, *Met. Ions Biol. Syst.*, 1979, **8**, 57–124.

44 D. B. Davies, P. Rajani and H. Sadikot, *J. Chem. Soc., Perkin Trans. 2*, 1985, 279–285.

45 R. Tribolet and H. Sigel, *Eur. J. Biochem.*, 1987, **163**, 353–363.

46 K. Aoki, *Met. Ions Biol. Syst.*, 1996, **32**, 91–134.

47 N. A. Corfù, R. Tribolet and H. Sigel, *Eur. J. Biochem.*, 1990, **191**, 721–735.

48 N. A. Corfù and H. Sigel, *Eur. J. Biochem.*, 1991, **199**, 659–669.

49 H. Sigel, R. Tribolet, R. Malini-Balakrishnan and R. B. Martin, *Inorg. Chem.*, 1987, **26**, 2149–2157.

50 B. Knobloch and H. Sigel, *J. Biol. Inorg. Chem.*, 2004, **9**, 365–373.

51 H. Sigel, *Chem. Soc. Rev.*, 2004, **33**, 191–200.

52 R. B. Martin, *Acc. Chem Res.*, 1985, **18**, 32–38.

53 J. D. Watson, *The Double Helix*; Atheneum Publisher; New York; 1968, Chapter 26.

54 G. Kampf, L. E. Kapinos, R. Griesser, B. Lippert and H. Sigel, *J. Chem. Soc., Perkin Trans. 2*, 2002, 1320–1327. There is a typing error in this ref.: It should read on page 1324 in the right hand column in line 8 "showing that the amino form of adenosine is strongly favored".

55 H. Sigel, *Pure Appl. Chem.*, 2004, **76**, 1869–1886.

56 B. Lippert, H. Schöllhorn and U. Thewalt, *Inorg. Chim. Acta*, 1992, **198–200**, 723–732.

57 (*a*) B. Lippert, (ed), *Cisplatin, Chemistry and Biochemistry of a Leading Anticancer Drug*; VHCA and Wiley-VCH; Zürich and Weinheim; 1999, pp. 1–563; (*b*) A. Sigel and H. Sigel, (eds), *Metal Complexes in Tumor Diagnosis and as Anticancer Agents*; **Vol. 42** of *Metal Ions in Biological Systems*; Marcel Dekker, Inc.; New York; 2004, pp. 1–534.

58 P. O. P. Ts'o, I. S. Melvin and A. C. Olson, *J. Am. Chem. Soc.*, 1963, **85**, 1289–1296.

59 P. R. Mitchell and H. Sigel, *Eur. J. Biochem.*, 1978, **88**, 149–154.

60 M. P. Schweizer, A. D. Broom, P. O. P. Ts'o and D. P. Hollis, *J. Am. Chem. Soc.*, 1968, **90**, 1042–1055.

61 K. J. Neurohr and H. H. Mantsch, *Can. J. Chem.*, 1979, **57**, 1986–1994.

62 (*a*) R. Bretz, A. Lustig and G. Schwarz, *Biophys. Chem.*, 1974, **1**, 237–241; (*b*) H. Sapper and W. Lohmann, *Biophys. Struct. Mechanism*, 1978, **4**, 327–335; (*c*) D. M. Cheng, L. S. Kan, P. O. P. Ts'o, C. Giessner-Prettre and B. Pullman, *J. Am. Chem. Soc.*, 1980, **102**, 525–534.

63 K. H. Scheller, F. Hofstetter, P. R. Mitchell, B. Prijs and H. Sigel, *J. Am. Chem. Soc.*, 1981, **103**, 247–260.

64 K. H. Scheller and H. Sigel, *J. Am. Chem. Soc.*, 1983, **105**, 5891–5900.

65 (*a*) R. B. Martin, *Chem. Rev.*, 1996, **96**, 3043–3064; (*b*) R. B. Martin, *Biophys. Chem.*, 2004, **109**, 59–61.

66 (*a*) J.-L. Dimicoli and C. Hélène, *J. Am. Chem. Soc.*, 1973, **95**, 1036–1044; (*b*) M. P. Heyn and R. Bretz, *Biophys. Chem.*, 1975, **3**, 35–45.

67 P. R. Mitchell, *J. Chem. Soc., Dalton Trans.*, 1980, 1079–1086.

68 R. Tribolet and H. Sigel, *Eur. J. Biochem.*, 1988, **170**, 617–626.

69 R. Tribolet and H. Sigel, *Biophys. Chem.*, 1987, **27**, 119–130.

70 H. Sigel, *Chimia*, 1987, **41**, 11–26.

71 H. Sigel, K. H. Scheller, V. Scheller-Krattiger and B. Prijs, *J. Am. Chem. Soc.*, 1986, **108**, 4171–4178.

72 T. A. Kaden, K. H. Scheller and H. Sigel, *Inorg. Chem.*, 1986, **25**, 1313–1315.

73 K. H. Scheller and H. Sigel, *Eur. J. Biochem.*, 1986, **157**, 147–153.

74 V. Scheller-Krattiger and H. Sigel, *Inorg. Chem.*, 1986, **25**, 2628–2634.

75 P. K. Glasoe and F. A. Long, *J. Phys. Chem.*, 1960, **64**, 188–190.

76 R. B. Martin, *Science*, 1963, **139**, 1198–1203.

77 H. Sigel, K. H. Scheller and R. M. Milburn, *Inorg. Chem.*, 1984, **23**, 1933–1938.

78 H. Sigel, S. S. Massoud and N. A. Corfù, *J. Am. Chem. Soc.*, 1994, **116**, 2958–2971.

79 O. Yamauchi, A. Odani, H. Masuda and H. Sigel, *Met. Ions Biol. Syst.*, 1996, **32**, 207–270.

80 H. Sigel, E. M. Bianchi, N. A. Corfù, Y. Kinjo, R. Tribolet and R. B. Martin, *J. Chem. Soc., Perkin Trans. 2*, 2001, 507–511.

81 (*a*) Z.-y. Zhao, A. McLeod, S. Harusawa, L. Araki, M. Yamaguchi, T. Kurihara and D. M. J. Lilley, *J. Am. Chem. Soc.*, 2005, **127**, 5026–5027; (*b*) R. K. O. Sigel, *Eur. J. Inorg. Chem.*, 2005, 2281–2292.

82 H. Sigel and N. A. Corfù, *Naturwissenschaften*, 1995, **82**, 237–238.

83 (*a*) A. Pletscher, M. Da Prada, K. H. Berneis, H. Steffen, B. Lütold and H. G. Weder, *Adv. Cytopharmacol.*, 1974, **2**, 257–264; (*b*) J. H. Phillips, Y. P. Allison and S. J. Morris, *Neuroscience*, 1977, **2**, 147–152; (*c*) H. Winkler and E. Westhead, *Neuroscience*, 1980, **5**, 1803–1823.

84 M.-a. Morikawa, M. Yashihara, T. Endo and N. Kimizuka, *J. Am. Chem. Soc.*, 2005, **127**, 1358–1359.

85 R. Tribolet, R. Malini-Balakrishnan and H. Sigel, *J. Chem. Soc., Dalton Trans.*, 1985, 2291–2303.

86 C. Meiser, B. Song, E. Freisinger, M. Peilert, H. Sigel and B. Lippert, *Chem. Eur. J.*, 1997, **3**, 388–398.

87 M. Bastian and H. Sigel, *Biophys. Chem.*, 1997, **67**, 27–34.

88 R. B. Martin, *Met. Ions Biol. Syst.*, 1996, **32**, 61–89.

89 (*a*) J. J. Christensen, J. H. Rytting and R. M. Izatt, *J. Chem. Soc. B*, 1970, 1643–1646 and 1646–1648; (*b*) J. J. Christensen, J. H. Rytting and R. M. Izatt, *Biochemistry*, 1970, **9**, 4907–4913.
90 S. S. Massoud and H. Sigel, *Inorg. Chem.*, 1988, **27**, 1447–1453.
91 H. Sigel, A. D. Zuberbühler and O. Yamauchi, *Analyt. Chim. Acta*, 1991, **255**, 63–72.
92 H. Sigel, S. S. Massoud and R. Tribolet, *J. Am. Chem. Soc.*, 1988, **110**, 6857–6865.
93 Y. Kinjo, L.-n. Ji, N. A. Corfù and H. Sigel, *Inorg. Chem.*, 1992, **31**, 5588–5596.
94 S. S. Massoud and H. Sigel, *Eur. J. Biochem.*, 1989, **179**, 451–458.
95 C. F. Moreno-Luque, E. Freisinger, B. Costisella, R. Griesser, J. Ochocki, B. Lippert and H. Sigel, *J. Chem. Soc., Perkin Trans. 2*, 2001, 2005–2011.
96 A. Fernández-Botello, R. Griesser, A. Holý, V. Moreno and H. Sigel, *Inorg. Chem.*, 2005, **44**, 5104–5117.
97 H. M. Irving and R. J. P. Williams, *J. Chem. Soc.*, 1953, 3192–3210.
98 H. Sigel and D. B. McCormick, *Acc. Chem. Res.*, 1970, **3**, 201–208.
99 H. Sigel, C. P. Da Costa and R. B. Martin, *Coord. Chem. Rev.*, 2001, **219–221**, 435–461.
100 (*a*) R. B. Martin, *Met. Ions Biol. Syst.*, 1986, **20**, 21–65; (*b*) R. B. Martin, in *Encyclopedia of Inorganic Chemistry*, (R. B. King, ed); **Vol. 4**; John Wiley; Chichester; 1994, pp. 2185–2196; (*c*) R. B. Martin, in *Encyclopedia of Molecular Biology and Molecular Medicine*, (R. A. Meyer, ed); **Vol. 1**; VCH; Weinheim; 1996, pp. 125–134.
101 R. B. Martin and H. Sigel, *Comments Inorg. Chem.*, 1988, **6**, 285–314.
102 L. E. Kapinos and H. Sigel, *Inorg. Chim. Acta*, 2002, **337**, 131–142.
103 L. E. Kapinos, A. Holý, J. Günter and H. Sigel, *Inorg. Chem.*, 2001, **40**, 2500–2508.
104 (*a*) R. Griesser, G. Kampf, L. E. Kapinos, S. Komeda, B. Lippert, J. Reedijk and H. Sigel, *Inorg. Chem.*, 2003, **42**, 32–41; (*b*) M. Garijo Añorbe, M. S. Lüth, M. Roitzsch, M. Morell Cerdà, P. Lax, G. Kampf, H. Sigel and B. Lippert, *Chem. Eur. J.*, 2004, **10**, 1046–1057; (*c*) B. Knobloch, R. K. O. Sigel, B. Lippert and H. Sigel, *Angew. Chem. Int. Ed.*, 2004, **43**, 3793–3795.
105 (*a*) H. Sigel, *Eur. J. Biochem.*, 1968, **3**, 530–537; (*b*) H. Sigel, *J. Am. Chem. Soc.*, 1975, **97**, 3209–3214.
106 H. Sigel and K. H. Scheller, *Eur. J. Biochem.*, 1984, **138**, 291–299.
107 H. Takeuchi, H. Murata and I. Harada, *J. Am. Chem. Soc.*, 1988, **110**, 392–397.
108 H. Brintzinger and G. G. Hammes, *Inorg. Chem.*, 1966, **5**, 1286–1287.
109 (*a*) G. P. P. Kuntz and G. Kotowycz, *Biochemistry*, 1975, **14**, 4144–4150; (*b*) In ref. 109a it is concluded that, in about 11% of the $Mn(ITP)^{2-}$ species, Mn^{2-}–nucleobase innersphere binding occurs. The problem with this study is that high concentrations of ITP were used (up to 0.3 M), and this means that under these conditions self-stacking of ITP also occurs (ref. 63).
110 R. Tribolet, R. B. Martin and H. Sigel, *Inorg. Chem.*, 1987, **26**, 638–643.
111 H. Sigel, R. Tribolet and O. Yamauchi, *Comments Inorg. Chem.*, 1990, **9**, 305–330.
112 P. R. Mitchell, B. Prijs and H. Sigel, *Helv. Chim. Acta*, 1979, **62**, 1723–1735.
113 H. Sigel, F. Hofstetter, R. B. Martin, R. M. Milburn, V. Scheller-Krattiger and K. H. Scheller, *J. Am. Chem. Soc.*, 1984, **106**, 7935–7946.
114 H. Sigel, B. E. Fischer and E. Farkas, *Inorg. Chem.*, 1983, **22**, 925–934.
115 (*a*) P. Orioli, R. Cini, D. Donati and S. Mangani, *J. Am. Chem. Soc.*, 1981, **103**, 4446–4452; (*b*) W. S. Sheldrick, *Angew., Chem. Int. Ed. Engl.*, 1981, **20**, 460–461; (*c*) W. S. Sheldrick, *Z. Naturforsch. B*, 1982, **37B**, 863–871.
116 H. Sigel, *Angew. Chem., Int. Ed. Engl.*, 1975, **14**, 394–402.
117 H. Sigel, in *Coordination Chemistry–20*, (D. Banerjea, ed); IUPAC through Pergamon Press; Oxford, 1980, pp. 27–45.
118 B. E. Fischer and H. Sigel, *J. Am. Chem. Soc.*, 1980, **102**, 2998–3008.
119 P. R. Mitchell and H. Sigel, *J. Am. Chem. Soc.*, 1978, **100**, 1564–1570.
120 R. B. Gómez-Coca, L. E. Kapinos, A. Holý, R. A. Vilaplana, F. González-Vílchez and H. Sigel, *J. Inorg. Biochem.*, 2001, **84**, 39–46.
121 (*a*) H. Sigel, B. E. Fischer and B. Prijs, *J. Am. Chem. Soc.*, 1977, **99**, 4489–4496; (*b*) H. Sigel, *Inorg. Chem.*, 1980, **19**, 1411–1413; (*c*) C. P. Da Costa, B. Song, F. Gregáň and H. Sigel, *J. Chem. Soc., Dalton Trans.*, 2000, 899–904.
122 H. Sigel and R. B. Martin, *Chem. Soc. Rev.*, 1994, **23**, 83–91.
123 B. E. Fischer, U. K. Häring, R. Tribolet and H. Sigel, *Eur. J. Biochem.*, 1979, **94**, 523–530.
124 K. H. Scheller, T. H. J. Abel, P. E. Polanyi, P. K. Wenk, B. E. Fischer and H. Sigel, *Eur. J. Biochem.*, 1980, **107**, 455–466.
125 N. A. Corfù, B. Song and L.-n. Ji, *Inorg. Chim. Acta*, 1992, **192**, 243–251.
126 N. E. Good, G. D. Winget, W. Winter, T. N. Connolly, S. Izawa and R. M. M. Singh, *Biochemistry*, 1966, **5**, 467–477.
127 N. Saha and H. Sigel, *J. Am. Chem. Soc.*, 1982, **104**, 4100–4105.
128 H. Sigel, *Coord. Chem. Rev.*, 1993, **122**, 227–242.
129 H. Sigel, K. H. Scheller and B. Prijs, *Inorg. Chim. Acta*, 1982, **66**, 147–155.
130 E. M. Bianchi, S. A. A. Sajadi, B. Song and H. Sigel, *Inorg. Chim. Acta*, 2000, **300–302**, 487–498.
131 (*a*) B. Albert, D. Bray, J. Lewis, M. Raff, K. Roberts and J. D. Watson, *Molecular Biology of the Cell*, (3rd edn); Garland; New York; 1994, p. 89; (*b*) O. Yamauchi, A. Odani and M. Takani, *J. Chem. Soc., Dalton Trans.*, 2002, 3411–3421.
132 (*a*) R. K. O. Sigel, E. Freisinger, M. Abbate and B. Lippert, *Inorg. Chim. Acta*, 2002, **339**, 355–365; (*b*) R. K. O. Sigel, S. M. Thompson, E. Freisinger, F. Glahé and B. Lippert, *Chem. Eur. J.*, 2001, **9**, 1968–1980.
133 (*a*) H. Suezawa, T. Yoshida, Y. Umezawa, S. Tsuboyama and M. Nishio, *Eur. J. Inorg. Chem.*, 2002, 3148–3155; (*b*) Y. Umezawa and M. Nishio, *Nucleic Acids Res.*, 2002, **30**, 2183–2192.
134 C. F. Naumann and H. Sigel, *FEBS Lett.*, 1974, **47**, 122–124.
135 H. Sigel and C. F. Naumann, *J. Am. Chem. Soc.*, 1976, **98**, 730–739.
136 J. B. Orenberg, B. E. Fischer and H. Sigel, *J. Inorg. Nucl. Chem.*, 1980, **42**, 785–792.
137 (*a*) J.-J. Toulmé, *Bioinorg. Chem.*, 1978, **8**, 319–329; (*b*) R. Basosi, E. Gaggelli and E. Tiezzi, *J. Chem. Res. (S)*, 1977, 278–279.
138 G. Arena, R. Calì, V. Cucinotta, S. Musumeci, E. Rizzarelli and S. Sammartano, *J. Chem. Soc., Dalton Trans.*, 1984, 1651–1658.
139 H. Sigel, *Pure Appl. Chem.*, 1989, **61**, 923–932.
140 C. F. Naumann and H. Sigel, *J. Am. Chem. Soc.*, 1974, **96**, 2750–2756.
141 D. Chen, M. Bastian, F. Gregáň, A. Holý and H. Sigel, *J. Chem. Soc., Dalton Trans.*, 1993, 1537–1546.
142 Y. Fukuda, P. R. Mitchell and H. Sigel, *Helv. Chim. Acta*, 1978, **61**, 638–647.
143 S. A. A. Sajadi, B. Song and H. Sigel, *Inorg. Chim. Acta*, 1998, **283**, 193–201.
144 (*a*) S. S. Massoud and H. Sigel, *Chimia*, 1990, **44**, 55–57; (*b*) M. S. Lüth, L. E. Kapinos, B. Song, B. Lippert and H. Sigel, *J. Chem. Soc., Dalton Trans.*, 1999, 357–365; (*c*) M. S. Lüth, B. Song, B. Lippert and H. Sigel, *Inorg. Chem.*, 2000, **39**, 1305–1310.
145 S. S. Massoud, R. Tribolet and H. Sigel, *Eur. J. Biochem.*, 1990, **187**, 387–393.
146 M. Bastian and H. Sigel, *Inorg. Chem.*, 1997, **36**, 1619–1624.
147 H. Sigel, *Coord. Chem. Rev.*, 1995, **144**, 287–319.
148 (*a*) R. B. Gómez-Coca, L. E. Kapinos, A. Holý, R. A. Vilaplana, F. González-Vílchez and H. Sigel, *Metal-Based Drugs*, 2000, **7**, 313–324; (*b*) R. B. Gómez-Coca, A. Holý, R. A. Vilaplana, F. González-Vílchez and H. Sigel, *Eur. J. Inorg. Chem.*, 2003, 2937–2947.
149 (*a*) D. C. Rees, *J. Mol. Biol.*, 1980, **141**, 323–326; (*b*) N. K. Rogers, G. R. Moore and M. J. E. Sternberg, *J. Mol. Biol.*, 1985, **182**, 613–616.
150 H. Sigel, R. B. Martin, R. Tribolet, U. K. Häring and R. Malini-Balakrishnan, *Eur. J. Biochem.*, 1985, **152**, 187–193. See also the comment in the note on page 258 in ref. 151.
151 M. Bastian and H. Sigel, *Inorg. Chim. Acta*, 1990, **178**, 249–259.
152 J. G. Watterson, *Biochem. J.*, 1987, **248**, 615–617.
153 L. De Meis, *Biochim. Biophys. Acta*, 1989, **973**, 333–349.

154 (*a*) G. Åkerlöf and O. A. Short, *J. Am. Chem. Soc.*, 1936, **58**, 1241–1243; (*b*) F. E. Critchfield, J. A. Gibson, Jr. and J. L. Hall, *J. Am. Chem. Soc.*, 1953, **75**, 1991–1992; (*c*) G. Åkerlöf and O. A. Short, *J. Am. Chem. Soc.*, 1953, **75**, 6357.
155 M. M. Yamashita, L. Wesson, G. Eisenman and D. Eisenberg, *Proc. Natl. Acad. Sci. USA*, 1990, **87**, 5648–5652.
156 G. Liang, N. A. Corfù and H. Sigel, *Z. Naturforsch. B*, 1989, **44**, 538–542.
157 G. Liang and H. Sigel, *Inorg. Chem.*, 1990, **29**, 3631–3632.
158 D. Chen, F. Gregáň, A. Holý and H. Sigel, *Inorg. Chem.*, 1993, **32**, 5377–5384.
159 (*a*) H. Sigel, R. Malini-Balakrishnan and U. K. Häring, *J. Am. Chem. Soc.*, 1985, **107**, 5137–5148; (*b*) G. Liang and H. Sigel, *Z. Naturforsch. B*, 1989, **44**, 1555–1566.
160 (*a*) H. Sigel and P. E. Amsler, *J. Am. Chem. Soc.*, 1976, **98**, 7390–7400; (*b*) H. Sigel and F. Hofstetter, *Eur. J. Biochem.*, 1983, **132**, 569–577; (*c*) H. Sigel, *Pure Appl. Chem.*, 1983, **55**, 137–144.
161 (*a*) H. Pelletier, M. R. Sawaya, A. Kumar, S. H. Wilson and J. Kraut, *Science*, 1994, **264**, 1891–1903; (*b*) H. Pelletier, *Science*, 1994, **266**, 2025–2026; (*c*) H. Pelletier, M. R. Sawaya, W. Wolfle, S. H. Wilson and J. Kraut, *Biochemistry*, 1996, **35**, 12762–12777.
162 (*a*) T. A. Steitz, *Nature*, 1998, **391**, 231–232; (*b*) C. A. Brautigam and T. A. Steitz, *Curr. Opinion Struct. Biol.*, 1998, **8**, 54–63.
163 H. Sigel and R. Tribolet, *J. Inorg. Biochem.*, 1990, **40**, 163–179.
164 H. Sigel, *Pure Appl. Chem.*, 1999, **71**, 1727–1740.
165 E. De Clercq, *Collect. Czech. Chem. Commun.*, 1998, **63**, 449–479.
166 A. Holý, J. Günter, H. Dvořáková, M. Masojídková, G. Andrei, R. Snoeck, J. Balzarini and E. De Clercq, *J. Med. Chem.*, 1999, **42**, 2064–2086.
167 Information regarding EMEA as downloaded from the World Wide Web in December 2003 and February 2005: http//www.emea.eu.int/humandocs/PDFs/EPAR/hepsera/610202en1.pdf.
168 *Chem. Rundsch.*, (CH-4501 Solothurn, Switzerland), No 19; Oct. 8, 2002; p 68.
169 (*a*) B. L. Robbins, J. Greenhaw, M. C. Connelly and A. Fridland, *Antimicrob. Agents Chemother.*, 1995, **39**, 2304–2308; (*b*) R. Krejcová, K. Horska, I. Votruba and A. Holý, *Collect. Czech. Chem. Commun.*, 2000, **65**, 1653–1668.
170 A. Holý, E. De Clercq and I. Votruba, *ACS Symp. Series*, 1989, **401**, 51–71.
171 S. A. Foster, J. Černý and Y.-c. Cheng, *J. Biol. Chem.*, 1991, **266**, 238–244.
172 P. Kramata, I. Votruba, B. Otová and A. Holý, *Molec. Pharmacol.*, 1996, **49**, 1005–1011.
173 G. Birkuš, I. Votruba, A. Holý and B. Otová, *Biochem. Pharmacol.*, 1999, **58**, 487–492.
174 A. Holý, I. Votruba, A. Merta, J. Černý, J. Veselý, J. Vlach, K. Sedivá, I. Rosenberg, M. Otmar, H. Hřebabecký, M. Trávníček, V. Vonka, R. Snoeck and E. De Clercq, *Antiviral Res.*, 1990, **13**, 295–311.
175 H. Sigel, B. Song, C. A. Blindauer, L. E. Kapinos, F. Gregáň and N. Prónayová, *Chem. Commun.*, 1999, 743–744.
176 (*a*) H. Sigel, C. P. Da Costa, B. Song, P. Carloni and F. Gregáň, *J. Am. Chem. Soc.*, 1999, **121**, 6248–6257; (*b*) B. Song, J. Zhao, F. Gregáň, N. Prónayová, S. A. Sajadi and H. Sigel, *Metal-Based Drugs*, 1999, **6**, 321–328.
177 J. Balzarini, Z. Hao, P. Herdewijn, D. G. Johns and E. De Clercq, *Proc. Natl. Acad. Sci. USA*, 1991, **88**, 1499–1503.
178 H. Sigel, D. Chen, N. A. Corfù, F. Gregáň, A. Holý and M. Strašák, *Helv. Chim. Acta*, 1992, **75**, 2634–2656.
179 H. Sigel and L. E. Kapinos, *Coord. Chem. Rev.*, 2000, **200–202**, 563–594.
180 V. L. Pecoraro, J. D. Hermes and W. W. Cleland, *Biochemistry*, 1984, **23**, 5262–5271.
181 C. P. Da Costa, D. Krajewska, A. Okruszek, W. J. Stec and H. Sigel, *J. Biol. Inorg. Chem.*, 2002, **7**, 405–415.
182 C. P. Da Costa, A. Okruszek and H. Sigel, *ChemBioChem.*, 2003, **4**, 593–602.
183 (*a*) R. K. O. Sigel, B. Song and H. Sigel, *J. Am. Chem. Soc.*, 1997, **119**, 744–755; (*b*) B. Song, R. K. O. Sigel and H. Sigel, *Chem. Eur. J.*, 1997, **3**, 29–33.
184 (*a*) F. Eckstein, *Antisense & Nucleic Acid Drug Develop.*, 2000, **10**, 117–121; (*b*) S. T. Crooke, *Biochim. Biophys. Acta*, 1999, **1489**, 31–44; (*c*) U. Galderisi, A. Cascino and A. Giordano, *J. Cell. Physiol.*, 1999, **181**, 251–257.

