Interactions of Metal Ions with Nucleotides and Nucleic Acids and their Constituents

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1 Introduction

Enzymatic reactions involving nucleotides have a general dependence on metal ions; this also applies to the biosynthesis of nucleic acids. For example, all enzyme-catalysed reactions with 5′-ATP,† including those involving DNA and RNA polymerases, need divalent cations, usually Mg^{2+}. An additional divalent metal ion may also be sometimes required,[1,2] Hence it is not surprising that nucleotide–metal-ion interactions have fascinated coordination chemists for more than three decades, especially since Szent-Györgyi[3] postulated not only phosphate binding to 5′-ATP for Mg^{2+}, but also an interaction with the nucleic base moiety.

As a result of their ambivalent properties,[4] nucleotides (see Figure 1)[5a,6] present a true challenge to coordination chemists.[4,5,7–10] A metal ion may interact with the phosphate group(s), the sugar moiety or the base residue of a nucleotide. Moreover, such a base residue is itself ambivalent; for example, an adenine residue offers the N-1, N-3, and N-7 sites to a metal ion for binding.

It is the aim of this short review to clarify the binding properties, of the various constituents, which are of course largely identical for both (low molecular weight) nucleotides and (high molecular weight) nucleic acids. Some composites of these constituents, in particular mono-nucleotides, will also be considered. The phosphate group(s) will be dealt with first because their interaction with metal ions determines to a large part the stability of nucleotide–metal-ion complexes.

At this point it is worth mentioning that only a few acidity constants (of protonated ligands) and stability constants (of the corresponding complexes) will be summarized. However, the literature citations for these equilibrium constants will be given where appropriate. Any constant actually presented is defined as given below:

$$HL^{+} \rightleftharpoons H^{+} + L \qquad (1a)$$

$$K^{H}_{HL} = [H^{+}][L]/[HL^{+}] \qquad (1b)$$

$$M^{2+} + L \rightleftharpoons ML^{2+} \qquad (2a)$$

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

2 Stability of Phosphate–Metal Ion Complexes

2.1 Phosphate Monoesters and Phosphonate Derivatives

In simple phosphate monoesters RMP^{2-}, where R represents a non-coordinating organic residue, R indirectly affects the metal ion binding properties of the phosphate group by altering its basicity. It is well known that for families of structurally related ligands straight lines are observed if log K^{M}_{ML} is plotted *versus* pK^{H}_{HL},[11] and the same is to be expected for phosphate monoester ligands. Indeed, the data pairs log $K^{M}_{M(RMP)}$/$pK^{H}_{H(RMP)}$ for phenyl phosphate, 4-nitrophenyl phosphate, methyl phosphate, n-butyl phosphate, and even hydrogen phosphate, for a given metal-ion complex, all fit on a straight line.[12] Some examples are shown in Figure 2[12–14] and it is worthwhile emphasizing that simple phosphonate ligands, like methyl phosphonate and ethyl phosphonate, also fit on the same straight lines.[15]

For the metal ions Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, or Cd^{2+} and their complexes with phosphate monoesters and phosphonates, straight line equations have been established.[15] Hence, it is possible to calculate the stability constants of the corresponding metal ion complexes for any phosphate or phosphonate derivative with a non-interacting group R if the pK_a value is known.

An interesting result, borne out from these studies, is that 5′-CMP^{2-}, 5′-UMP^{2-}, and 5′-$dTMP^{2-}$ form complexes with the mentioned divalent metal ions and for these the data pairs fit on the reference lines defined above (see Figure 2).[12] This means that cytidine, uridine, and thymidine residues do not participate in complex formation. For 5′-CMP^{2-} this may seem particularly surprising because it is well known that cytidine can bind metal ions *via* N-3.[16] However, 5′-CMP^{2-}, like 5′-UMP^{2-} and 5′-$dTMP^{2-}$, exists predominantly in the *anti* conformation in aqueous solution[6a] such that N-3 is pointing away from the phosphate group (see Figure 1), and therefore simultaneous coordination of the metal ion to both the phosphate residue and N-3 is only possible through adoption of the less favoured *syn* conformation (*cf.* also reference 17). The uracil and thymine residues become attractive for metal ions after deprotonation of the H(N-3) unit (see also Sections 4.1 and 5.1).

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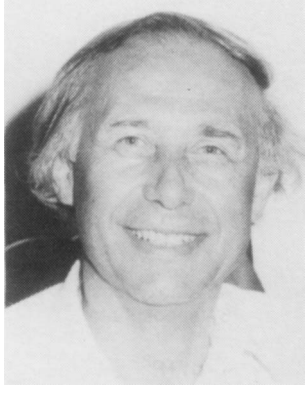
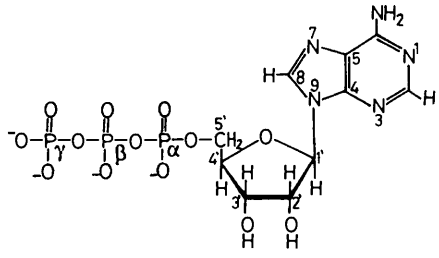


† Abbreviations: Note, listed below are only those abbreviations which do not logically follow from either the definitions given in Figure 1 and its legend or in the legend of Figure 2. Ado = adenosine; 2′-AMP^{2-} = adenosine 2′-monophosphate; 3′-AMP^{2-} = adenosine 3′-monophosphate; Cyd = cytidine; $DHAP^{2-}$ = dihydroxyacetone phosphate = $HOCH_2C(O)CH_2OPO_3^{2-}$; dThd = thymidine; ϵ = dielectric constant; 5′-GMP^{2-}, 5′-GDP^{3-}, 5′-GTP^{4-} = guanosine 5′-monophosphate, 5′-diphosphate, 5′-triphosphate; $G1P^{2-}$ = glycerol 1-phosphate = $HOCH_2CH(OH)CH_2OPO_3^{2-}$; Guo = guanosine; I = ionic strength of a solution; Ino = inosine; K_a = acidity constant (see also equation 1); L = general ligand with an undefined charge; M^{n+} = general metal ion; RMP^{2-} = phosphate monoester (R may be any organic residue, *e.g.*, phenyl or nucleosidyl; in some instances also phosphonate derivatives, RPO_3^{2-}, are included in this abbreviation); RNA = ribonucleic acid; TuMP^{2-} = tubercidin 5′-monophosphate (= 7-deaza-5′-AMP^{2-}; *i.e.*, N-7 of the adenine residue is replaced by a CH unit); Urd = uridine.

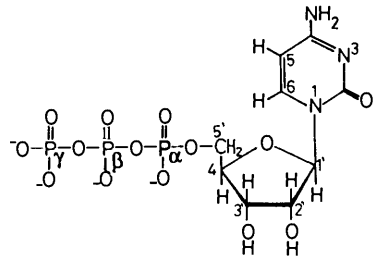
Figure 1 Chemical structures of various nucleosides (Ns) and their corresponding nucleoside 5′-mono-, 5′-di-, and 5′-triphosphates (5′-NMP^{2-}, 5′-NDP^{3-}, and 5′-NTP^{4-}) As examples, at the top are shown adenosine 5′-triphosphate (5′-ATP^{4-}) and cytidine 5′-triphosphate (5′-CTP^{4-}) in their dominating *anti* conformation,[5a,6] together with the labelling system for the triphosphate chain, note, the phosphate groups in the NTPs are labelled α, β and γ, where γ refers to the terminal phosphate group The analogous NMPs and NDPs have the corresponding structures with one or two phosphate groups, respectively The adenine and cytosine residues in the structures given at the top for 5′-ATP^{4-} and 5′-CTP^{4-}, respectively, may be replaced by one of the other nucleic base residues shown above, if this substitution is done in the way the bases are depicted within the plane then the *anti* conformation will also result for the corresponding nucleoside 5′-phosphates For reasons of clarity the 5′ label is used only in the top two examples, however, in the text this specification is always given when necessary

2.2 Diphosphate and Triphosphate Monoester Ligands

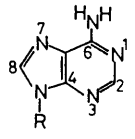
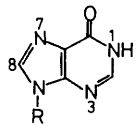
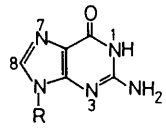
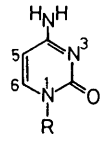
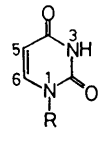
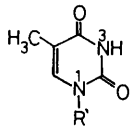
In nucleoside 5′-triphosphates (5′-NTP^{4-}) the terminal γ-phosphate group is relatively far removed from the nucleosidyl residue and consequently its basicity is largely unaffected by the base residues Indeed, $pK^{H}_{H(NTP)}$ = 6 50 ± 0 05 in aqueous solution (I = 0 1 M, $NaNO_3$ or $NaClO_4$, 25 °C) for H(5′-ATP)$^{3-}$, H(5′-ITP)$^{3-}$, H(5′-GTP)$^{3-}$, H(5′-CTP)$^{3-}$, H(5′-UTP)$^{3-}$, and H(5′-dTTP)$^{3-}$,[17,18] the pK_a value for monoprotonated methyl triphosphate is also within the given limits [18c] Again, ^{1}HNMR shift experiments,[19] in agreement with spectrophotometric[20] and kinetic studies,[21] reveal that for 5′-CTP^{4-}, 5′-UTP^{4-}, and 5′-$dTTP^{4-}$ the affinity for the divalent metal ions mentioned in Section 2 1 is solely determined by the properties of the triphosphate residue (the single exception being the Cu(5′-CTP)^{2} complex, *cf* reference 17)



5′-ATP^{4-}



5′-CTP^{4-}

R =	–ribose	–ribosyl 5′-monophosphate	–ribosyl 5′-diphosphate	–ribosyl 5′-triphosphate
	Ado	AMP^{2-}	ADP^{3-}	ATP^{4-}
	Ino	IMP^{2-}	IDP^{3-}	ITP^{4-}
	Guo	GMP^{2-}	GDP^{3-}	GTP^{4-}
	Cyd	CMP^{2-}	CDP^{3-}	CTP^{4-}
	Urd	UMP^{2-}	UDP^{3-}	UTP^{4-}
R′ =	–2′-deoxyribose	–2′-deoxyribosyl 5′-monophosphate	–2′-deoxyribosyl 5′-diphosphate	–2′-deoxyribosyl 5′-triphosphate
	dThd	$dTMP^{2-}$	$dTDP^{2-}$	$dTTP^{2-}$

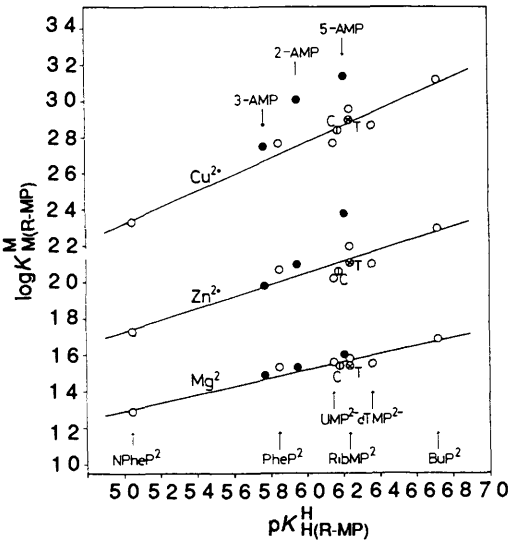


Figure 2 Relationship between log $K^{M}_{M(RMP)}$ and $pK^{H}_{H(RMP)}$ for the 1 1 complexes of Mg^{2+}, Zn^{2+}, and Cu^{2+} with some simple phosphate monoester ligands (RMP^{2-}) 4-nitrophenyl phosphate ($NPheP^{2-}$), phenyl phosphate ($PheP^{2-}$), uridine 5′-monophosphate (UMP^{2-}), D-ribose 5′-monophosphate ($RibMP^{2-}$), thymidine 5′-monophosphate ($dTMP^{2-}$), and n-butyl phosphate (BuP^{2-}) (from left to right((○) The least-squares lines are drawn through the corresponding six data sets, which are taken from ref 12, the equations for these base lines are taken from Table V of ref 12 (see also Table I of ref 13) The points due to the complexes formed with 2′-AMP^{2-}, 3′-AMP^{2-}, and 5′-AMP^{2-} (●) are inserted for comparison and they provide evidence for an enhanced stability of several of the M(AMP) complexes, the corresponding equilibrium constants are taken from Tables 2 and 3 and ref 14 All points for the complexes with 5′-CMP^{2-} (= C) (⊕) (see ref 12) and $TuMP^{2-}$ (= T) (⊗) fall within the error limits on the reference lines, the log stability constants of the M(TuMP) complexes are plotted *versus* the microconstant $pk^{TuMP}_{TuMP\,H}$ = 6 24 and these data are taken from Table III and Figure 2 of ref 13, respectively All plotted equilibrium constants refer to aqueous solutions at 25 °C and I = 0 1 M ($NaNO_3$)

Our knowledge of nucleoside 5′-diphosphates (5′-NDP^{3-}) is considerably scarcer [22] It appears that the acidity constants for various H(5′-NDP)$^{2-}$ species vary somewhat, *i e* $pK^{H}_{H(NDP)}$ = 6 2 to 6 4 [22 23] This variation may be taken as an indication that the nucleosidyl residue affects the basicity of the β-phosphate group in the 5′-NDP^{3-} species to some extent However, from ^{1}HNMR shift experiments in D_2O (I = 0 1 M, $NaNO_3$, 27 °C)[23] it is clear that in the M(5′-CDP)$^-$ and M(5′-UDP)$^-$ complexes with Mg^{2+}, Zn^{2+}, or Cd^{2+} no metal-ion–base interaction occurs, this is probably also true for most other divalent metal ions

The observations for the M(5′-CTP)$^{2-}$, M(5′-UTP)$^{2-}$, M(5′-dTTP)$^{2-}$, M(5′-CDP)$^-$, and M(5′-UDP)$^-$ complexes may be explained as discussed in Section 2 1 in nucleotide complexes containing the uracil or thymine residues no metal ion interaction with the nucleic base moiety is feasible as long as the H(N-3) site is not deprotonated (*cf* also Section 4 1), aside from the fact that the *anti* conformation dominates, as is also true for 5′-CTP^{4-} and 5′-CDP^{3-} [5a] In this *anti* conformation the N-1– C-6 bond of pyrimidines projects onto or near to the ribose ring, and N-3 is directed away from the phosphate moiety The energy barrier between the *syn* and *anti* conformations of 5′-CTP^{4-} has been estimated[17] to be about 6 kJ mol^{-1}

Recommended stability constants for M(5′-NTP)$^{2-}$ complexes are listed in reference 17, values for M(5′-NDP)$^-$ species are given in references 22 and 23

2.3 Structural Aspects of Phosphate-Metal Ion Interactions

How do metal ions interact with phosphate groups? For the complexes of poly(cytidylate) [= poly(C)], which contains phosphate diester groups with a single negative charge on the phosphate unit, with Mg^{2+}, Co^{2+}, Ni^{2+}, or Zn^{2+} very similar stability constants have been observed [24] This observation is taken as evidence that these metal ions are bound to poly(C) mainly by electrostatic interactions with little or no inner-sphere coordination, an outer-sphere coordination with water between the metal ion and the phosphate oxygens is suggested [24] Such an outer-sphere interaction is also proposed for Mg^{2+}/poly(adenylate([= poly(A)] complexes, while metal ions like Co^{2+}, Ni^{2+}, and Zn^{2+} also form inner-sphere species with poly(A) (see also Sections 5 2 and 5 5)

For doubly negatively charged phosphate monoester ligands (RMP^{2-}) it has been tentatively concluded[12] by considering the slopes of the aforementioned reference lines (see also Figure 2) that in aqueous solution four-membered chelate rings are rarely formed – despite their (albeit infrequent) occurrence in the solid state [8b 25] – and that the dominating binding mode is monodentate, inner-sphere phosphate oxygen coordination (see also Section 5 2) possibly together with a six-membered 'semi-chelate' ring involving both a metal ion-coordinated water molecule and a hydrogen bond [12] This conclusion agrees with a recent examination[25b] of phosphate–metal ion interactions in the solid state based on the Cambridge Structural Database metal ions display preferentially a monodentate, out-of-plane coordination stereochemistry However, one has to add that in aqueous solution pure outer-sphere complexation may also play a role [26] Moreover, a crystal structure study of the barium–adenosine 5′-monophosphate heptahydrate complex[27] revealed that Ba^{2+} is coordinated to eight water molecules without direct interaction with 5′-AMP^{2-}, and that seven of the eight water molecules from the Ba^{2+} hydration shell are hydrogen bonded to phosphate groups, three of these water molecules are also hydrogen bonded to other suitable acceptor sites on the base (N-1 and N-7) and ribose (O-3′) entities Consequently 'isomeric' equilibria, regarding the phosphate–metal ion binding mode, have to be expected in solution, the position of which will also depend on *the kind of metal ion* involved [12] Indeed, for complexes of methyl phosphate it has previously been concluded that the extent of outer-sphere complexation depends on the metal ion [26a] However, clearly more research is needed on the degree of formation of these various species

For di- and triphosphates the formation of inner-sphere complexes will certainly be more pronounced owing to the increased negative charge of these ligands (see also Section 5 5) This conclusion agrees with an earlier one,[26b] namely 'the lower the charge, the more predominant are outer-sphere complexes'

2.4 The Effect of a Decreasing Solvent Polarity on Complex Stability

Nowadays it is well established that in proteins[28] and the active-site cavities of enzymes[29] the 'effective' or 'equivalent solution' dielectric constant is reduced compared to the situation in bulk water, *i e* , the activity of water is decreased[30] due to the presence of aliphatic and aromatic amino acid side chains at the protein–water interface Estimates for the dielectric constants (ϵ) in such locations range from about 30 to 70 [28 29] Therefore, it should be emphasized in the present context that metal ion-phosphate group interactions increase considerably with decreasing solvent polarity This effect is well established for both phosphate[31] and triphosphate monoester[32] ligands, for example, the stability of the Cu^{2+} complex of phenyl phosphate ($PheP^{2-}$) increases by nearly a factor of ten in going from water (ϵ = 78 5,[33] log $K^{Cu}_{Cu(PheP)}$ = 2 77) to an aqueous solution containing 30% (v/v), 1,4-dioxane (ϵ = 52 7,[33] log $K^{Cu}_{Cu(PheP)}$ = 3 72) [31]

Straight-line plots were constructed of log $K^{Cu}_{Cu(RMP)}$ *versus* $pK^{H}_{H(RMP)}$ for the Cu^{2+} complexes of simple phosphate monoester ligands in water containing 20, 30, 40, or 50% (v/v) 1,4-dioxane (I = 0 1M, $NaNO_3$, 25°C) (see also Figure 3 in Section 3) For the solvents containing 30 and 50% 1,4-dioxane it was also shown that the data pairs for the complexes of simple

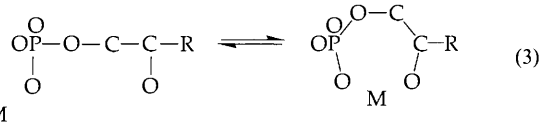
phosphonate ligands fit on these straight lines[34] (see also Section 2 1) As before, the stability constant for the corresponding Cu^{2+} complex can be calculated from these straight-line equations[31b,c] and the pK_a value of a phosphate monoester or a phosphonate derivative

3 Interactions of Metal Ions with Sugar Residues

Complexes between carbohydrates or sugar-type ligands and metal ions have recently been reviewed [35] Structural studies showed [35] that simple carbohydrates can bind Ca^{2+} only if they can provide three or more hydroxy groups in a geometrical arrangement fitting the coordination sphere of calcium [36] Another example involving a transition metal ion stems from an *X*-ray structure study of a polymeric Cu^{2+} complex of guanosine 2'-monophosphate that revealed an axial Cu–O(5') bond with the ribose [37] In this case Cu^{2+} has a distorted [4 + 2]-octahedral coordination sphere in which the Cu-O(5') bond (2 474 Å) is 0 138 Å longer than the opposite Cu–O bond to a coordinated water molecule with a bond length of 2 336 Å

The two examples clearly indicate that the interactions between divalent metal ions and simple sugar residues are weak This is different, of course, for sugars containing an amino or carboxylate group as well as for macromolecular carbohydrates [35]

In line with the above conclusions are the results of a recent study[38] dealing with dihydroxyacetone phosphate ($DHAP^{2-}$) and glycerol-1-phosphate (GIP^{2-}) By employing the straight-line equations mentioned in Section 2 1, it was established for aqueous solutions that the stability of the M(DHAP) and M(GIP) complexes, where $M^{2+} = Mg^{2+}$, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, or Cd^{2+}, is governed by the basicity of the phosphate group of $DHAP^{2-}$ and GIP^{2-}, there are no indications for the participation of the oxygen atom of either the carbonyl or hydroxyl groups at C-2 of these ligands in complex formation, which would be possible on steric grounds However, measurements with Cu^{2+} and $DHAP^{2-}$ or GIP^{2-} in water containing 30 or 50% (v/v) 1,4-dioxane show – as is nicely seen in Figure 3 from the increased complex stabilities – that to some extent seven-membered chelates involving these oxygen atoms may be formed [38] This may also be surmised for the other divalent metal ions listed above under appropriate conditions By using the results summarized in Figure 3 it was possible to quantify the position of the following intramolecular equilibrium



(3)

The degree of formation of each of the chelated species of Cu(DHAP) and Cu(GIP) is approximately 0, 15, and 45% in water, water/30% (v/v) 1,4-dioxane, and water/50% (v/v) 1,4-dioxane, respectively [38] These results nicely demonstrate that the weak binding sites of sugar residues become important when the solvent has poorer solvating properties than water, a condition that exists in the active-site cavities of enzymes, as indicated in Section 2 4

4 Metal Ion Binding Sites in Nucleoside Complexes

4.1 Some Considerations on Uridine/Thymidine and Inosine/Guanosine

Scanning the six nucleic base structures shown in Figure 1 reveals that the base moieties of uridine (Urd) and thymidine (dThd) offer to metal ions no strong binding sites – aside from the weakly coordinating carbonyl groups – as long as the H(N-3) unit is not deprotonated, a reaction that will occur only exceptionally in the physiological pH range under the influence of metal ions because the acidity constants are high

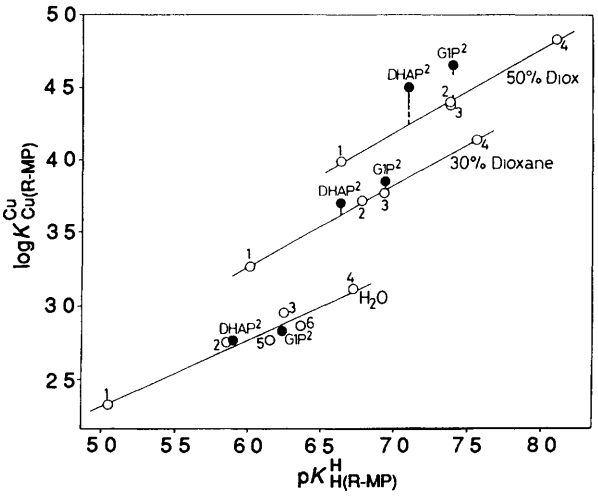


Figure 3 Evidence for an enhanced stability of Cu(DHAP) and Cu(GIP) in mixed dioxane–water solvents based on the relationship between log $K^{M}_{M(RMP)}$ and $pK^{H}_{H(RMP)}$ for the Cu^{2+} 1 1 complexes of 4-nitrophenyl phosphate (1), phenyl phosphate (2), D-ribose 5'-monophosphate (3), *n*-butyl phosphate (4), uridine 5'-monophosphate (5), and thymidine 5'-monophosphate (6) in water and in water containing 30 or 50% (v/v) 1,4-dioxane The least-squares lines are drawn in each case through the data sets shown, the equations of these reference lines are available [12,31b,c] The points due to the Cu^{2+} 1 1 complexes formed with $DHAP^{2-}$ and GIP^{2-} (●) in the three mentioned solvents are inserted for comparison, these data are from Table III of ref 38 The vertical broken lines emphasize the stability differences to the corresponding reference lines, these differences equal log $\Delta_{Cu(RMP)}$ as defined in Section 5 2 by equation (7) All the plotted constants refer to 25 °C and I = 0 1 M ($NaNO_3$)

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($pK^{H}_{H(Urd)}$ = 9 19, $pK^{H}_{H(dThd)}$ = 9 69, I = 0 1 M, $NaNO_3$, 25°C, H Sigel, results to be published)

The situation with inosine (Ino) and guanosine (Guo) (see Figure 1) is quite similar The H(N-1) sites are in general not available at a pH of about 7 ($pK^{H}_{H(Ino)}$ = 8 76, $pK^{H}_{H(Guo)}$ = 9 22, H Sigel, results to be published) for metal ion binding, this leaves N-3 and N-7 as binding sites Among these N-7 is the predominant site,[7c] while binding to N-3 is observed only exceptionally (see Section 5 3)

The two base entities that show a large degree of ambivalent behaviour are clearly adenine and, maybe more surprisingly, cytosine Therefore we shall consider adenosine and cytidine in somewhat more detail

4.2 The N-1 *versus* N-7 Dichotomy of Adenosine

The adenine residue offers metal ions N-1, occasionally N-3 (see Section 5 3), and N-7 as binding sites (*cf* Figure 1) Consequently for adenosine (Ado) this leaves a dichotomy in metal ion binding of N-1 *versus* N-7, a problem first addressed by Kim and Martin [5b,39] The development of log K^{M}_{ML} *versus* pK^{H}_{HL} reference lines for imidazole-like, or N-7 type, ligands, and for pyridine-like, or N-1 type, ligands,[40] as well as the evaluation of the steric inhibitory effect of the *o*-amino group on the complexation tendency of the N-1 site in adenosine, by studying the complex forming properties of tubercidin (Tu)[41] (in Tu the N-7 of Ado is replaced by a CH unit), brought some further progress to the resolution of the problem [42]

Indeed, this information, together with an improved estimate for the pK_a value of the H^+(N-7) site in *monoprotonated* adenosine (which is not directly accessible by experiments because N-7 is less basic than N-1), allowed the conclusion that Ni^{2+} and Cu^{2+} (to approximately 70%), and most probably also Co^{2+} and Cd^{2+}, prefer to coordinate to adenosine *via* the N-7 site, for Zn^{2+} a more even distribution between the N-1 and N-7 sites appears to occur, while Mn^{2+} possibly prefers the N-1 site which is indubitably strongly predominant for the binding of

H^+. For further details reference 42 should be consulted, where the corresponding equilibrium constants are also listed. It may be added that similar treatment has been carried out for the dichotomy present in H(N-1) deprotonated xanthosine ($pK_a = 5.47$).[40]

4.3 The Ambivalent Properties of Cytidine

The establishment of reference lines (log K^M_{ML} *versus* pK^H_{HL})[42] for *o*-amino pyridine-like ligands (Section 4.2) allowed a quantification of the coordination tendency of N-3 of cytidine (Cyd) as well as of the stability enhancing effect of the neighbouring 2-carbonyl group in $M(Cyd)^+$ complexes.[16] This effect increases within the series $Co^{2+} \sim Ni^{2+}$ (no effect) < $Mn^{2+} \sim Zn^{2+}$ (0.25 log unit) < Cd^{2+} (~ 0.55) < Cu^{2+} (~ 1.05); a positive effect is also observable for the $M(Cyd)^{2+}$ complexes of Mg^{2+} and Ca^{2+}. However, in most instances the coordination tendency of the *o*-carbonyl group is not able to compensate completely for the steric inhibitory effect of the *o*-amino group (see Figure 1).

It is concluded[16] that in $Co(Cyd)^{2+}$ and $Ni(Cyd)^{2+}$ the 2-carbonyl group does not participate to any appreciable extent in metal ion binding; in the $M(Cyd)^{2+}$ systems with Zn^{2+}, Cd^{2+}, or Cu^{2+} chelates involving N-3 and a more weakly bound O-2 are formed, at least in equilibrium . These chelates could be four-membered, such as those observed in the solid state (see citations in reference 16), but a metal ion-bound water molecule might also participate in aqueous solution, and six-membered chelates would result from this partial outer-sphere coordination; a binding type sometimes termed semi-chelation. In contrast it should be pointed out that the stabilities of the $M(Cyd)^{2+}$ complexes with Mn^{2+}, Mg^{2+}, and Ca^{2+} are apparently determined to a large part by the metal ion affinity of the O-2 of the carbonyl group.

For further details, especially the related stability constants, reference 16 should be consulted.

4.4 Conclusions Regarding Nucleic Acids

Some provisional conclusions regarding the metal ion binding properties of single-stranded RNA or DNA, based on studies of the indicated type, are given in reference 7c. For the neutral pH range the following overall order of affinity for the metal ion-binding of the sites available in the base residues in single-stranded nucleic acids may be tentatively proposed: N-7; Guo ≳ N-3; Cyd ≳ N-7; Ado ≳ N-1; Ado > N-3; Ado, Guo.

Some metal ions, *e.g.* Cu^{2+}, may also be able to partially replace H^+ from the neutral H(N-1) unit in the guanine moiety (*cf.* Figure 1) or from the H(N-3) site of the uracyl or thymine residues in the neutral pH range [5b,19,43] (see also Section 4.1). The position of these negatively charged N sites in the preceding series of metal ion affinities is as yet undetermined. Regarding the consequences of metal ion binding to base residues upon certain degradation reactions see Section 7.

Of course, the so-called *hard* or *class a* metal ions,[2,44] like Na^+, Mg^{2+}, Al^{3+}, Mn^{2+} or Fe^{3+}, preferably interact with the *hard* oxygen sites of the phosphate groups of nucleic acids; these metal ions have only a low affinity for the base residues. Clearly, the *soft* or *class b*, *e.g.*, Cd^{2+}, Hg^{2+}, Pd^{2+}, Pt^{2+}, as well as the *borderline*, *e.g.*, Fe^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, metal ions have a rather pronounced affinity for the *borderline* aromatic N-sites of the nucleic base residues; however, it should be noted that many of these metal ions, *e.g.* Cu^{2+}, Zn^{2+}, Cd^{2+} or Pb^{2+}, also bind significantly to phosphate groups (see also Sections 5 and 7).

5 Nucleotide–Metal Ion Interactions

5.1 Some Introductory Remarks

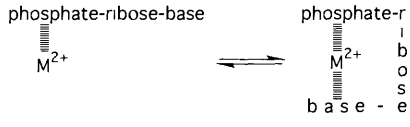
It was pointed out in Sections 2.1 and 2.2 that, in the case of the pyrimidine nucleoside phosphates, the stability of the resulting complexes is determined by the metal ion affinity of the phosphate group(s) and that there is no significant metal ion–nucleic base interaction (at least as long as the H(N-3) site of the uracil or thymine bases[19,43] is not ionized);[17] the only exception known so far is $Cu(CTP)^{2-}$, of which about 30% exist in the form of a macrochelated isomer involving N-3 (see also equilibrium 4 below).[17] Therefore the following parts in this section will concentrate on the complexes of purine nucleoside phosphates.

There is one important aspect that needs to be emphasized, and that has to be kept in mind when dealing with purine derivatives. They all show a pronounced tendency for self-association which occurs *via* stacking of the purine rings.[19,23,45] As a result, experiments aimed at determining the properties of monomeric metal ion complexes of purine nucleosides, or their phosphates, should not be carried out in concentrations higher than 10^{-3} M; in fact, to be on the safe side, it is recommended that a maximum concentration of only 5×10^{-4} M is used (*cf.*, *e.g.*, references 7*c*, 13–15, 41). Consequently, most of the results to be discussed in the following sections were obtained by potentiometric pH titrations of solutions that had a nucleotide concentration of 3×10^{-4} M.

To prevent erroneous conclusions[7c] it is very important to be aware of the aforementioned self-stacking properties,[45] which may be favoured *via* either metal ion[7c,19,23] or proton[46] binding. Moreover, such self-stacking also affects the acid–base qualities of nucleosides and nucleotides, which can be studied as a function of concentration.[47]

5.2 Macrochelate Formation Involving N-7 in Metal Ion Complexes of Adenosine 5′-Monophosphate (5′-AMP^{2-})

The biologically most important compound among the adenosine monophosphates (see Figure 4 in Section 5.3) is probably 5′-AMP^{2-}. In the case when a metal ion is coordinated to the phosphate group of this nucleotide the ion may also interact with N-7 of the adenine residue because in the dominating *anti* conformation N-7 is orientated towards the phosphate group (while N-1 is pointing away; see Figure 1). This then gives rise to the following intramolecular equilibrium:



(4)

The degree of formation of the macrochelated or 'closed' species, which we designate as $M(NMP)_{cl}$, is independent of the total concentration of complex present because the intramolecular equilibrium constant K_I, as defined by equation 5 where $M(NMP)_{op}$ refers to the 'open' species in equilibrium 4, is dimensionless:

$$K_I = [M(NMP)_{cl}]/[M(NMP)_{op}] \quad (5)$$

K_I may be calculated[11,13,19] *via* equation 6,

$$K_I = \frac{K^M_{M(NMP)}}{K^M_{M(NMP)_{op}}} - 1 = 10^{\log \Delta} - 1 \quad (6)$$

where log Δ is defined by equation 7:

$$\log \Delta = \log K^M_{M(NMP)} - \log K^M_{M(NMP)_{op}} \quad (7)$$

If a further identification of log Δ is necessary, it is given by indices; *e.g.*, log $\Delta_{M(5'\text{-AMP})}$ refers to the stability difference as expressed in equation 7 for M(5′-AMP) complexes. The remaining definitions are given in equations 8 through 10:

$$M^{2+} + NMP^{2-} \rightleftharpoons M(NMP)_{op} \rightleftharpoons M(NMP)_{cl} \quad (8)$$

$$K^M_{M(NMP)_{op}} = [M(NMP)_{op}]/([M^{2+}][NMP^{2-}]) \quad (9)$$

$$K^M_{M(NMP)} = \frac{[M(NMP)]}{[M^{2+}][NMP^{2-}]} = \frac{([M(NMP)_{op}] + [M(NMP)_{cl}])}{[M^{2+}][NMP^{2-}]} \quad (10a)$$

Table 1 Comparison of the measured stability, $K^{M}_{M(5\ AMP)}$, of the M(5′-AMP) complexes[a] with the calculated stability, $K^{M}_{M(5\ AMP)_{op}}$, for an isomer with only M^{2+}/phosphate coordination,[b] and extent of the intramolecular macrochelate formation (equilibrium 4) in the M(5′-AMP) complexes at 25 °C and $I = 0.1$ M ($NaNO_3$)

M^{2+}	log $K^{M}_{M(5\ AMP)}$ (Eq 10)[a]	log $K^{M}_{M(5\ AMP)_{op}}$ (Eq 9)[b]	log Δ (Eq 7)	K_I (Eq 6)	% M(5′-AMP)$_{cl}$ (Eq 4 11)
Mg^{2+}	1 60 ± 0 02	1 57 ± 0 04	0 03 ± 0 04	0 07 ± 0 10	0 (7 ± 9/≤ 15)
Ca^{2+}	1 46 ± 0 01	1 46 ± 0 05	0 00 ± 0 05	0 00 ± 0 12	0 (≤ 11)
Sr^{2+}	1 24 ± 0 01	1 24 ± 0 05	0 00 ± 0 05	0 00 ± 0 12	0 (≤ 11)
Ba^{2+}	1 17 ± 0 02	1 16 ± 0 05	0 01 ± 0 05	0 02 ± 0 13	0 (≤ 13)
Mn^{2+}	2 23 ± 0 01	2 16 ± 0 07	0 07 ± 0 07[e]	0 17 ± 0 19[e]	15 ± 14[e]
Co^{2+}	2 23 ± 0 02	1 94 ± 0 07	0 29 ± 0 07	0 95 ± 0 33	49 ± 9
Ni^{2+}	2 49 ± 0 02	1 95 ± 0 06	0 54 ± 0 06	2 47 ± 0 50	71 ± 4
Cu^{2+}	3 14 ± 0 01	2 87 ± 0 08	0 27 ± 0 08	0 86 ± 0 35	46 ± 10
Zn^{2+}	2 38 ± 0 07[d]	2 12 ± 0 08	0 26 ± 0 11	0 82 ± 0 45	45 ± 13
Cd^{2+}	2 68 ± 0 02	2 44 ± 0 06	0 24 ± 0 06	0 74 ± 0 25	43 ± 8

Determined in aqueous solution by potentiometric pH titrations The errors given are either 3 times the standard error of the mean or the sum of the probable systematic errors whichever is larger The acidity constants of H_2(5′-AMP)$^{+}$ are $pK^{H}_{H\ 5\ AMP}$ = 3 84 ± 0 02 and $pK^{H}_{H\ 5\ AMP}$ = 6 21 ± 0 01 [13] [b] Calculated with $pK^{H}_{H\ 5\ AMP}$ = 6 21 and the baseline equations given in Table 1 of ref 13 [12] the error limits correspond to 3 times the standard deviations (SD) given in the column at the right in Table 1 of ref 13 See also the information summarized in Section 2 1 The errors given here and in the other two columns at the right were calculated according to the error propagation after Gauss by using the errors listed in the second and third columns In this connection the in part increased stability of several M(5′-AMP) complexes shown in Figure 2 should also be noted the vertical distance of a datum point to the reference line corresponds in each case to log $\Delta_{M\ 5\ AMP}$ [d] Regarding experimental difficulties see ref 13 The log $K^{Zn}_{Zn\ 5\ AMP}$ values determined in aqueous solution with $NaNO_3$ and $NaClO_4$ as background electrolyte ($I = 0.1$ M) were 2 41 ± 0 10 and 2 34 ± 0 06 respectively the value given above is the overall average This result is in all probability significant with 2 σ as error limits the data are log $\Delta_{Mn\ 5\ AMP}$ = 0 07 ± 0 04 K_I = 0 17 ± 0 11 and % M(5′-AMP)$_{cl}$ = 15 ± 8 [13]
(Reprinted by permission from *J Am Chem Soc* 1988 **110** 6857)

$$= K^{M}_{M(NMP)_{op}} + K_I K^{M}_{M(NMP)_{op}} = K^{M}_{M(NMP)_{op}} (1 + K_I) \quad (10b)$$

Equation 6 follows from equation 10b The overall stability constant $K^{M}_{M(NMP)}$ (equation 10a) is experimentally accessible and, *e g* for M(5′-AMP) systems, values for $K^{M}_{M(5\ AMP)_{op}}$ (equation 9) are calculated by employing the reference-line equations described in Section 2 1 and the acidity constant of H(5′-AMP)$^{-}$ ($pK^{H}_{H(5\ AMP)}$ = 6 21),[13] hence, log $\Delta_{M(5\ AMP)}$ becomes known and thus *via* K_I the position of the intramolecular equilibrium 4, *i e* the percentage of the closed or macrochelated species, follows from equation 11

$$\%\ M(NMP)_{cl} = 100\ K_I/(1 + K_I) \quad (11)$$

This calculation procedure, outlined now for M(NMP)$_{cl}$ applies to all corresponding systems discussed in Section 5 Furthermore, to provide the reader with all the data involved in such a calculation in a single example, those for various M(5′-AMP) systems are summarized in Table 1

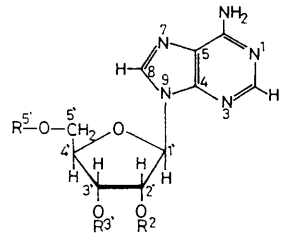
From column 4 of Table 1 it is evident that the stability increase log $\Delta_{M(5\ AMP)}$ is zero, within the error limits, for various M(5′-AMP) complexes, and therefore at the most only traces of base-backbound isomers can occur The increased complex stability with the 3*d* metal ions and Zn^{2+} or Cd^{2+} demonstrates that the macrochelated isomers (equilibrium (4)) are formed in appreciable amounts (column 6 of Table 1) The smaller stability enhancement observed for Cu(5′-AMP) in comparison with that for Ni(5′-AMP) indicates that the geometry of the coordination sphere of the metal ion is playing a role [13] Assuming that Cu^{2+} adopts a Jahn-Teller distorted octahedral coordination sphere with a strong tendency to coordinate donor atoms equatorially,[48] there are three equatorial positions left in a phosphate-coordinated Cu^{2+}, but for steric reasons only the *two cis* positions are able to interact with N-7 In the octahedral coordination sphere of Ni^{2+} *four* of the five positions left after phosphate coordination are sterically accessible Hence, Ni^{2+} backbinding to N-7 is statistically favoured by a factor of 2 corresponding to 0 3 log units, and indeed this is comparable to the larger stability enhancement of 0 27 log units (= log $\Delta_{Ni(5\ AMP)}$ − log $\Delta_{Cu(5\ AMP)}$ = 0 54 − 0 27, Table 1) for the Ni^{2+} complex The near identity of the stability enhancements (log Δ) of the complexes with Co^{2+}, Zn^{2+}, and Cd^{2+} (Table 1) reflects the similar affinity of these ions toward imidazole-type nitrogen donors [49]

That the observed increased stabilities of various M(5′-AMP) complexes (see Figure 2 in Section 2 1 and Table 1) are actually due to the formation of macrochelates involving N-7 was proved by studying the M^{2+} complexes of tubercidin 5′-monophosphate (TuMP^{2-} = 7-deazaAMP^{2-} , *i e* N-7 is replaced by a CH group) [13] Indeed, M(TuMP) complexes show no increased stability, *i e* , TuMP^{2-} behaves as a simple phosphate monoester ligand and its data pairs fit, within the error limits, on the reference lines (see also Figure 2)

In the previously mentioned M(5′-AMP)$_{cl}$ macrochelates both binding sites, *i e* the phosphate group and N-7 may bind to a metal ion in an inner-sphere manner [13] This conclusion is also supported by kinetic studies (mainly with Ni^{2+} and Co^{2+}),[21 50] and agrees with suggestions based on space-filling molecular models [51] Moreover, the following results for other purine nucleoside 5′-monophosphates are also in line kinetic and product studies[52] for the reaction between *cis*-$Pt(NH_3)_2^{2+}$ and 5′-(2′-deoxy)GMP^{2-} or 5′-GMP^{2-} indicate a direct coordination, a suggestion also confirmed in NMR studies[53] for 5′-IMP^{2-} and other related purine nucleotides Furthermore there is evidence that in D_2O the Mo^{IV} of the $(\eta^5\text{-}C_5H_5)_2Mo^{2+}$ unit coordinates directly to both N-7 and the phosphate group of 5′-(2′-deoxy)AMP^{2-} , thus forming a macrochelate [54] In agreement with these results are the properties observed for the M^{2+} complexes of 1,N^6-ethenoadenosine 5′-monophosphate (ϵ-AMP^{2-}) [7a 13] Of course, these summarized proofs for inner-sphere binding in these macrochelates do not exclude the possibility that in aqueous solution macrochelates with a (partial) outer-sphere binding also occur to a certain degree (see also Section 5 5)

5.3 Complexes of 2′-AMP^{2-} and 3′-AMP^{2-} : Evidence for Metal Ion–(N-3) Interactions

Figure 2 in Section 2 1 demonstrates that the stability of some M(2′-AMP) and possibly also, though certainly to a lesser extent, M(3′-AMP) complexes is enhanced What types of macrochelates are possible with 2′-AMP^{2-} and 3 -AMP^{2-} ? By considering the structures of the ligands shown in Figure 4 it is evident that N-7, though crucial for the properties of the M(5 -AMP) complexes, is for steric reasons clearly not accessible to a metal ion already bound to either the 2′- or 3′-phosphate group, instead one might be tempted to postulate chelate formation with the neighbouring OH groups of the ribose ring for both AMPs However, this is apparently not the case the steric conditions for 2′-AMP^{2-} and 3′-AMP^{2-} to form such seven-



2′-AMP^{2-} : $R^{2'} = -PO_3^{2-}$, $R^{3'} = R^{5'} = -H$

3′-AMP^{2-} : $R^{3'} = -PO_3^{2-}$, $R^{2'} = R^{5'} = -H$

5′-AMP^{2-} : $R^{5'} = -PO_3^{2-}$, $R^{2'} = R^{3'} = -H$

Figure 4 Chemical structures of the adenosine monophosphates (AMPs) considered in Section 5.3. The AMPs are shown in their dominating *anti* conformation.[6b]

membered chelates are identical (Figure 4) and therefore equivalent properties for both series of complexes are expected, but this is not observed as can be seen from Figure 2, as well as from the results listed in columns 2 and 3 of Table 2.

Table 2 Extent of intramolecular macrochelate formation according to equilibrium (4) in M(AMP) complexes expressed as the percentage of $M(AMP)_{cl}$ formed in aqueous solution at 25 °C and $I = 0.1$ M ($NaNO_3$)[a]

M^{2+}	% $M(2'\text{-AMP})_{cl}$	% $(M(3'\text{-AMP})_{cl}$	% $M(5'\text{-AMP})_{cl}$
Mg^{2+}	0	0	7 ± 9 (≤ 15)
Ca^{2+}	0	0	0
Sr^{2+}	0	0	0
Ba^{2+}	0	0	0
Mn^{2+}	11 ± 15 (< 24)	0	15 ± 14[b]
Co^{2+}	11 ± 16 (< 24)	0	49 ± 9
Ni^{2+}	13 ± 13 (< 25)	13 ± 13 (< 25)	71 ± 4
Cu^{2+}	45 ± 10	17 ± 16 (< 30)	46 ± 10
Zn^{2+}	15 ± 17 (< 29)	0	45 ± 13
Cd^{2+}	13 ± 13 (< 25)	0	43 ± 9

[a] Abstracted from Table 4 of ref. 14. [b] See comment in footnote *e* of Table 1.

Different structural qualities of the ligands must be responsible for the different properties of the M(2′-AMP) and M(3′-AMP) complexes: the obvious conclusion is that in Cu(2′-AMP) (see column 2 of Table 2), as well as possibly in some of the other M(2′-AMP) species, macrochelates are formed by an interaction of the phosphate-coordinated metal ion with N-3 of the adenine residue. Indeed, 2′-AMP^{2-} in its preferred *anti* conformation (Figure 4) is perfectly suited for this type of macrochelate formation.[14] That metal ions may interact with N-3 of a purine moiety has become more and more clear during the past few years through *X*-ray structure studies of Pt^{II} complexes of guanine derivatives,[55] of Rh^{I} complexes of 8-azaadenine derivatives,[56] and also of Ni^{II} complexes formed with neutral adenine.[57] It may be added that very recently chelate formation involving N-3 has also been proposed for M^{2+} complexes of the dianion of the antiviral AMP^{2-} analogue 9-(2-phosphonylmethoxyethyl)adenine (PMEA^{2-})[15] and that a Rh^{III} binding to N-3 of adenine residues of DNA was tentatively assigned.[58]

The presented conclusions regarding the M(2′-AMP) complexes also explain why the tendency to form chelates is further reduced for 3′-AMP^{2-} (see column 3 of Table 2).[14] In M(3′-AMP) complexes an interaction of the phosphate-coordinated metal ion with N-3 only becomes possible when the nucleotide adopts the less favoured *syn* conformation. Though it appears highly likely that the stability increases observed for M(2′-AMP) and (possibly also) M(3′-AMP) systems[14] (Figure 2) are due to base-backbinding of the phosphate-coordinated metal ion to N-3, as discussed above, the additional occurrence of even smaller concentrations of seven-membered chelates involving the phosphate-coordinated metal ion and the neighbouring OH group of the ribose ring cannot be ruled out completely; however, in the light of the results presented in Section 3 such chelation appears to be rather unlikely. In any case it is clear that the degree of formation of the closed species (equilibrium 4) decreases in the order: $M(5'\text{-AMP})_{cl} > M(2'\text{-AMP})_{cl} > M(3'\text{-AMP})_{cl}$ (see Table 2).

Preliminary results have also been obtained for M^{2+} complexes of 2′-GMP^{2-}, 3′-GMP^{2-}, and 5′-GMP^{2-}.[59] They parallel those described for 2′-, 3′-, and 5′-AMP^{2-}: the maximum degree of formation of macrochelated species for all metal ions occurs with 5′-GMP^{2-}; *i.e.* % $(M(5'\text{-GMP})_{cl} >$ % $M(2'\text{-GMP})_{cl} >$ % $M(3'\text{-GMP})_{cl}$; 3′-GMP^{2-} shows at most only a small tendency to form chelates. In $M(5'\text{-GMP})_{cl}$ base-backbinding occurs to N-7 (see Section 5.4) and it is now also suggested that it occurs in M(2′-GMP), most probably to N-3 of the guanosine residue.

5.4 The N-7 Backbinding Properties in Complexes of Inosine (5′-IMP^{2-}) and Guanosine 5′-Monophosphate (5′-GMP^{2-})

The results summarized in Table 3 for various M^{2+} complexes of purine nucleoside 5′-monophosphates show that macrochelation according to equilibrium 4 is also important for the M(5′-IMP) and M(5′-GMP) complexes.[60] In addition, a closer comparison of the results reveals that the percentages of the closed species increase from the M(5′-AMP) to the M(5′-IMP), and further to the M(5′-GMP) complexes.

Table 3 Extent of intramolecular macrochelate formation according to equilibrium 4 in M(5′-AMP), M(5′-IMP), and M(5′-GMP) species expressed as the percentage of $M(NMP)_{cl}$ formed in aqueous solution at 25 °C and $I = 0.1$ M ($NaNO_3$)[a]

M^{2+}	% $M(5'\text{-AMP})_{cl}$	% $M(5'\text{-IMP})_{cl}$	% $M(5'\text{-GMP})_{cl}$
Mg^{2+}	7 ± 9 (≤ 15)	21 ± 8	26 ± 8
Ca^{2+}	0	9 ± 11	15 ± 10
Mn^{2+}	15 ± 14	29 ± 12	40 ± 10
Co^{2+}	49 ± 9	78 ± 4	83 ± 3
Ni^{2+}	71 ± 4	89 ± 2	93 ± 1
Cu^{2+}	46 ± 10	69 ± 6	81 ± 4
Zn^{2+}	45 ± 13	62 ± 7	72 ± 5
Cd^{2+}	43 ± 8	64 ± 5	70 ± 4

[a] The data for M(5′-AMP) are from Table 1, those for M(5′-IMP) and M(5′-GMP) from references 60*a* and 60*b*,*c* respectively.

Though further studies are necessary for the 5′-IMP^{2-} and 5′-GMP^{2-} systems,[60] the stability increase log $\Delta_{M(NMP)}$ as defined by equation 7 is accessible *via* the experimentally measured stability constant $K^{M}_{M(NMP)}$, which quantifies the overall stability of the M(NMP) complexes, and $K^{M}_{M(NMP)_{op}}$ which describes the stability of the complex with a sole phosphate coordination; this latter value is calculated *via* the straight reference-line equations, as indicated in Section 2.1.

As metal ion backbinding occurs to N-7 in M(5′-AMP), M(5′-IMP), and M(5′-GMP) complexes, one might expect that the extent depends on the basicity of this site. In other words, a plot of the stability increase log $\Delta_{M(NMP)}$ *versus* the pK_a of the N-7 site in these three 5′-NMPs might reveal further insights.[42] In a relative sense the basicity of the N-7 site in 5′-AMP^{2-}, 5′-IMP^{2-}, and 5′-GMP^{2-} should be reflected by the pK_a values of the corresponding nucleosides (Ns), adenosine (Ado), inosine (Ino), and guanosine (Guo), *i.e.* by $pk^{H}_{H(N\text{-}7/Ns)}$. The values $pK^{H}_{H(Ino)} = pk^{H}_{H(N\text{-}7/Ino)} = 1.06 \pm 0.04$ and $pK^{H}_{H(Guo)} = pk^{H}_{H(N\text{-}7/Guo)} = 2.11 \pm 0.04$ were measured,[47] and the one for $pk^{H}_{H(N\text{-}7/Ado)} = -0.2$ was recently estimated.[42]

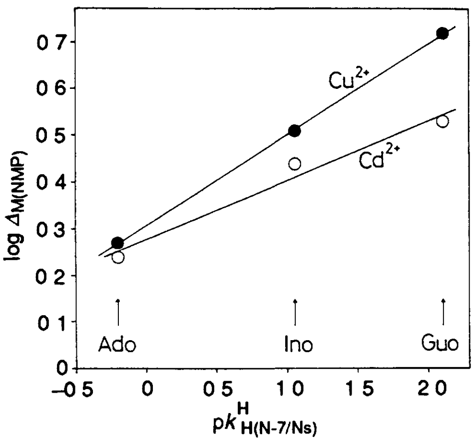


Figure 5 Relationship between log $\Delta_{M(NMP)}$ (equation (7)) for the Cu^{2+} (●) and Cd^{2+} (○) 1 1 complexes of 5′-AMP^{2-}, 5′-IMP^{2-} or 5′-GMP^{2} and $pk^{H}_{H(N\,7\,Ns)}$ of the corresponding nucleosides (Ns), adenosine (Ado), inosine (Ino), and guanosine (Guo) For details, including the origin of the data (25 °C, I = 0 1 M, $NaNO_3$), see ref 42
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Though the data for only a few metal ion systems are as yet available, it is clear from the results shown in Figure 5,[13 60 61] where log $\Delta_{M(NMP)}$ *versus* the mentioned $pk^{H}_{H(N\,7/Ns)}$ values are plotted for the Cu(5′-NMP) and Cd(5′-NMP) systems, that straight lines are obtained [42] This observation allows two conclusions (i) macrochelate formation in the M(5′-NMP) complexes depends on the basicity of N-7, (ii) the carbonyl oxygen at C-6 in 5′-IMP^{2-} and 5′-GMP^{2-} (see Figure 1) has apparently very little effect on the stability increase log $\Delta_{M(NMP)}$, this observation argues against the formation of five-membered chelates involving N-7 and O(C-6) next to the mentioned macrochelates However, the structural aspects of the formation of macrochelates (see Table 3) with alkaline earth ions warrant further studies

In the present context a recent IUPAC publication[22] on 'Stability Constants for Nucleotide Complexes with Protons and Metal Ions' has to be mentioned This compilation is 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, *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′-CMP), for Cd(5′-UMP), and for Cd(5′-dTMP) The value of (reference) for Cd(5′-GMP) *is much larger than the above values* and is not recommended" For the reader who 'digested' Section 2 1 and the results presented above in this section (as well as those described in Sections 5 2 and 5 3) the apparent discrepancy is quite clear the stability of the Cd^{2+} complexes with the aforementioned three pyrimidine nucleoside 5′-monophosphates is solely determined by the basicity of the corresponding phosphate groups, *i e* there is *no* nucleic base-metal ion interaction while the stability of Cd(5′-GMP) is significantly increased, *i e* log $\Delta_{Cd(5\,GMP)}$ = 0 53 ± 0 06 (see Figure 5),[61] owing to considerable base-backbinding to N-7 (see also Section 5 2) of the phosphate-coordinated Cd^{2+}, indeed, $Cd(5'\text{-}GMP)_{cl}$ is formed to 70 ± 4% (Table 3) It is evident that, most unfortunately, users of the IUPAC compilation[22] have to make their own judgements in selecting stability constants to prevent being misguided!

5.5 Isomeric Equilibria in Complexes of Adenosine 5′-Triphosphate (5′-ATP^{4-})

Stability constants measured by potentiometric pH titration for $M(5'\text{-}ATP)^{2-}$ complexes are in a number of instances larger than those of the corresponding complexes formed with pyrimidine nucleoside 5′-triphosphates ($PNTP^{4-}$) [17] As the stability of $M(PNTP)^{2-}$ species is solely determined by the binding properties of the triphosphate chain (see also Section 2 2) this allowed the definition of log $\Delta_{M(ATP)}$ (equation 7) and the calculation of the percentages of the closed form, $M(5'\text{-}ATP)^{2-}_{cl}$, according to equilibrium 4 in the way described in Section 5 2 The detailed results of potentiometric measurements, including those of various research groups, are given in references 7*b* and 17

In the present context it is important to note that other determinations of the percentages of $M(5'\text{-}ATP)^{2}_{cl}$ by UV difference spectrophotometry[18c 20] and ^1H NMR shift experiments[19] gave smaller values for % $M(5'\text{-}ATP)^{2}_{cl}$ than those obtained *via* potentiometric measurements This apparent discrepancy is especially clear-cut for $Mg(5'\text{-}ATP)^{2-}$ and $Ni(5'\text{-}ATP)^{2-}$ where approximately 0 and 30%, respectively, for the closed species were deduced in contrast to the 11 ± 6% and 56 ± 4% obtained *via* the potentiometric pH titrations

Taking into account that ultraviolet absorption and nuclear magnetic resonance spectroscopy techniques, which detect perturbations in the adenine ring, are sensitive mainly to inner-sphere coordination of the ring by a metal ion, the above observation has led to the suggestion[7b 17] that outer-sphere chelates are also formed It is evident that the stability increase detected by potentiometric pH titrations encompasses both inner-sphere and outer-sphere coordination of N-7, *i e* $M(5'\text{-}ATP)^{2}_{cl/tot}$ (see column 2 of Table 4), hence, the difference between % $M(5'\text{-}ATP)^{2-}_{cl/tot}$ and the percentage determined by the spectroscopic methods sensitive mainly to inner-sphere coordination, *i e* to $M(5'\text{-}ATP)^{2-}_{cl/i}$, should provide the percentage of the N-7 outer-sphere coordinated species, $M(5'\text{-}ATP)^{2-}_{cl/o}$ Of course, the difference between 100% and % $M(5'\text{-}ATP)^{2}_{cl\,tot}$ (= % $M(5'\text{-}ATP)^{2-}_{cl/i}$ + % $M(5'\text{-}ATP)^{2-}_{cl/o}$) gives the percentage of the open complex $M(5'\text{-}ATP)^{2-}_{op}$ Tentative, and simplified, structures of the two macrochelated species are shown in Figure 6

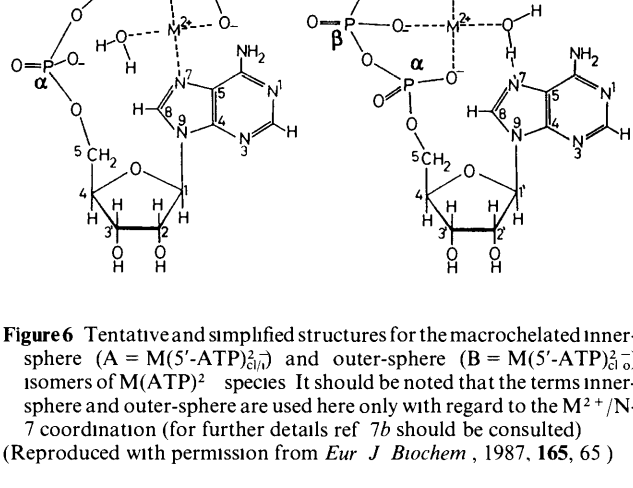

Figure 6 Tentative and simplified structures for the macrochelated inner-sphere (A = $M(5'\text{-}ATP)^{2-}_{cl/i}$) and outer-sphere (B = $M(5'\text{-}ATP)^{2-}_{cl\,o}$) isomers of $M(ATP)^{2}$ species It should be noted that the terms inner-sphere and outer-sphere are used here only with regard to the M^{2+}/N-7 coordination (for further details ref 7*b* should be consulted)
(Reproduced with permission from *Eur J Biochem* , 1987, **165**, 65)

The results summarized in Table 4 are the 'best' values presently available At this time only indirect evidence for the formation of N-7 outer-sphere macrochelates can be presented However, it should also be emphasized that in some of the examples[7b 17] the differences between $M(5'\text{-}ATP)^{2-}_{cl/tot}$ and $M(5'\text{-}ATP)^{2-}_{cl/i}$ are certainly beyond the experimental error limits, *e g* for the complexes with Ni^{2+} and Mg^{2+} Interestingly, the percentages of the inner-sphere closed forms (third column in Table 4) follow the usual stability series for dispositive 3*d* metal ions[2 62] including the stability constants for imidazole binding[44a 49] and the relative placements of Zn^{2+} and Cd^{2+}

It may be added that recent NMR evidence has been presented[53b] that in dilute neutral D_2O solutions *cis*-$Pt(ND_2CH_3)_2^{2+}$ coordinates to purine nucleoside 5′-triphosphates *via* N-7 and

Table 4 Estimates for the degree of formation of N-7 inner-sphere, $M(5'\text{-ATP})^{2}_{cl/i}^{-}$, and outer-sphere macrochelates, $M(5'\text{-ATP})^{2}_{cl/o}^{-}$, as well as for the 'open' species $M(5'\text{-ATP})^{2}_{cl/op}^{-}$ in aqueous solution (~ 25 °C, $I \approx 0.1$ M) The degree of formation for $M(5'\text{-ATP})^{2}_{cl/tot}^{-}$, which encompasses both inner-sphere and outer-sphere coordination to N-7, as determined from potentiometric pH titrations is given for comparison[a]

		Estimates for		
M^{2+}	% $M(5'\text{-ATP})^{2}_{cl/tot}^{-}$	% $M(5'\text{-ATP})^{2}_{cl\,i}^{-}$	% $M(5'\text{-ATP})^{2}_{cl\,o}^{-}$	% $M(5'\text{-ATP})^{2}_{op}^{-}$
Mg^{2+}	11 ± 6/13 ± 6	0	10	90
Ca^{2+}	2 ± 6	0	~0	100
Mn^{2+}	17 ± 10	~10	~10	80
Co^{2+}	38 ± 9/35 ± 10	~25	~15	60
Ni^{2+}	56 ± 4/58	30	25	45
Cu^{2+}	67 ± 2/68 ± 4	67	~0	33
Zn^{2+}	28 ± 7/26 ± 5	15	15	70
Cd^{2+}	46 ± 4/50 ± 6/52	30	20	50

These data are abstracted from Table 4 of ref 7*b* The data listed in the second column resulted from potentiometric pH titrations where two or more values are given in a row they are based on independent determinations [7*b*] For further details references 7*b* and 17 should be consulted

the γ-phosphate group in forming a macrochelate A further interesting result is the evidence[63] that in $Mg(5'\text{-ATP})^{2-}$ phosphate binding occurs as a mixture of β,γ-bidentate and α,β,γ-tridentate complexation (see also Section 2 3) Finally, there are indications[64] that for Cu^{2+} in strongly alkaline media the hydroxy groups in the ribose residue – due to deprotonation – become important binding sites

That N-7 coordination is the 'weak' point in macrochelate formation of the $M(5'\text{-ATP})^{2-}$ complexes is not surprising [7*c*] Indeed, the release of N-7 upon mixed ligand complex formation in solution has been demonstrated with ligands as different as OH^{-},[65] NH_3,[65*b*] imidazole,[65*b* 66] 2,2'-bipyridyl,[65*a* 67] 1,10-phenanthroline,[67*a*] and tryptophanate,[67*a* 68] and has also been confirmed for the solid state [69] These results suggest that the binary $M(5'\text{-ATP})^{2-}$ complex bound to an enzyme may exist as a closed macrochelate only when no enzyme groups coordinate directly to the metal ion

5.6 Comparison of the Extent of Macrochelate Formation in M(5'-AMP), M(5'-ADP) , $M(5'\text{-ATP})^{2-}$, $M(5'\text{-ITP})^{2-}$, and $M(5'\text{-GTP})^{2-}$ Systems

For the M(5'-AMP) species it appears that macrochelate formation to N-7 predominantly occurs in an inner-sphere fashion (Section 5 2) [13] However, for the other complexes mentioned in the above headline, aside from $M(5'\text{-ATP})^{2-}$, practically no such information is available and therefore all the following comparisons rely on results for $M(N)_{cl}$ ($= M(N)_{cl/tot}$), where N = nucleotide, as obtained *via* potentiometric pH titrations, though there are indications that in the case of $M(5'\text{-ADP})^{-}$ (*cf* references 20 and 23) similar isomeric equilibria exist, as discussed in Section 5 5 for $M(5'\text{-ATP})^{2-}$ systems

In columns 2, 3, and 4 of Table 5 the available data for % $M(5'AMP)_{cl}$, $M(5'\text{-ADP})^{-}_{cl}$, and $M(5'\text{-ATP})^{2-}_{cl}$, respectively, are listed The data for $M(5'\text{-ADP})^{-}$ are incomplete and it may be mentioned that the percentages for $M(5'\text{-ADP})^{-}_{cl}$ were calculated such that rather lower limits resulted [23] However, despite all shortcomings these results suggest that the total extent of macrochelate formation for the metal ions studied depends on the number of phosphate groups, and varies in the series % $M(5'\text{-AMP})_{cl}$ < % $M(5'\text{-ADP})^{-}_{cl}$ > % $M(5'\text{-ATP})^{2-}_{cl}$ This order possibly indicates that the macrochelates of $M(5'\text{-ADP})^{-}$ are less strained than those of M(5'-AMP), and that they also form more easily than those of $M(5'\text{-ATP})^{2-}_{cl}$, a result that may be due to the denticity of the different phosphate residues

For $M(5'\text{-ITP})^{2-}_{cl}$ and $M(5'\text{-GTP})^{2-}_{cl}$ the preliminary results available are listed in columns 5 and 6 of Table 5, respectively [60*a c* 70] Surprisingly, the degree of formation of the macrochelates for a given metal ion with the three purine nucleoside 5'-triphosphates, including $M(5'\text{-ATP})^{2-}_{cl}$, do not vary considerably (columns 4–6 of Table 5) This is different from the observations made with the corresponding monophosphates (Table 3) which were discussed in Section 5 4 At this stage it is difficult to provide a conclusive explanation for the different properties of the M(5'-NMP) and M(5'-NTP) complexes, maybe the higher basicities of N-7 in the inosine and guanosine residues are better suited for an inner-sphere coordination, whereas the adenosine residue (possibly due to the 6-NH_2 group) allows a higher degree of outer-sphere complex formation and this leads to similar overall percentages for the $M(5'\text{-NTP})^{2-}_{cl}$ species of a given metal ion

6 Solvent Influence on Metal Ion–(N-7) Base-Backbinding

The importance of a decreasing solvent polarity on the metal ion binding properties of phosphate groups has already been pointed out in Section 2 4 (*cf* also Figure 3) a decreasing solvent polarity considerably favours phosphate-complex stability! Of course, with the results on macrochelate formation discussed in

Table 5 Comparison of the extent of macrochelate formation according to equilibrium 4 for various complexes of 5'-nucleotides (N) expressed as the percentage of $M(N)_{cl}$ formed in aqueous solution at 25 °C and $I = 0.1$ M ($NaNO_3$)

M^{2+}	% $M(5'\text{-AMP})_{cl}$	% $M(5'\text{-ADP})^{-}_{cl}$	% $M(5'\text{-ATP})^{2-}_{cl}$	% $M(5'\text{-ITP})^{2-}_{cl}$	% $M(5'\text{-GTP})^{2-}_{cl}$
Mg^{2+}	7 ± 9	0	11 ± 6	0 (≤ 5)	9 ± 13
Ca^{2+}	0		2 ± 6	0 (≤ 3)	0 (≤ 7)
Mn^{2+}	15 ± 14	55	17 ± 10	37 ± 15	38 ± 14
Co^{2+}	49 ± 9	60	38 ± 9	41 ± 4	52 ± 5
Ni^{2+}	71 ± 4	80	56 ± 4	60 ± 4	74 ± 3
Cu^{2+}	46 ± 10	94	67 ± 2	55 ± 7	67 ± 7
Zn^{2+}	45 ± 13	67	28 ± 7	26 ± 9	28 ± 10
Cd^{2+}	43 ± 8		46 ± 4	55 ± 6	51 ± 4
Ref	13	23	17	60*a*	60*c*

Section 5 in mind, the question arises is macrochelate formation also affected by a decreasing solvent polarity?

So far only a very limited amount of data is available[71] and these refer to Cu(5′-AMP) and Cu(5′-ATP)$^{2-}$ complexes in water–dioxane mixtures The corresponding results are depicted in Figure 7 The surprising result is certainly the observation that the degree of formation of Cu(5′-AMP)$_{cl}$ passes through a minimum with increasing concentrations of 1,4-dioxane in water The same observation has also been reported recently for the Cu^{2+} complex formed with the dianion of 9-(2-phosphonylmethoxyethyl)adenine (PMEA^{2-})[34] Even though three isomeric complexes occur in this system,[15 34] the chelated isomer with a Cu^{2+}–adenine interaction also passes through a minimum upon the addition of increasing amounts of 1,4-dioxane to an aqueous solution containing Cu(PMEA) [34] Of course, in accord with previous experience (see Section 2 4), the overall stability of the Cu(5′-AMP), Cu(PMEA), and Cu(5′-ATP)^{2} complexes, which is mainly determined by the metal ion affinity of the phosphate residues, increases considerably for all three complexes with increasing amounts of 1,4-dioxane despite the evident changes in the degree of formation of the macrochelates

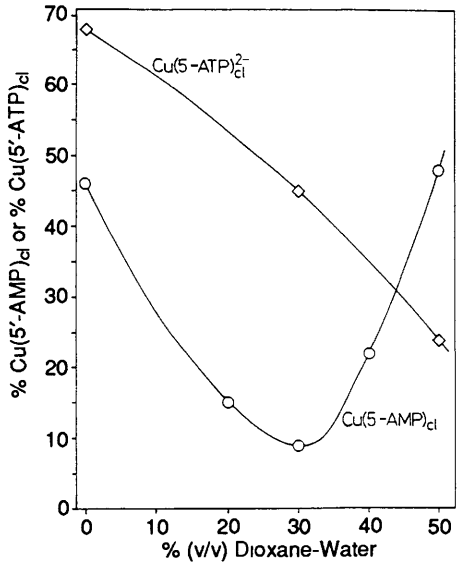

Figure 7 Degree of formation of the macrochelates (equilibrium 4) in the Cu(5′-AMP) (○) and Cu(5′-ATP)$^{2-}$ (◇) complex systems as a function of the percentage of 1,4-dioxane added to the aqueous reagent mixtures at 25 °C and I = 0 1 M ($NaNO_3$)
(Reproduced by permission from *Inorg Chem* , 1990, **29**, 3631)

Why does the degree of formation of the macrochelate for Cu(5′-AMP) (see Figure 7), and also for Cu(PMEA),[34] pass through a minimum? This observation is difficult to explain, but there have to be two opposing effects which result from the addition of 1,4-dioxane to an aqueous solution containing the complexes It could be, for example, that low amounts of 1,4-dioxane lead to a hydrophobic (lipophilic) solvation of the purine fraction of the nucleotides by the ethylene groups of 1,4-dioxane, and that in this way the binding site N-7 is shielded to some extent Upon addition of larger amounts of 1,4-dioxane to the aqueous solution no further shielding occurs but the activity of water decreases to the point where poor solvation results for those metal-ion sites not occupied by the phosphate group(s) of the nucleotides, consequently this poorer solvation leads to an increased affinity of these metal ion sites for other ligating groups, *i e* for N-7 Along these lines the observations made with Cu(5′-AMP), and also Cu(PMEA), could be explained Clearly, should this explanation be correct then for Cu(5′-ATP)$^{2-}_{cl}$ such a minimum at higher 1,4-dioxane concentrations should also be observable, unfortunately solubility problems prevent such a study

In any case, the above observations are meaningful – and they are also to be expected for other metal ion–nucleotide complexes – considering the substrate and product structures in enzymic reactions it is evident that at a protein–water interface subtle polarity changes are enough to favour either one of the structures shown in equilibrium 4 [72] Moreover, one wonders how far metal ion binding to nucleic acids[10 73] (Section 4 4), *e g* [74] of Pt^{2+}, is also affected by changes in the polarity of the surrounding solvent? In this respect it should be noted that not only the nucleic base parts alter their coordinating properties, but, as pointed out above, the metal ion affinity of phosphate residues also increases drastically with a decreasing solvent polarity (Section 2 4) Finally, the influence of such solvent changes on the reactivity has already been proven[9a 75] for the metal ion facilitated dephosphorylation of 5′-ATP

7 Some Reflections on Reactions Involving Metal Ion–Nucleic Base Interactions

The role of metal ions and their interaction with N-7, as well as the importance of self-stacking for the promoted dephosphorylation of 5′-ATP, has recently been reviewed [9] This reaction proceeds in the presence of, *e g* , Cu^{2+} or Zn^{2+} *via* a dimeric species, in which one 5′-ATP orientates, *via* N-7, the metal ion and the other 5′-ATP, such that a reactive state is achieved In other words, 5′-ATP can be considered as its own 'enzyme' in the metal ion facilitated hydrolysis The role of the structuring 5′-ATP[4] can also be taken over by 5′-AMP^{2-} These observations have led to the question [9b] have 5′-ATP^{4-} and related purine nucleotides played a role in early evolution? Clearly, in contemporary biochemistry 5′-ATP is still the most important energy-rich intermediate in metabolic processes Maybe 5′-ATP has conserved its eminent role for life over billions of years

It should also be emphasized in the present context that intramolecular equilibria of the type discussed in Sections 5 and 6 often involve only small changes in free energy (ΔG^0) The existence of 20% of a certain species, in rapid equilibrium with the other isomers, may be more than enough for a given enzymic reaction to proceed, however, the connected energy change is very small, *i e* , $\Delta G^0 = -0\,6$ kJ mol^{-1} [7c 11] It is evident that here Nature has a tool to achieve high selectivity by connecting various such equilibria without creating high energy barriers [72]

Another interesting aspect is the following one more than 25 years ago it was shown that the copper-catalysed disproportionation of H_2O_2 does not proceed *via* free HO radicals, but occurs[76 77] in the coordination sphere of Cu^{2+} Consequently, peroxidase-like reactions can be carried out in the coordination sphere of Cu^{2+} (*cf* reference 78) and the Cu^{2+}/H_2O_2 system can be used to probe the structures of complexes in solution [77 79] For example, evidence for base-backbinding in monomeric Cu^{2+} complexes of nucleoside 5′-triphosphates was provided by this method many years ago [77 80] Quantification of the peroxidase-like activity in such systems, *i e* degradation of the nucleic base residues within the coordination sphere of Cu^{2+} by H_2O_2, leads to the bell-shaped curves shown in Figure 8 In a certain pH range the activity increases with increasing pH due to backbinding of the nucleic base moiety to Cu^{2+} and then it decreases again at higher pH, due to the formation of hydroxo complexes which lead to a release of the base moiety from the coordination sphere of the metal ion (see Section 5 5) The bell-shaped curve in Figure 8 for the Cu^{2+}/5′-ATP/H_2O_2 system, passing through a maximum at a pH of about 8 5, can be easily understood in this way The observation of the reactivity maximum at a pH of about 9 5 in the corresponding systems with 5′-ITP and 5′-GTP is connected with a deprotonation of the H(N-1) group [43] Similarly, 5′-UTP and 5′-dTTP also undergo base backbinding at pH > 8 due to deprotonation of the H(N-3) unit [43] Finally, in the case of Cu(5′-CTP)$^{2-}$ only small amounts of a macrochelate are formed[17] and consequently the degradation of the base residue is not very pronounced in this case (see Figure 8)

The Cu^{2+}/H_2O_2 systems can also be used to probe macromolecules [77] For example, native DNA coordinates Cu^{2+} in neutral or slightly alkaline aqueous solution, preferentially *via*

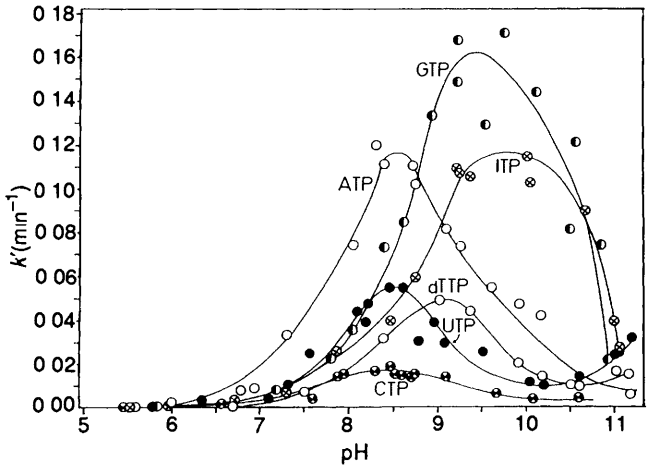

Figure 8 Peroxidase-like activity ($[H_2O_2] = 8 \times 10^{-3}$ M) for the Cu^{2+} complexes (4×10^{-4} M) of 5'-ATP (⊙), 5'-ITP (⊗), 5'-GTP (◐), 5'-CTP (⊖), 5'-UTP (●), and 5'-dTTP (○) ([NTP] = 4×10^{-4} M) at 22 °C and natural ionic strength The degradation of the nucleic bases was followed spectrophotometrically by measuring the decreasing absorption at the maximum, or in the case of pH dependent spectra at the isosbestic point, and by quantifying the reactivity *via* the calculation of the pseudo-first order rate constant k' (min^{-1}) For details see ref 80 and in part also 77
(Redrawn by permission from *Helv Chim Acta*, 1967, **50**, 582)

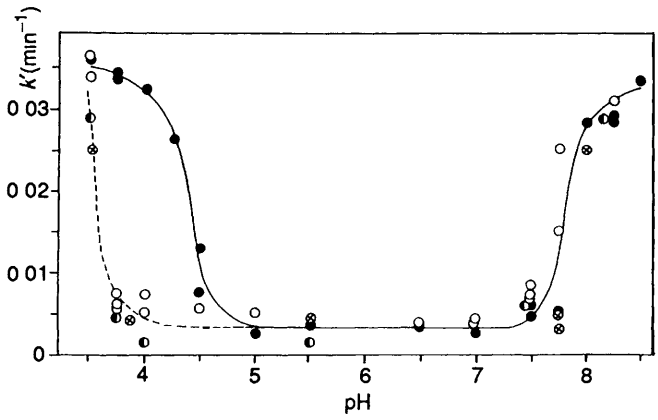

Figure 9 Denaturation of DNA under the influence of the pH of the solution (slow stirring for 16 hours) at 22 °C and natural ionic strength (●), and the stabilizing effect of Li^+ (⊗), Na^+ (○), and K^+ (◐) The peroxidase-like activity was used to characterize the extent of denaturation of DNA under the various conditions ([DNA] = 0 01% *i e*, 7 5 mg DNA were solved in 15 mL H_2O), *i e*, after the addition of Cu^{2+} (10^{-4} M) and H_2O_2 (8×10^{-3} M) the rate of degradation of the nucleic bases was measured at 260 nm in 2 mm quartz cells and expressed as the pseudo-first order rate constant k' (min^{-1}) The alkali ions (0 05 M), when present, were added as chlorides For details see references 83 and 77
(Redrawn by permission from *Biochim Biophys Acta*, 1968, **157**, 637)

the phosphate groups Addition of H_2O_2 gives rise to a catalase-like activity as well as to the formation of ternary peroxo complexes as observed by spectrophotometry,[81] but there is no evident degradation of the nucleic bases under these conditions However, if Cu^{2+} is added to DNA and the solution is kept at room temperature for 1 5 days, Cu^{2+} penetrates into DNA and coordinates to the base residues (*cf* also Section 4 4) Addition of H_2O_2 to such a solution leads then not only to a catalase-like activity but also to a peroxidase-like degradation of the nucleic base residues This can be followed by measuring the decreasing absorption at 260 nm [82] By such experiments, both native and denatured DNA can be distinguished RNA with its less complete base-pairing offers, even in the native form, nucleic base sites for Cu^{2+} binding, and consequently base degradation occurs in the presence of H_2O_2 [77 82]

DNA may be denatured in acidic solutions due to protonation of certain sites of the nucleic bases, similarly, in the alkaline pH range deprotonation of certain nucleic base sites occurs that again leads to denaturation and opening of the double helix These various events may also be nicely probed with the Cu^{2+}/H_2O_2 system, as seen in Figure 9 [83] Moreover, metal ion binding to the phosphate groups inhibits the *protonation* of the base residues and consequently stabilizes the double helix, whereas this kind of metal ion binding hardly affects *deprotonation* of the bases The corresponding effects of Li^+, Na^+, and K^+ are also seen in Figure 9 It may be added that a 100 times lower Mg^{2+} concentration (5×10^{-4} M) has the same stabilizing effect on DNA as the mentioned monovalent ions in 0 05 M solutions, an observation which corresponds approximately to the expected differences in phosphate-complex stability between the alkaline M^+ ions and Mg^{2+} [75] The indicated stabilizing effect of Mg^{2+} on DNA is well known [10] In this context a recent study[84] on the interaction of Cu^{2+} with DNA and the observed antagonism of various other metal ions should also be mentioned

A final point of interest which is indirectly connected with self-stacking properties of nucleic bases (see Section 5 1) is their interaction with other aromatic entities The stabilization of such interactions by a metal ion bridge was first shown[85] for the mixed ligand complex formed between Cu^{2+}, 2,2'-bipyridyl, and 5'-ATP^{4-} Meanwhile many more systems with such intramolecular stacks have been studied and in various instances the positions of the connected intramolecular equilibria quantified [72 86] Such interactions between nucleic base residues and amino acid side chains can also occur in mixed ligand–metal ion complexes containing a nucleotide and an amino acid residue In all these cases the nucleic base moiety is released from the coordination sphere of the metal ion upon formation of the intramolecular stack in the mixed ligand complex (Section 5 5) [66 86] Moreover, this type of interaction shows a significant degree of selectivity, for example, the affinity of M(5'-ATP)$^{2-}$ for the following amino acids decreases in the order tryptophanate > leucinate > alaninate [68 72] The importance of such interactions regarding the selectivity observed in Nature is evident

Acknowledgements The technical assistance of Ms Rita Baumbusch in the preparation of the manuscript, and the financial support for the research of my group on the complexes of phosphates, nucleosides, and nucleotides by the Swiss National Science Foundation are gratefully acknowledged

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