Stabilities and Isomeric Equilibria in Aqueous Solution of Monomeric Metal Ion Complexes of Adenosine 5′-Diphosphate (ADP^{3-}) in Comparison with Those of Adenosine 5′-Monophosphate (AMP^{2-})**

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Abstract: Under experimental conditions in which the self-association of the adenine phosphates (AP), that is, of adenosine 5′-monophosphate (AMP^{2-}) and adenosine 5′-diphosphate (ADP^{3-}), is negligible, potentiometric pH titrations were carried out to determine the stabilities of the M(H;AP) and M(AP) 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+} (25 °C; $I = 0.1$ M, $NaNO_3$). It is concluded that in the $M(H;AMP)^+$ species M^{2+} is bound at the adenine moiety and in the M(H;ADP) complexes at the diphosphate unit; however, the proton resides in both types of monoprotonated complexes at the phosphate residue. The stabilities of nearly all the M(AMP) and $M(ADP)^-$ complexes are significantly larger than what is expected for a sole coordination of M^{2+} to the phosphate residue. This increased complex stability is attributed, in agreement with previous ^1H NMR shift studies and further information existing in the literature, to the formation of macrochelates of the phosphate-coordinated metal ions with N7 of the adenine residues. On the basis of recent measurements with simple phosphate monoesters and phosphonate ligands ($R\text{-}MP^{2-}$) as well as with diphosphate monoesters ($R\text{-}DP^{3-}$), where R is a noncoordinating and noninhibiting residue, the increased stabilities of the M(AMP) and $M(ADP)^-$ complexes due to the M^{2+}–N7 interaction could be evaluated and the extent of macrochelate formation calculated. The results show that the formation degrees of the macrochelates for the complexes of the alkaline earth ions are small (about 15 % at the most), whereas for the 3d metal ions as well as for Zn^{2+} and Cd^{2+} the formation degrees vary between about 15 % (Mn^{2+}) and 75 % (Ni^{2+}) with values of about 40 and 50 % for Zn^{2+} and Cu^{2+}, respectively. It is interesting to note, taking earlier results for $M(ATP)^{2-}$ complexes also into account (ATP^{4-} = adenosine 5′-triphosphate), that for a given metal ion in nearly all instances the formation degrees of the macrochelates are within the error limits the same for M(AMP), $M(ADP)^-$ and $M(ATP)^{2-}$ complexes; except for Co^{2+} and Ni^{2+} it holds $M(AMP) > M(ADP)^- \sim M(ATP)^{2-}$. This result is astonishing if one considers that the absolute stability constants of these complexes, which are determined largely by the affinity of the phosphate residues, can differ by more than two orders of magnitude. The impact and conclusions of these observations for biological systems are shortly lined out.

Keywords: isomeric equilibria · macrochelates · metal-ion complexes · nucleotides · stability constants

1. Introduction

Nucleotides, especially the adenine nucleotides being substrates for a large number of enzymes, are at the crossroad of many biological reactions[1, 2] and the transfer of phosphoryl or nucleotidyl groups occurs in the presence of divalent metal ions.[3–5] For adenosine 5′-triphosphate (ATP^{4-}), the most prominent member of this class of compounds, Boyer has

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[**] Abbreviations and definitions (see also Figure 1): Ado, adenosine; AP, adenosine phosphate, i.e., AMP^{2-} and ADP^{3-}; ATP^{4-}, adenosine 5′-triphosphate; CDP^{3-}, cytidine 5′-diphosphate; I, ionic strength; IMP^{2-}, inosine 5′-monophosphate; K_a, general acidity constant; L, general ligand, including $R\text{-}MP^{2-}$ and $R\text{-}DP^{3-}$; M^{2+}, divalent metal ion; $MeMP^{2-}$, methyl monophosphate; NDP^{3-}, nucleoside 5′-diphosphate; NMP^{2-}, nucleoside 5′-monophosphate; NTP^{4-}, nucleoside 5′-triphosphate; $R\text{-}DP^{3-}$, diphosphate monoester with a non-coordinating residue R; $R\text{-}MP^{2-}$, monophosphate monoester including phosphonate ligands, R being a non-coordinating residue; UDP^{3-}, uridine 5′-diphosphate. Species written in the text without a charge either 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;ADP), the H^+ and ADP^{3-} 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.

  0947-6539/03/0904-0881 $ 20.00+.50/0

estimated,[6] based on known metabolic pathways and the extent of the world's biomass, that ATP and ADP and inorganic phosphate (P_i) from which it is formed participate in more chemical reactions than any other compound on the Earth's surface except water. This example demonstrates how closely the fates of ATP and ADP are interwoven with each other. Since ATP is used in the generation of cell components, in muscle contractions, transmission of nerve messages and many other functions,[1, 7] ADP (Figure 1)[8–10] is involved here as well.

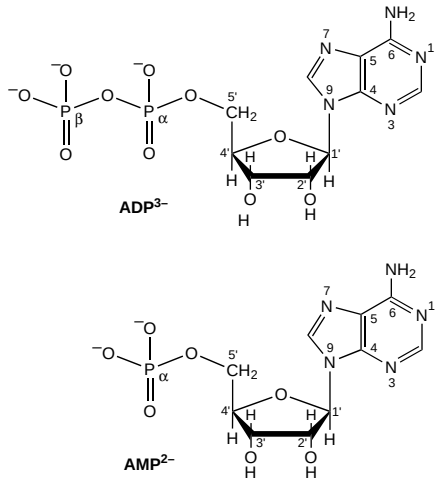
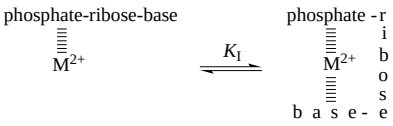


Figure 1. Chemical structure of adenosine 5′-diphosphate (ADP^{3-}) and adenosine 5′-monophosphate (AMP^{2-}) in their dominating *anti* conformation.[8–10]

A further example is the hydrolysis of $Mg(ATP)^{2-}$ to $Mg(ADP)^{-}$ which is coupled to the electron transfer from the Fe protein to the MoFe protein of Mo-containing nitrogenases which are two-component enzyme systems.[11] The presence of Mg^{2+} is also required for ADP-ribosylactin hydrolase which is suggested to have the function of polymerizing actin for signal transduction in the cytosol of nerve cells and synaptosomes.[12]

Considering the indicated interrelations between metal ions and nucleotides, it is not surprising that a remarkable amount of thermodynamic data exist on the metal ion-binding properties of nucleotides in solution[13–16] and there is also significant information available on complexes in the solid state.[10, 17, 18] However, the available literature data[19–21] for solutions concern so far mostly complexes of nucleoside monophosphates (NMP^{2-})[16, 22] and nucleoside triphosphates (NTP^{4-}).[16, 23–25] As far as the metal-ion binding properties of nucleoside diphosphates (NDP^{3-}) are concerned, the information is scarce[26, 27] and for ADP in practice only a single comprehensive study exists[28] with most of the stability constants being labelled as "tentative".[20] That macrochelate formation of a phosphate-coordinated metal ion (M^{2+}) by interacting in addition with N7 of the purine moiety may occur, an idea that goes back to Szent-Györgyi,[29, 30] has been proven for M(NMP) (cf.[31–33]) and $M(NTP)^{2-}$ complexes[34–36] of several divalent metal ions and it has also been shown by ^1H NMR shift measurements to occur[37] in the ADP^{3-} systems with Zn^{2+} and Cd^{2+}, but a comprehensive evaluation regarding the position of the intramolecular Equilibrium (1)[38] for $M(NDP)^{-}$ complexes is missing.



(1)

Since the structural variation of complexes, which are potential substrates in enzymatic reactions, are important to be known, we have now endeavored to measure in aqueous solution (25 °C; $I = 0.1$ M, $NaNO_3$) a comprehensive set of stability constants for $M(ADP)^{-}$ complexes as well as of its protonated form, M(H;ADP), for the alkaline earth metal ions and for the 3d ions Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} as well as for Cd^{2+}. The corresponding M(AMP) complexes have been studied previously[31] but the formation of $M(H;AMP)^{+}$ species had been ignored;[39] therefore we have reinvestigated also the AMP (Figure 1) systems.[39] These results, in combination with recently published data for simple diphosphate monoesters and their M^{2+} complexes,[26] are then used to calculate the position of Equilibrium (1). The surprising result is that despite significant differences in the stability constants between the M(AMP) and $M(ADP)^{-}$ complexes (≤ 1.2 log units) the formation degree of the macrochelated isomers according to Equilibrium (1) is astonishingly similar.

2. Results and Discussion

Purine derivatives undergo self-association due to stacking of their nucleobase ring systems.[40, 41] Therefore, all potentiometric pH titrations (25 °C; $I = 0.1$ M, $NaNO_3$), the results of which are summarised below, were carried out at ligand concentrations of 0.3 and 0.6 mM. Under these conditions self-stacking of ADP is negligible;[37] this is also true for AMP[31] (see also ref. [22]). In fact, with the self-association constant $K = 15\ M^{-1}$ (which holds for adenosine)[42] one calculates that in a 1 mM solution about 97% of the species are present in their monomeric form. This means, the low nucleotide concentrations employed in this study guarantee that the properties of the monomeric species were being studied indeed.

2.1. Acidity constants of $H_4(ADP)^{+}$ and $H_3(AMP)^{+}$: The deprotonated nucleotide ADP^{3-} is a tetrabasic species: It can accept three protons at the diphosphate group and one at the N1 site of the adenine moiety[8] to give the acid $H_4(ADP)^{+}$. First, one of the two primary protons of the diphosphate residue is released; its pK_a is very low (<1). The next proton is the second primary proton from the diphosphate group and its acidity was measured [Eq. (2)]; next, deprotonation of the $(N1)H^{+}$ site (see Figure 1) occurs [Eq. (3)] which is followed by the release of the secondary proton from the terminal β-phosphate group [Eq. (4)].

$$H_3(ADP)^{\pm} \rightleftharpoons H_2(ADP)^{-} + H^{+} \quad (2a)$$

$$K^{H}_{H_3(ADP)} = [H_2(ADP)^{-}][H^{+}]/[H_3(ADP)^{\pm}] \quad (2b)$$

0947-6539/03/0904-0882 $ 20.00+.50/0 *Chem. Eur. J.* **2003**, *9*, No. 4

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

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

The analogous equations hold for the deprotonation reactions of $H_3(AMP)^+$, the first proton released being the primary one from the phosphate group [Eq. (2)], and so on.

The measured acidity constants are summarised in Table 1 together with some values of related compounds.[43, 44] The pK_a values determined now for $H_2(AMP)^\pm$ are identical with those measured previously in our laboratory[31] and that for $H(ADP)^{2-}$ agrees well with the "recommended" value given in ref. [20]. All values are also in accord with those obtained for $H_3(ATP)^-$ and other $H_3(NTP)^-$ species (see the discussion below).[45]

A view on the data listed in Table 1 by including the results for adenosine (Ado) and methyl monophosphate ($MeMP^{2-}$) confirms immediately that the site attributions given above for the release of the various protons according to Equilibria (2a), (3a), and (4a), are correct. In addition, several further comparisons are possible with the constants given in Table 1, a few follow: The basicity-enhancing effect of a second phosphate group on the release of the final primary proton, which is possibly distributed between the α- and β-phosphate groups in the case of ADP, is evident from Equation (5):

Table 1. Negative logarithms of the acidity constants of $H_3(AMP)^+$ and $H_3(ADP)^\pm$ [Eqs. (2)–(4)], as determined by potentiometric pH titrations in aqueous solution (25 °C; $I = 0.1$ M, $NaNO_3$), together with some further related data.[a]

Acid	pK_a for the sites		
	prim. phos. site[b]	$(N1)H^+$	$-P(O)_2(OH)^-$
$H(Ado)^+$		3.61 ± 0.03[9]	
$H_2(MeMP)$	1.1 ± 0.2[43]		6.36 ± 0.01[43]
$H_3(AMP)^+$	0.4 ± 0.2[9]	3.84 ± 0.02	6.21 ± 0.01
$H_3(ADP)^\pm$	1.02 ± 0.20	3.92 ± 0.02	6.40 ± 0.01
$H_3(ATP)^-$	1.7 ± 0.1[44]	4.00 ± 0.01[23]	6.47 ± 0.01[23]

[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. So-called practical (or mixed) acidity constants are listed; see Section 4.2. [b] Primary phosphate site, i.e., $-P(O)(OH)_2$, $-P_2(O)_4(OH)_2^-$, and $-P_3(O)_7(OH)_2^{2-}$ for MeMP and AMP, ADP, and ATP, respectively.

$$\Delta pK_{a/5} = pK^H_{H_3(ADP)} - pK^H_{H_3(AMP)} = (1.02 \pm 0.20) - (0.4 \pm 0.2) = 0.62 \pm 0.28 \quad (5)$$

Furthermore, the fact that $\Delta pK_{a/5}$ is of the same size as for the comparison made in Equation (6),

$$\Delta pK_{a/6} = pK^H_{H_3(ATP)} - pK^H_{H_3(ADP)} = (1.7 \pm 0.1) - (1.02 \pm 0.20) = 0.68 \pm 0.22 \quad (6)$$

suggests that the proton released from $H_3(ADP)^\pm$ is actually largely located at the α-phosphate group and therefore addition of a further phosphate group leads to an effect corresponding to that seen in Equation (5). This is different regarding the comparisons for the release of the proton from the terminal phosphate group, where a decrease in ΔpK_a is observed because the additional negative charge is further away [Eqs. (7) and (8)]:

$$\Delta pK_{a/7} = pK^H_{H(ADP)} - pK^H_{H(AMP)} = (6.40 \pm 0.01) - (6.21 \pm 0.01) = 0.19 \pm 0.01 \quad (7)$$

$$\Delta pK_{a/8} = pK^H_{H(ATP)} - pK^H_{H(ADP)} = (6.47 \pm 0.01) - (6.40 \pm 0.01) = 0.07 \pm 0.01 \quad (8)$$

That the protonated $(N1)H^+$ site facilitates the release of the primary proton in $H_3(AMP)^+$ ($pK^H_{H_3(AMP)} = 0.4 \pm 0.2$) follows from a comparison with the $pK^H_{H_2(MeMP)}$ value ($= 1.1 \pm 0.2$) of $H_2(MeMP)$. However, the effect of a further phosphate group, as described by Equation (5), remains within the error limits the same as follows from a comparison with methyl diphosphate ($MeDP^{3-}$) as given in Equation (9):

$$\Delta pK_{a/9} = pK^H_{H_2(MeDP)} - pK^H_{H_2(MeMP)} = (1.62 \pm 0.09/\text{from ref. [26]}) - (1.1 \pm 0.2) = 0.52 \pm 0.22 \quad (9)$$

The influence that an additional phosphate group exerts on the acid–base properties of the $(N1)H^+$ site is also constant as follows from Equations (10) and (11):

$$\Delta pK_{a/10} = pK^H_{H_2(ADP)} - pK^H_{H_2(AMP)} = (3.92 \pm 0.02) - (3.84 \pm 0.02) = 0.08 \pm 0.03 \quad (10)$$

$$\Delta pK_{a/11} = pK^H_{H_2(ATP)} - pK^H_{H_2(ADP)} = (4.00 \pm 0.01) - (3.92 \pm 0.02) = 0.08 \pm 0.02 \quad (11)$$

To conclude, the results given in Equations (5)–(11) prove the 'inner' consistency of the data assembled in Table 1 and they also provide confidence for extrapolations towards values for systems which have not yet been studied.

2.2. Stability constants of M^{2+} complexes of AMP and ADP: The experimental data of the potentiometric pH titrations of the M^{2+}/ADP systems, where $M^{2+} = Mg^{2+}$, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} or Cd^{2+}, are completely described by Equilibria (3), (4), (12) and (13),

$$M^{2+} + H(ADP)^{2-} \rightleftharpoons M(H;ADP) \quad (12a)$$
$$K^M_{M(H;ADP)} = [M(H;ADP)]/([M^{2+}][H(ADP)^{2-}]) \quad (12b)$$

$$M^{2+} + ADP^{3-} \rightleftharpoons M(ADP)^- \quad (13a)$$
$$K^M_{M(ADP)} = [M(ADP)^-]/([M^{2+}][ADP^{3-}]) \quad (13b)$$

if the evaluation is not carried into the pH range where formation of hydroxo complexes occurs (see Section 4.4). The acidity constant of the connected Equilibrium (14) may be calculated with Equation (15).

$$M(H;ADP) \rightleftharpoons M(ADP)^- + H^+ \quad (14a)$$
$$K^H_{M(H;ADP)} = [M(ADP)^-][H^+]/[M(H;ADP)] \quad (14b)$$

$$pK^H_{M(H;ADP)} = pK^H_{H(ADP)} + \log K^M_{M(H;ADP)} - \log K^M_{M(ADP)} \quad (15)$$

The analogous equations hold for the M^{2+}/AMP systems. The results obtained for Equilibria (12a), (13a) and (14a), and their analogues with AMP, concerning the M^{2+} complexes of

Table 2. Logarithms of the stability constants of M(H;AP) [Eq. (12)] and M(AP) complexes [Eq. (13)], where AP = AMP^{2-} or ADP^{3-}, as determined by potentiometric pH titrations in aqueous solution, together with the negative logarithms of the acidity constants [Eqs. (14) and (15)] of the corresponding M(H;AP) complexes (25 °C; $I = 0.1$ M, $NaNO_3$).[a]

AP	M^{2+}	$\log K^{M}_{M(H;AP)}$	$\log K^{M}_{M(AP)}$	$pK^{H}_{M(H;AP)}$
AMP^{2-}	Mg^{2+}	0.0 ±0.3[b]	1.62 ± 0.04	4.6 ±0.3
	Ca^{2+}	−0.2 ±0.3[b]	1.48 ± 0.03	4.5 ±0.3
	Sr^{2+}	−0.3 ±0.3[b]	1.26 ± 0.02	4.7 ±0.3
	Ba^{2+}	−0.4 ±0.3[b]	1.18 ± 0.04	4.6 ±0.3
	Mn^{2+}	0.3 ±0.3	2.23 ± 0.02	4.3 ±0.3
	Co^{2+}	0.88 ± 0.15	2.30 ± 0.04	4.79 ± 0.16
	Ni^{2+}	1.05 ± 0.15	2.55 ± 0.04	4.71 ± 0.16
	Cu^{2+}	1.5 ±0.2	3.17 ± 0.02	4.54 ± 0.20
	Zn^{2+}	0.8 ±0.3[b]	2.38 ± 0.07	4.63 ± 0.31
	Cd^{2+}	1.15 ± 0.10	2.74 ± 0.05	4.62 ± 0.11
ADP^{3-}	Mg^{2+}	1.68 ± 0.10	3.36 ± 0.03	4.72 ± 0.10
	Ca^{2+}	1.5 ±0.25[b]	2.95 ± 0.02	4.95 ± 0.25
	Sr^{2+}	1.2 ±0.25[b]	2.42 ± 0.03	5.18 ± 0.25
	Ba^{2+}	1.12 ± 0.16	2.37 ± 0.06	5.15 ± 0.17
	Mn^{2+}	2.38 ± 0.22	4.22 ± 0.02	4.56 ± 0.22
	Co^{2+}	2.07 ± 0.14	3.92 ± 0.02	4.55 ± 0.14
	Ni^{2+}	2.26 ± 0.15	3.93 ± 0.02	4.73 ± 0.15
	Cu^{2+}	2.77 ± 0.16	5.61 ± 0.03	3.56 ± 0.16
	Zn^{2+}	2.31 ± 0.20	4.28 ± 0.05	4.43 ± 0.21
	Cd^{2+}	2.57 ± 0.12	4.63 ± 0.04	4.34 ± 0.13

[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 present case for column 5, were calculated according to the error propagation after Gauss. [b] These values are estimates.

AMP and ADP are listed in columns 3, 4 and 5 of Table 2, respectively.

The agreement between the present results for the M(AMP) complexes and the previous ones[31] is excellent despite the fact that now the formation of $M(H;AMP)^+$ complexes has been considered: only for the Co(AMP) complex now a stability constant was measured which is 0.07 log unit larger; similarly, Ni(AMP) and Cd(AMP) are now 0.06 log unit more stable; in all other instances the deviations are less than 0.03 log unit. For the log stability constant of the $Ni(H;AMP)^+$ complex values between 1.0 and 1.2 are listed in ref. [14], which is in accord with the present result; however, the values given there[14] for $Co(H;AMP)^+$ and $Cu(H;AMP)^+$ are by more than 0.5 log unit too large. No other such values have apparently been determined before.

If one compares earlier results obtained for the stabilities of the $M(ADP)^-$ complexes, based especially on refs. [14] and [20], one may conclude that the early values of Taqui Kahn and Martell[28a] agree in several instances well with the present results: For the complexes Mg(H;ADP), Ca(H;ADP), Co(H;ADP), Ni(H;ADP), $Ca(ADP)^-$, $Ba(ADP)^-$, $Mn(ADP)^-$ and $Zn(ADP)^-$, the log stability constants agree within ±0.1 log unit; however, for other complexes rather large deviations are observed; especially the former value[28a] for the stability of $Ni(ADP)^-$ is 0.57 log unit too high. On the other hand the value of Frey and Stuehr,[46] $\log K^{Ni}_{Ni(ADP)} = 4.18$, which was determined at 15 °C ($I = 0.1$ M, KNO_3) is in reasonable accord with the present result if one takes into account the difference in temperature at which the experiments were carried out; this also holds for $\log K^{Ni}_{Ni(H;ADP)} = 2.30$ from the same source.[46] The very recently published stability constants of M(AMP) and $M(ADP)^-$ complexes[47] should be ignored; in this study the formation of protonated complexes was not considered.

2.3. Structural considerations on the monoprotonated complexes in solution. The proton is at the phosphate group! Potentiometric pH titrations allow determination of the stability constants of the $M(H;AMP)^+$ and M(H;ADP) complexes, but in order to locate the binding sites of the proton and the metal ion in these species, further information is needed. At first one best considers the proton because binding of a metal ion to a protonated ligand commonly leads to an acidification of the ligand-bound proton.[48] Indeed, the acidity constants of the $M(H;AMP)^+$ complexes given in column 5 of Table 2 ($pK^{H}_{M(H;AMP)} \cong 4.6$) are on average 1.6 p$K$ units smaller than the value listed in column 4 of Table 1 for the $H(AMP)^-$ species ($pK^{H}_{H(AMP)} = 6.21$), but the acidity constants of the $M(H;AMP)^+$ complexes are on average also about 0.8 pK units *larger* than the $pK^{H}_{H_2(AMP)}$ value (= 3.84) which quantifies the release of the proton from the $(N1)H^+$ site; hence, the proton must be located at the phosphate group of AMP^{2-} in the $M(H;AMP)^+$ complexes.

The corresponding considerations also hold for the M(H;ADP) complexes: Here the average value for the deprotonation of the M(H;ADP) complexes ($pK^{H}_{M(H;ADP)} = 4.7 \pm 0.4$), ignoring the one for Cu(H;ADP), is also about 1.7 pK units below $pK^{H}_{H(ADP)}$ (= 6.40; Table 1) and about 0.8 pK unit *above* $pK^{H}_{H_2(ADP)}$ (= 3.92). Hence, in all the M(H;ADP) species, except for Cu(H;ADP) which will be discussed below, the proton must evidently also be located at the diphosphate residue and here at the terminal β-phosphate group because this is the most basic site in this residue.

However, where is the metal ion? Tentatively one might argue that if the proton is at the phosphate group then it appears likely that M^{2+} is at the nucleobase residue, at least in the case of the $M(H;AMP)^+$ complexes. In fact, that the stabilities of the $M(H;AMP)^+$ complexes (Table 2, column 3) *follow* the Irving–Williams sequence[49] (in contrast to phosph(on)ate complexes)[16, 26, 43, 50] also supports[51] the suggestion that metal ion binding occurs preferably at a nitrogen atom. At the same time the irregular order observed for the stabilities of the M(H;ADP) complexes (see Table 2, column 3) suggests that in these instances the metal ion is located at the monoprotonated diphosphate residue. These tentative reasonings are confirmed by the following evaluations.

2.4. Considerations on the location of M^{2+} in the $M(H;AMP)^+$ complexes: For the location of M^{2+} in principle two possibilities exist: i) the metal ion is at the phosphate group like the proton, symbolised by $(AMP \cdot M \cdot H)^+$, and ii) it is at the nucleobase, symbolised by $(M \cdot AMP \cdot H)^+$. Hence, Equation (12b) may be rewritten for $M(H;AMP)^+$ in the form of Equation (16):

$$K^{M}_{M(H;AMP)} = \frac{[(M{\cdot}AMP{\cdot}H)^+] + [(AMP{\cdot}M{\cdot}H)^+]}{[M^{2+}][H(AMP)^-]} \quad (16a)$$

$$= k^{M}_{M{\cdot}AMP{\cdot}H} + k^{M}_{AMP{\cdot}M{\cdot}H} \quad (16b)$$

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Estimations of the micro stability constant $k^{M}_{M\cdot AMP\cdot H}$ may be made in some cases by using available stability constants of $M(Ado)^{2+}$ complexes.[52] The known[52, 53] N1 versus N7 dichotomy for metal ion binding to the adenine residue is not of relevance in the present context, though there are indications that binding to N7 dominates.[53]

For the AMP systems, the stability constant $\log K^{M}_{M(Ado)}$ of $M(Ado)^{2+}$ needs to be corrected i) for the different basicities of the N1 site in $H(AMP)^{-}$ and Ado, and ii) for the charge effect that the $-P(O)_2(OH)^{-}$ group exerts on the M^{2+} bound at the adenine residue in $(M\cdot AMP\cdot H)^{+}$.[54, 55] In addition, iii) one has to consider that part of the M^{2+} ions in the $(M\cdot AMP\cdot H)^{+}$ species may form a macrochelate, most likely outersphere, with the $-P(O)_2(OH)^{-}$ group; for such outersphere interactions also crystal structure data exist.[56] Hence, the stability of the $(M\cdot AMP\cdot H)^{+}$ species will be further enhanced.[57] To give an example, this estimation results[58] for $(Cu\cdot AMP\cdot H)^{+}$ in $\log k^{Cu}_{Cu\cdot AMP\cdot H} = 1.44 \pm 0.24$ and this value is evidently identical within the error limits with the measured value, $\log K^{M}_{M(H;AMP)} = 1.5 \pm 0.2$, meaning that the stability of the $Cu(H;AMP)^{+}$ species is determined by the stability of the $(Cu\cdot AMP\cdot H)^{+}$ isomer [cf. Eq. (16)] which carries Cu^{2+} at the adenine residue and the proton at the phosphate group and that the formation of the $(AMP\cdot Cu\cdot H)$ isomer with both Cu^{2+} and H^{+} at the phosphate group is negligible.

To a first approximation the above conclusion is certainly correct and it also holds for the other $(M\cdot AMP\cdot H)^{+}$ species for which an evaluation could be carried out, that is for Co^{2+}, Ni^{2+}, Zn^{2+} and Cd^{2+} (see Table 3). However, a closer look at the values in Table 3 seems to indicate, despite the large error limits, that the estimates for $\log k^{M}_{M\cdot AMP\cdot H}$, if compared with the measured values for $\log K^{M}_{M(H;AMP)}$ are somewhat too small. If this indication should be correct, then there are two possible explanations, i) the extent of chelate formation in the $(M\cdot AMP\cdot H)^{+}$ species is somewhat larger than assumed[57] or ii) the second term in Equation (16b) is also of some relevance.

Table 3. Comparison of the estimated micro stability constants, $\log k^{M}_{M\cdot AMP\cdot H}$ [Eq. (16)], for the $(M\cdot AMP\cdot H)^{+}$ species with the measured stability constants, $\log K^{M}_{M(H;AMP)}$, for the $M(H;AMP)^{+}$ complexes.[a]

M^{2+}	$\log K^{M}_{M(Ado)}$[b]	$\Delta pK_{a/cor}$[d]	$\log k^{M}_{M\cdot AMP\cdot H}$[e]	$\log K^{M}_{M(H;AMP)}$[f]
Co^{2+}	0.2 ±0.3	0.03 ± 0.02	0.73 ± 0.36	0.88 ± 0.15
Ni^{2+}	0.41 ± 0.22	0.04 ± 0.02	0.95 ± 0.30	1.05 ± 0.15
Cu^{2+}	0.85 ± 0.12	0.09 ± 0.05	1.44 ± 0.24	1.5 ±0.2
Zn^{2+}	0.24 ± 0.30	0.04 ± 0.02	0.78 ± 0.36	0.8 ±0.3
Cd^{2+}	0.60 ± 0.10[c]	0.05 ± 0.03	1.15 ± 0.23	1.15 ± 0.10

[a] The error limits are either 3σ or estimates; the limits of the derived data were calculated according to the error propagation after Gauss. [b] Values (mostly based on the work of Lönnberg and Arpalahti)[59] taken from the compilation given in ref. [52]. [c] L. E. Kapinos and H. Sigel, preliminary result; details to be published. [d] Correction for the basicity difference of N1 in $H(AMP)^{-}$ and Ado, i.e., $\Delta pK_a = pK^{H}_{H_2(AMP)} - pK^{H}_{H(Ado)} = (3.84 \pm 0.02) - (3.61 \pm 0.03) = 0.23 \pm 0.04$ (see Table 1), by applying the following slopes m for $\log K$ versus pK_a straight-line plots (averages of the slopes obtained for series of benzimidazole-type[60] and *ortho*-substituted pyridine-type[61] ligands): $m = 0.126$ (Co^{2+}), 0.158 (Ni^{2+}), 0.394 (Cu^{2+}), 0.194 (Zn^{2+}) and 0.212 (Cd^{2+}). The values $\Delta pK_{a/cor}$ (error limits estimated) result from $\Delta pK_a \cdot m$. [e] Addition of $\Delta pK_{a/cor}$ to $\log K^{M}_{M(Ado)}$ plus the correction for the charge and chelate effects (0.50 ± 0.20),[57] which the $-P(O)_2(OH)^{-}$ group exerts on M^{2+} at the adenine residue including some backbinding to the protonated phosphate group, gives the value for $\log k^{M}_{M\cdot AMP\cdot H}$. For a complete calculation example see note [58]. [f] From column 3 in Table 2.

It seems worthwhile to investigate the second possibility somewhat further and to attempt to make an estimate for the stability of the $(AMP\cdot M\cdot H)^{+}$ isomer because this should quite generally and independent of the present cases provide some further insights in intramolecular equilibria of this kind (which are analogous to Equilibrium (1)).

To make an estimate for the stability constants of the $(AMP\cdot M\cdot H)^{+}$ species in which both M^{2+} and H^{+} are bound to the phosphate group,[62] and which thus correspond to the open isomer in Equilibrium (1), is difficult because no stability constants for a $-P(O)_2(OH)^{-}/M^{2+}$ interaction are available. The only way we can see at present is to apply the stability constants of complexes formed with monoprotonated and free diphosphate monoesters.[26] The estimates obtained[63] for the stability constants of the $(AMP\cdot M\cdot H)^{+}$ species are rather upper limits because of the assumptions made.[63] The values are $\log k^{M}_{AMP\cdot M\cdot H} = 0.26$ (Co^{2+}), 0.64 (Ni^{2+}), 0.1 (Cu^{2+}), 0.36 (Zn^{2+}), and 0.72 (Cd^{2+}) (error limits ±0.3). Hence, application of these values plus those given in column 4 of Table 3 allows with Equation (16b) to calculate stability constants, $\log K^{M}_{M(H;AMP)_{calcd}}$, for the M(H;AMP) complexes. The results are:

$$K^{M}_{M(H;AMP)_{calcd}} = k^{M}_{M\cdot AMP\cdot H} + k^{M}_{AMP\cdot M\cdot H} \quad (16b)$$

$$Co^{2+}: \quad K^{Co}_{Co(H;AMP)_{calcd}} = 10^{(0.73\pm0.36)} + 10^{(0.26\pm0.3)} \quad \log K^{Co}_{Co(H;AMP)_{calcd}} = 0.86 \pm 0.28 \quad (16c)$$

$$Ni^{2+}: \quad K^{Ni}_{Ni(H;AMP)_{calcd}} = 10^{(0.95\pm0.30)} + 10^{(0.64\pm0.3)} \quad \log K^{Ni}_{Ni(H;AMP)_{calcd}} = 1.12 \pm 0.22 \quad (16d)$$

$$Cu^{2+}: \quad K^{Cu}_{Cu(H;AMP)_{calcd}} = 10^{(1.44\pm0.24)} + 10^{(0.1\pm0.3)} \quad \log K^{Cu}_{Cu(H;AMP)_{calcd}} = 1.46 \pm 0.23 \quad (16e)$$

$$Zn^{2+}: \quad K^{Zn}_{Zn(H;AMP)_{calcd}} = 10^{(0.78\pm0.36)} + 10^{(0.36\pm0.3)} \quad \log K^{Zn}_{Zn(H;AMP)_{calcd}} = 0.92 \pm 0.27 \quad (16f)$$

$$Cd^{2+}: \quad K^{Cd}_{Cd(H;AMP)_{calcd}} = 10^{(1.15\pm0.23)} + 10^{(0.72\pm0.3)} \quad \log K^{Cd}_{Cd(H;AMP)_{calcd}} = 1.29 \pm 0.19 \quad (16g)$$

Evidently, these calculated stability constants, given in Equations (16c) – (16g), for the $M(H;AMP)^{+}$ complexes also agree well within the error limits with the measured ones as a comparison with the final column in Table 3 demonstrates.

A further insight is gained by the application of the two estimated micro stability constants in the calculation of the ratio R for the two isomeric species:

$$R_M = \frac{[(M\cdot AMP\cdot H)^{+}]}{[(AMP\cdot M\cdot H)^{+}]} = \frac{k^{M}_{M\cdot AMP\cdot H}}{k^{M}_{AMP\cdot M\cdot H}} \quad (17a)$$

$$R_{Co} = \frac{10^{(0.73\pm0.36)}}{10^{(0.26\pm0.3)}} = 10^{(0.47\pm0.47)} \cong \frac{3.0}{1} = \frac{75}{25} \quad (17b)$$

$$R_{Ni} = \frac{10^{(0.95\pm0.30)}}{10^{(0.64\pm0.3)}} = 10^{(0.31\pm0.42)} \cong \frac{2.0}{1} = \frac{67}{33} \quad (17c)$$

$$R_{Cu} = \frac{10^{(1.44\pm0.24)}}{10^{(0.1\pm0.3)}} = 10^{(1.34\pm0.38)} \cong \frac{22}{1} = \frac{97}{3} \quad (17d)$$

$$R_{Zn}=\frac{10^{(0.78\pm0.36)}}{10^{(0.36\pm0.3)}}=10^{(0.42\pm0.47)}\cong\frac{2.6}{1}=\frac{72}{28} \quad (17e)$$

$$R_{Cd}=\frac{10^{(1.15\pm0.23)}}{10^{(0.72\pm0.3)}}=10^{(0.43\pm0.38)}\cong\frac{2.7}{1}=\frac{73}{27} \quad (17f)$$

Of course, the results of Equation (17), that is, the percentages given at the right, must be considered as rough estimates, but they confirm the initial conclusion that $(M\cdot AMP\cdot H)^+$ is the dominating species. This is even more so if one recalls that the values used in the numerator of Equation (17) are rather too small[57, 58] and those in the denominator are rather upper limits[63] because this then means that the given ratios are too small and have to be considered as lower limits. Hence, despite all uncertainties, it is safe to conclude that in the $M(H;AMP)^+$ complexes the species with the metal ion at the adenine residue and the proton at the phosphate group, that is, $(M\cdot AMP\cdot H)^+$ are the dominating ones. On the other hand, the above reasonings also suggest that the concentration of the species with both the metal ion and the proton at the phosphate group, $(AMP\cdot M\cdot H)$ is most likely not zero.

2.5. Where is the metal ion in the M(H;ADP) complexes located? In Section 2.3 it was already concluded that the proton, with the possible exception of Cu(H;ADP), is at the terminal β-phosphate group in the M(H;ADP) complexes. Where is the metal ion? At the adenine moiety or also at the diphosphate residue? Are macrochelates of relevance?

We begin the analysis with Cu(H;ADP) because in this case $pK^{H}_{Cu(H;ADP)}=3.56\pm0.16$ (Table 2) is lower than $pK^{H}_{H_2(ADP)}=3.92\pm0.02$ (Table 1) which means that an isomer with the proton at N1 is a possibility. In principle, four isomeric species are possible: i) In $ADP\cdot Cu\cdot H$ the proton and Cu^{2+} are located at the diphosphate group, ii) $H\cdot ADP\cdot Cu$ carries the proton at N1 and Cu^{2+} at the diphosphate, whereas iii) in $Cu\cdot ADP\cdot H$ the metal ion is at the nucleobase residue and the proton at the terminal β-phosphate group of the diphosphate residue. Finally, iv) the $ADP\cdot Cu\cdot H$ species (or the one of $Cu\cdot ADP\cdot H$ with Cu^{2+} at N7) could to some extent form a closed (=cl) or macrochelated species involving N7, thus giving rise to the $(ADP\cdot Cu\cdot H)_{cl}$ isomer. Hence, Equation (12b) may be redefined as given in Equation (18),

$$K^{Cu}_{Cu(H;ADP)}=\frac{[ADP\cdot Cu\cdot H]+[H\cdot ADP\cdot Cu]+[Cu\cdot ADP\cdot H]+[(ADP\cdot Cu\cdot H)_{cl}]}{[Cu^{2+}][H(ADP)^{2-}]} \quad (18)$$

and therefore, the experimentally accessible overall equilibrium constant $K^{Cu}_{Cu(H;ADP)}$ is actually composed of the four microconstants given in Equation (19):

$$K^{Cu}_{Cu(H;ADP)}=k^{Cu}_{ADP\cdot Cu\cdot H}+k^{Cu}_{H\cdot ADP\cdot Cu}+k^{Cu}_{Cu\cdot ADP\cdot H}+k^{Cu}_{(ADP\cdot Cu\cdot H)_{cl}} \quad (19)$$

In a recent analysis for mixed ligand complexes[64] it has been shown that the micro stability constant for the $H\cdot ADP\cdot Cu$ isomer, $k^{Cu}_{H\cdot ADP\cdot Cu}$, is zero within the error limits; in other words, this species does not occur in significant amounts.

With this result in mind and by setting the right hand sides of Equations (12b) and (18) equal, one obtains Equation (20) and the results derived[64] for it recently:

$$[Cu(H;ADP)]=[ADP\cdot Cu\cdot H]+[Cu\cdot ADP\cdot H]+[(ADP\cdot Cu\cdot H)_{cl}] \quad (20a)$$

$$10^{(2.77\pm0.16)}=10^{(2.4\pm0.25)}+10^{(1.64\pm0.24)}+10^{(2.48\pm0.27)} \quad (20b)$$

$$100\,\%=(43\pm29)\,\%+(7\pm5)\,\%+(51\pm37)\,\% \quad (20c)$$

Since all error limits refer to three times the standard error of the mean value (3σ) (see also footnote [a] of Table 2), it might be helpful to rewrite Equation (20c) with only 1σ:

$$100\,\%=(43\pm10)\,\%+(7\pm2)\,\%+(51\pm12)\,\% \quad (21)$$

From Equations (20c) and (21) it follows that the species $Cu\cdot ADP\cdot H$ occurs only in low concentration. The dominating isomers of the Cu(H;ADP) complex are clearly those where both the proton and the metal ion are bound to the diphosphate residue. This means, the two species of the intramolecular Equilibrium (1) dominate, both the open $ADP\cdot Cu\cdot H$ and the chelated isomer $(ADP\cdot Cu\cdot H)_{cl}$ occur in about equal concentrations with nearly 50 % each.

How is the situation with the M(H;ADP) complexes of the other metal ions studied (Table 2)? Clearly, here the $H\cdot ADP\cdot M$ isomer is of no relevance as already concluded in Section 2.3. The same is true for the $M\cdot ADP\cdot H$ species because it occurs already with Cu^{2+} only in very low concentration [$7\pm5\,\%$; Eq. (20c)] and this metal ion has by far the highest affinity toward the adenine residue of all the metal ions considered here. Hence, we are left with the open isomer $ADP\cdot M\cdot H$ and the closed one $(ADP\cdot M\cdot H)_{cl}$. For a conclusion, values for the stability constant of the open isomer, $k^{M}_{ADP\cdot M\cdot H}$, are needed. Since it has been proven by ^1H NMR studies[37] and by stability constant comparisons[26] that in the $M(UDP)^-$ complexes the uridine residue does not participate in metal ion binding, the same may be assumed for its monoprotonated complexes and hence, it holds $k^{M}_{ADP\cdot M\cdot H}=K^{M}_{M(H;UDP)}$. If one compares the previously estimated[26] stability constants of the M(H;UDP) complexes with those of the M(H;ADP) species listed in column 3 of Table 2, one sees that the values are identical within the error limits for all nine metal ions (except Cu^{2+}; see above). This then means that the open $ADP\cdot M\cdot H$ isomer is evidently the dominating species for these M(H;ADP) complexes. However, because the error limits of the considered stability constants are large (see Table 2 and ref. [26]), one cannot exclude that to some extent also the chelated isomer $(ADP\cdot M\cdot H)_{cl}$ occurs in an intramolecular equilibrium; for example, stability differences of 0.1 or 0.2 log unit (i.e., between $\log K^{M}_{M(H;ADP)}$ and $\log K^{M}_{M(H;UDP)}$), which are well within the error limits, correspond already to a formation degree of 21 and 37 %, respectively.[65]

2.6. Proof of an enhanced stability of several M(AMP) and $M(ADP)^-$ complexes: The existence of Equilibrium (1) for M(AMP) complexes is well established;[15] the increased stability observed due to macrochelate formation with N7 of

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the already phosphate-coordinated metal ion disappears, as expected, in all the corresponding complexes formed with tubercidin 5′-monophosphate (=7-deaza-AMP^{2-}) since this ligand lacks N7.[31] Indeed, any kind of chelation[66] must be reflected in an enhanced complex stability.[16, 50] Of course, macrochelates as indicated in Equilibrium (1) will hardly form to 100%. It is important to be aware that the formation degree of the macrochelated or ′closed′ species, which we designate for the complexes of adenosine phosphates (AP) as $M(AP)_{cl}$, is *independent* of the total complex concentration because the intramolecular equilibrium constant K_I, as defined by Equation (22), where $M(AP)_{op}$ refers to the ′open′ species in Equilibrium (1), is dimension-less:

$$K_I = [M(AP)_{cl}]/[M(AP)_{op}] \quad (22)$$

Taking this into account, Equilibrium (13a) may be rewritten as below (charges in part deleted):

$$M^{2+} + AP \rightleftharpoons M(AP)_{op} \rightleftharpoons M(AP)_{cl} \quad (23)$$

The corresponding equilibrium constant is then defined by Equation (24):

$$K^M_{M(AP)} = \frac{[M(AP)]}{[M^{2+}][AP]} \quad (24a)$$

$$= \frac{[M(AP)_{op}] + [M(AP)_{cl}]}{[M^{2+}][AP]} \quad (24b)$$

This expression contains as one term the stability constant of the open isomer shown in Equilibrium (1) which is defined in Equation (25):

$$K^M_{M(AP)_{op}} = [M(AP)_{op}]/([M^{2+}][AP]) \quad (25)$$

It is evident that any breakdown of the values for $K^M_{M(AP)}$, which has to reflect the contribution of the various terms necessary for a further interpretation, requires that values for $K^M_{M(AP)op}$, which cannot directly be measured, are obtainable. In contrast, $K^M_{M(AP)}$ [Eqs. (13b) and (24)] is experimentally accessible. However, the existence of a linear relationship for families of structurally closely related ligands between $\log K^M_{M(L)}$ and $pK^H_{H(L)}$ is well known[66] and exists also for $\log K^M_{M(R\text{-}MP)}$ versus $pK^H_{H(R\text{-}MP)}$ (cf.[67, 68]) and $\log K^M_{M(R\text{-}DP)}$ versus $pK^H_{H(R\text{-}DP)}$ plots,[26] where R-MP^{2-} represents a simple phosphate monoester or phosphonate ligand[67] and R-DP^{3-} a simple diphosphate monoester,[26] that is, R may be any residue which does not affect complex formation. The parameters for the corresponding straight-line equations, which are defined by Equation (26),

$$\log K^M_{M(L)} = m \cdot pK^H_{H(L)} + b \quad (26)$$

have been tabulated for L = R-MP^{2-} and R-DP^{3-}, that is, for M(R-MP)[16, 67, 68] and M(R-DP)$^-$ complexes.[26] Hence, with a known pK_a value for the deprotonation of a -P(O)$_2$(OH)$^-$ group an expected stability constant can be calculated for any phosph(on)ate- or diphosphate-metal ion complex.

Plots of $\log K^M_{M(R\text{-}MP)}$ versus $pK^H_{H(R\text{-}MP)}$ according to Equation (26) are shown in Figure 2 for the 1:1 complexes of Mg^{2+}, Zn^{2+} and Cu^{2+}, as examples, with the data points (empty circles) of the eight simple ligand systems used[67] for the determination of the straight reference line. The solid points refer to the corresponding M(AMP) complexes; those for the Zn^{2+} and Cu^{2+} species are above the reference lines, thus proving an increased stability for these two complexes, whereas the data point for the Mg(AMP) complex nearly fits on the line.

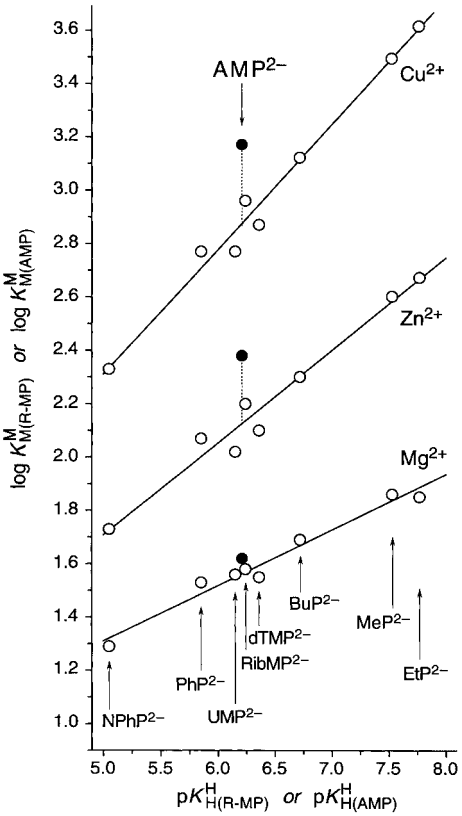

Figure 2. Evidence for an enhanced stability of the Cu(AMP) and Zn(AMP) complexes, the situation of Mg(AMP) being equivocal (●), based on the relationship between $\log K^M_{M(R\text{-}MP)}$ and $pK^H_{H(R\text{-}MP)}$ for M(R-MP) complexes of some simple phosphate monoester and phosphonate ligands (R-MP^{2-}) (○): 4-nitrophenyl phosphate (NPhP^{2-}), phenyl phosphate (PhP^{2-}), uridine 5′-monophosphate (UMP^{2-}), D-ribose 5-monophosphate (RibMP^{2-}), thymidine [=1-(2-deoxy-β-D-ribofuranosyl)thymine] 5′-monophosphate (dTMP^{2-}), *n*-butyl phosphate (BuP^{2-}), methanephosphonate (MeP^{2-}), and ethanephosphonate (EtP^{2-}) (from left to right). The least-squares lines[67a] [Eq. (26)] are drawn through the corresponding eight data sets (○) taken from ref. [67b] for the phosphate monoesters and from ref. [67a] for the phosphonates. The data points due to the M^{2+}/AMP systems (●) are based on the values listed in Tables 1 and 2. The vertical broken lines emphasize the stability differences from the reference lines; they equal log $\Delta_{M(AP)}$ as defined in Equation (29) (see also Table 4, column 5). All the plotted equilibrium constants refer to aqueous solutions at 25 °C and I = 0.1M ($NaNO_3$).

The situation in Figure 3 for the complexes of diphosphate monoesters (R-DP^{3-}) and ADP^{3-} is similar; for the three examples involving ADP^{3-} and Mg^{2+}, Co^{2+} or Zn^{2+} the data points are above the reference lines. Hence, the results of Figure 2 and Figure 3 prove that macrochelates form and Equilibrium (1) exists. However, because the vertical distances of the solid data points to their reference lines varies, this

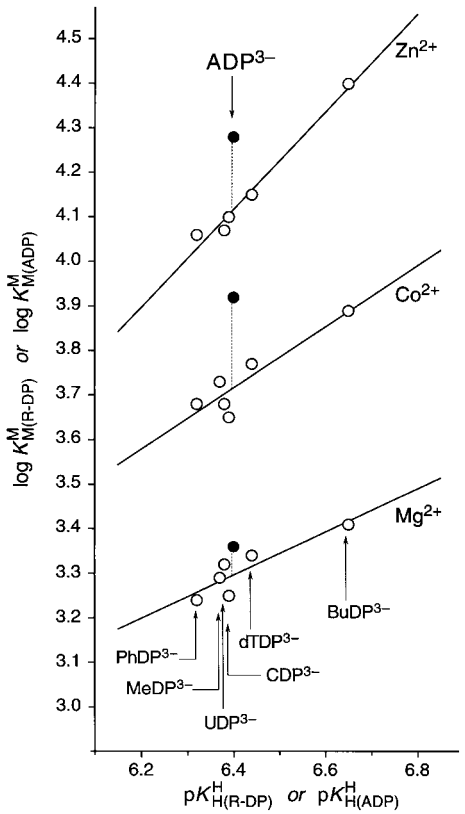

Figure 3. Evidence for an enhanced stability of the $Mg(ADP)^-$, $Co(ADP)^-$ and $Zn(ADP)^-$ complexes (●), based on the relationship between $\log K^{M}_{M(R\text{-}DP)}$ and $pK^{H}_{H(R\text{-}DP)}$ for the simple $M(R\text{-}DP)^-$ complexes (○), where $R\text{-}DP^{3-}$ = phenyl diphosphate ($PhDP^{3-}$), methyl diphosphate ($MeDP^{3-}$), uridine 5′-diphosphate (UDP^{3-}), cytidine 5′-diphosphate (CDP^{3-}), thymidine [=1-(2-deoxy-β-D-ribofuranosyl)thymine] 5′-diphosphate ($dTDP^{3-}$) and *n*-butyl diphosphate ($BuDP^{3-}$) (from left to right). The least-squares lines [Eq. (26)] are drawn through the indicated six (in the case of Zn^{2+} five) data sets; the corresponding equilibrium constants are from ref. [26]. The data points due to the M^{2+}/ADP systems (●) are based on the values listed in Tables 1 and 2. The vertical broken lines emphasize the stability differences from the reference lines; they equal $\log \Delta_{M(AP)}$ as defined in Equation (29) (see also Table 4, column 5). All the plotted equilibrium constants refer to aqueous solutions at 25 °C and $I = 0.1$ M ($NaNO_3$).

proves further that the extent of macrochelate formation differs for the various systems.

2.7. Extent of macrochelate formation in solution for M(AMP) and M(ADP)⁻ complexes: With the results depicted in Figures 2 and 3 in mind, it is evident that values for the intramolecular equilibrium constant K_I [Eq. (22)] have to be the aim. In fact, combination of Equations (22), (24), and (25) leads to Equation (27) which may be rearranged[31, 66] to yield a further definition for K_I [Eq. (28)] in which the stability difference $\log \Delta$ is defined by Equation (29).

$$K^{M}_{M(AP)} = K^{M}_{M(AP)_{op}} + K_I \cdot K^{M}_{M(AP)_{op}} \quad (27a)$$

$$= K^{M}_{M(AP)_{op}} (1 + K_I) \quad (27b)$$

$$K_I = \frac{K^{M}_{M(AP)}}{K^{M}_{M(AP)_{op}}} - 1 = 10^{\log \Delta} - 1 \quad (28)$$

$$\log \Delta = \log \Delta_{M(AP)} = \log K^{M}_{M(AP)} - \log K^{M}_{M(AP)_{op}} \quad (29)$$

The equilibrium constant K_I can now be calculated through Equations (28) and (29) as the values for $K^{M}_{M(AP)}$ are known (Table 2, column 4) and those for $K^{M}_{M(AP)_{op}}$ may be calculated with the acidity constants of $H(AMP)^-$ and $H(ADP)^{2-}$ (Table 1) and the corresponding straight reference line equations given in refs. [67a, 68] and [26], respectively.

The vertical distances indicated by dotted lines in Figure 2 and Figure 3 are identical with the stability differences $\log \Delta_{M(AP)}$ as defined in Equation (29). Of course, the reliability of any calculation for K_I depends on the accuracy of the difference $\log \Delta_{M(AP)}$ which becomes the more important the more similar the two constants in Equation (29) are. Therefore, only well defined error limits allow a quantitative evaluation of the extent of a possibly formed macrochelate. Finally, if K_I is known, the percentage of the closed or macrochelated species occurring in Equilibrium (1) follows from Equation (30):

$$\%\ M(AP)_{cl} = 100 \cdot K_I/(1 + K_I) \quad (30)$$

Application of this procedure[31, 66] yields the results of Table 4. The values in the final column show that macrochelate formation is zero or close to it within the error limits for the M(AMP) and $M(ADP)^-$ complexes of Ca^{2+}, Sr^{2+} and Ba^{2+}, yet for Mg(AMP) and $Mg(ADP)^-$ significant amounts in the order of 10% are formed; this agrees with results obtained for the M(NMP) (cf.[16, 22]) and $M(NTP)^{2-}$ (cf.[25]) complexes of the four alkaline earth ions where especially with the guanine residue the formation degree is remarkable in all instances. Evidently, for all 3d-transition elements, including Zn^{2+} and Cd^{2+}, the formation degree of the $M(AP)_{cl}$ species is high.

It is satisfying to note that the formation degrees of the $M(AMP)_{cl}$ species determined now (Table 4) are within the error limits identical with our earlier results,[22, 31] which were based on stability constants calculated under the assumption that no $M(H;AMP)^+$ complexes form, though in a few instances the formation degree increased a bit: the maximum increase is 8% for $Cd(AMP)_{cl}$. It is further comforting to see that Taylor and Diebler[32a, d] determined under similar conditions (25 °C; $I = 0.1$ M, $NaClO_4$) but employing the temperature-jump relaxation technique, a formation degree of 69% for $Ni(AMP)_{cl}$; considering the differences in the applied methodologies, the agreement with the present 75±4% (Table 4) is excellent. Similarly, Peguy and Diebler[32b, d] obtained for $Co(AMP)_{cl}$ 70% at 8 °C and $I = 0.2$ M ($NaClO_4$); considering the differences, especially in temperature as well as in *I* and the methodology, the agreement with the present 56 ± 7% is fair.

By ^1H NMR shift experiments it has previously been proven that macrochelates involving N7 form with the $Zn(ADP)^-$ and $Cd(ADP)^-$ systems in solution.[37] From experiments carried out by Frey and Stuehr[46] at 15 °C and $I = 0.1$ M (KNO_3) a formation degree of 80% follows for $Ni(ADP)^-_{cl}$; if one considers the difference in temperature, this result is in fair agreement with the 59 ± 6% determined now. More important, macrochelate formation for this system has also been proven by Mariam and Martin[38] by spectrophotometric experiments.

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Table 4. Comparison of the measured stability constants, $K^{M}_{M(AP)}$, of the M(AMP) and $M(ADP)^{-}$ complexes with the stability constants, $K^{M}_{M(AP)_{op}}$, of the isomers with a sole monophosphate or diphosphate coordination of M^{2+}, and extent of the intramolecular macrochelate formation according to Equilibrium (1) in the M(AMP) and $M(ADP)^{-}$ complexes in aqueous solution at 25 °C and $I = 0.1$ M ($NaNO_3$).[a]

AP	M^{2+}	$\log K^{M}_{M(AP)}$ [b] [Eqs. (13b),(24)]	$\log K^{M}_{M(AP)_{op}}$ [c] [Eq. (25)]	$\log \Delta_{M(AP)}$ [Eq. (29)]	K_I [Eqs. (22),(28)]	% $M(AP)_{cl}$ [Eq. (30)]
AMP^{2-}	Mg^{2+}	1.62 ± 0.04	1.56 ± 0.03	0.06 ± 0.05	0.15 ± 0.13	13 ± 10
	Ca^{2+}	1.48 ± 0.03	1.45 ± 0.05	0.03 ± 0.06	0.07 ± 0.14	7 ± 13
	Sr^{2+}	1.26 ± 0.02	1.24 ± 0.04	0.02 ± 0.04	0.05 ± 0.11	5 ± 10
	Ba^{2+}	1.18 ± 0.04	1.16 ± 0.04	0.02 ± 0.06	0.05 ± 0.14	5 ± 12
	Mn^{2+}	2.23 ± 0.02	2.16 ± 0.05	0.07 ± 0.05	0.17 ± 0.15	15 ± 11
	Co^{2+}	2.30 ± 0.04	1.94 ± 0.06	0.36 ± 0.07	1.29 ± 0.38	56 ± 7
	Ni^{2+}	2.55 ± 0.04	1.94 ± 0.05	0.61 ± 0.06	3.07 ± 0.60	75 ± 4
	Cu^{2+}	3.17 ± 0.02	2.87 ± 0.06	0.30 ± 0.06	1.00 ± 0.29	50 ± 7
	Zn^{2+}	2.38 ± 0.07	2.13 ± 0.06	0.25 ± 0.09	0.78 ± 0.38	44 ± 12
	Cd^{2+}	2.74 ± 0.05	2.44 ± 0.05	0.30 ± 0.07	1.00 ± 0.32	50 ± 8
ADP^{3-}	Mg^{2+}	3.36 ± 0.03	3.30 ± 0.03	0.06 ± 0.04	0.15 ± 0.11	13 ± 9
	Ca^{2+}	2.95 ± 0.02	2.91 ± 0.03	0.04 ± 0.04	0.10 ± 0.09	9 ± 8
	Sr^{2+}	2.42 ± 0.03	2.36 ± 0.04	0.06 ± 0.05	0.15 ± 0.13	13 ± 10
	Ba^{2+}	2.37 ± 0.06	2.30 ± 0.03	0.07 ± 0.07	0.17 ± 0.18	15 ± 13
	Mn^{2+}	4.22 ± 0.02	4.12 ± 0.03	0.10 ± 0.04	0.26 ± 0.10	21 ± 7
	Co^{2+}	3.92 ± 0.02	3.72 ± 0.05	0.20 ± 0.05	0.58 ± 0.20	37 ± 8
	Ni^{2+}	3.93 ± 0.02	3.54 ± 0.06	0.39 ± 0.06	1.45 ± 0.36	59 ± 6
	Cu^{2+}	5.61 ± 0.03	5.27 ± 0.04	0.34 ± 0.05	1.19 ± 0.25	54 ± 5
	Zn^{2+}	4.28 ± 0.05	4.12 ± 0.03	0.16 ± 0.06	0.44 ± 0.19	31 ± 9
	Cd^{2+}	4.63 ± 0.04	4.27 ± 0.03	0.36 ± 0.05	1.29 ± 0.26	56 ± 5

[a] For the error limits see footnote [a] of Table 2. [b] Values from column 4 in Table 2. [c] For the AMP systems calculated with $pK^{H}_{H(AMP)} = 6.21$ and the reference-line equations established previously[16, 22, 50] [see Eq. (26) and Figure 2]; for the ADP systems the calculations were done with $pK^{H}_{H(ADP)} = 6.40$ and the reference-line equations defined recently[26] [see Eq. (26) and Figure 3].

Considering the various techniques involved, it is clear that Equilibrium (1) exists and that macrochelates form in aqueous solution. The results assembled in Table 4 represent the first comprehensive and self-consistent data set which quantifies the formation degree of $M(AMP)_{cl}$ and $M(ADP)^{-}_{cl}$ for ten metal ions each. If one considers the results obtained for the complexes of the 3d series, it is evident from the $\log \Delta_{M(AP)}$ values (Table 4, column 5) that these follow the Irving–Williams series[49] as one would expect[51] for a metal ion-imidazole-type interaction. The fact that the maximum stability increase (see the $\log \Delta_{M(AP)}$ values in Table 4, column 5) and consequently the highest formation degree of the macrochelates is observed for Ni(AMP) and $Ni(ADP)^{-}$ and not for the corresponding Cu^{2+} complexes, has previously been explained[31] for the AMP^{2-} systems by the differences in the coordination spheres of Ni^{2+} and Cu^{2+} and by statistical considerations connected with these differences.

General Conclusions

For $M(ATP)^{2-}$ complexes considerable evidence has accumulated over the years[15, 16, 23–25] that (at least) two types of macrochelates can form, one in which the $\gamma,\beta,(\alpha)$-triphosphate-coordinated[4, 8, 15, 24] metal ion binds innersphere to N7 of the adenine residue and one in which this interaction is of the outersphere type, that is, with a water molecule between N7 and M^{2+} (see Figure 6 in ref. [15]). That such outersphere interactions are also of relevance for the $M(ADP)^{-}_{cl}$ species is evident from the results obtained for $Mg(ADP)^{-}$: ^{1}H NMR shift measurements[37] provide no evidence for a Mg^{2+}–N7 interaction, yet macrochelate formation in the order of about 10% is certain (Table 4) and must thus occur in an outersphere manner. This conclusion is in accord with the situation for $Mg(ATP)^{2-}$.[23, 24]

Similarly, from ^{1}H NMR shift measurements, which are sensitive to innersphere binding only, it was concluded[37] that $Zn(ADP)^{-}_{cl}$ and $Cd(ADP)^{-}_{cl}$ reach formation degrees of about 20 and 40%, respectively, yet the data from the potentiometric pH titrations, which measure the overall stability increase and which do not distinguish between inner- and outersphere binding, provide formation degrees of 30 and 55%, respectively. Hence, one has to conclude that roughly speaking about one third of $Zn(ADP)^{-}_{cl}$ and $Cd(ADP)^{-}_{cl}$ is formed by outersphere binding to N7 and the other two thirds by innersphere coordination. It is interesting that Mariam and Martin[38] concluded, based on their spectrophotometric measurements, that about one part of $Ni(ADP)^{-}_{cl}$ is outersphere and that about four parts are innersphere. It is evident that these additional equilibria deserve further study.

There is another, most fascinating aspect: If one compares the formation degrees of $M(AMP)_{cl}$ and $M(ADP)^{-}_{cl}$ as assembled in Table 4 with the corresponding values for $M(ATP)^{2-}_{cl}$ given in ref. [25], one makes the remarkable observation that for nearly all metal ions listed in Table 4 the formation degrees for 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 AP ligands and in the coordination spheres of the metal ions. There are only two exceptions; for Co^{2+} and Ni^{2+} one observes the series $M(AMP)_{cl} > M(ADP)^{-}_{cl} \cong M(ATP)^{2-}_{cl}$.

The above observation is even more surprising when one considers the overall stabilities of the M(AP) complexes (Table 4 and refs. [23, 24]; see also [26]), 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, whereas those between the M(ADP)$^-$ and M(ATP)$^{2-}$ 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. One may also recall in this context that 1 log unit of a stability constant corresponds approximately to a change in free energy (ΔG°) of 6 kJ mol^{-1}.[50] These high-energy binding sites of the phosphate residue are in contrast with the weak structuring interactions as they occur at N7 of the adenine moiety: A stability difference log $\Delta_{M(AP)}$ of 0.1 log unit gives rise to a formation degree of about 20% for the macrochelated $M(AP)_{cl}$ species, yet the change in the free energy involved, which creates the special structure, corresponds only to about 0.6 kJ mol^{-1}.[50] 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 these metal ions. Finally, one may mention that not only Pt^{II} species prefer N7 sites of purines for binding[69, 70] but that this also holds for Mn^{2+} and Zn^{2+},[10, 71] and that furthermore there are also indications[72] that N7 of ATP might interact with Zn^{2+} in a RNA polymerase reaction during the catalytic process.[73] Clearly, understanding the solution properties of metal ion-nucleotide complexes should help to appreciate their role in enzymic reactions.[74]

4. Experimental Section

4.1. Materials: The sodium salts of adenosine 5′-monophosphate (AMP) and adenosine 5′-diphosphate (ADP) were purchased from Sigma Chemical Co., St. Louis, MO (USA). A further lot of the Na^+ salt of ADP was obtained from Serva Feinbiochemica GmbH, Heidelberg (Germany). The results collected for ADP from the two sources did not differ. The concentration of free, inorganic phosphate was determined[75] via molybdate reagent; it amounted to about 3 mol% of ADP or less. The aqueous stock solutions of the ligands were freshly prepared daily and their pH was adjusted to about 8.2; their exact AP concentrations were newly determined each time by titrations with NaOH.

All other materials used in the experiments, including the CO_2-free water, were from the same sources as previously.[26]

4.2. Potentiometric pH titrations: The pH titrations were carried out with the same equipment and in the same way as described.[26]

As previously, the direct pH meter readings were used to calculate the acidity constants, that is, these constants are so-called practical, mixed or Brønsted constants.[76] Their negative logarithms given for aqueous solutions at $I = 0.1$M ($NaNO_3$) and 25 °C may be converted into the corresponding concentration constants by subtracting 0.02 from the given pK_a values;[76] this conversion term contains both the junction potential of the glass electrode and the hydrogen ion activity.[76, 77] No conversion term is necessary for the stability constants of the metal ion complexes. Always the *differences* in NaOH consumption between solutions with and without ligand[76] (see below) are evaluated.

4.3. Determination of the acidity constants: The acidity constants $K^H_{H_2(ADP)}$ and $K^H_{H(ADP)}$ of $H_2(ADP)^-$ [Eqs. (3) and (4)] were determined under N_2 in two different experimental settings for the titrations (25 °C; $I = 0.1$M, $NaNO_3$). In the first set 50 mL of aqueous 0.54 mM HNO_3 were titrated in the presence and absence of 0.3 mM ADP with 1 mL of 0.03 M NaOH, that is, under exactly the same experimental conditions previously used for the $H_2(CDP)^-$ system.[26] In the second set 50 mL of aqueous 2.2 mM HNO_3 were titrated in the presence and absence of 0.6 mM ADP with 2 mL of 0.06 M NaOH. The calculations were carried out (for the computer equipment see ref. [26]) by a curve-fitting procedure using a Newton-Gauss nonlinear-least-squares program. The pH range employed was from 3.0 to 8.1, corresponding initially to about 11% neutralization for the equilibrium $H_2(ADP)^-/H(ADP)^{2-}$ and at the end to about 98% neutralization for $H(ADP)^{2-}/ADP^{3-}$.

The acidity constant, $K^H_{H_3(ADP)}$ [Eq. (2)], of $H_3(ADP)^\pm$ was determined exactly under the same conditions as given previously for $H_3(CDP)^\pm$.[26] In this case the final result is the average of six independent pairs of titrations. The two acidity constants, $K^H_{H_2(ADP)}$ and $K^H_{H(ADP)}$ result from the averages of more than 100 independent pairs of titrations.

For $H_2(AMP)^\pm$ the acidity constants $K^H_{H_2(AMP)}$ and $K^H_{H(AMP)}$ were determined under the conditions given above for the first set (which also corresponds to the conditions previously used),[31] and in addition by titrating 50 mL of aqueous 1.08 mM HNO_3 and $NaNO_3$ (25 °C; $I = 0.1$M, $NaNO_3$) in the presence and absence of 0.6 mM AMP under N_2 with 1 mL of 0.06 M NaOH. The final constants are the averages of more than 20 independent pairs of titrations.

4.4. Determination of the stability constants: In the determination of the stability constants of the M(H;ADP) and M(ADP)$^-$ complexes [Eqs. (12) and (13)], where $M^{2+} = Mg^{2+}$, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and Cd^{2+}, the concentrations given above in Section 4.3 for the first and second set of experiments were applied. The M^{2+}:ADP ratios used in the experiments were usually 1:1 and 2:1 for all metal ion systems, except that in the case of the alkaline earth metal ions, because of the low stability of their complexes, also the ratios 3:1, 5:1 and 10:1 were used for the Mg^{2+} and Ca^{2+} systems, and the ratios for Sr^{2+} and Ba^{2+} were 5:1 and 10:1. In these instances part of $NaNO_3$ was replaced by $M(NO_3)_2$ to keep I at 0.1M.

The stability constants $K^M_{M(H;ADP)}$ and $K^M_{M(ADP)}$ [Eqs. (12) and (13)] were calculated with a curve-fitting procedure by taking into account the species H^+, $H_2(ADP)^-$, $H(ADP)^{2-}$, ADP^{3-}, M^{2+}, M(H;ADP) and M(ADP)$^-$.[78] The data were collected every 0.1 pH unit from either the lowest pH which could be reached in the experiment or from a formation degree of about 2% for M(ADP)$^-$ to a neutralization degree corresponding in total to about 90% for $H(ADP)^{2-}$, or to the beginning of the hydrolysis of $M(aq)^{2+}$ (e.g., with Cu^{2+} or Zn^{2+}), which was evident from the titrations without ligand. The formation degrees of the protonated M(H;ADP) complexes were usually small [about 6% for Ba^{2+} (10:1) and Co^{2+} (1:1) and about 10% for Cd^{2+} (maximum)] and therefore the errors of the corresponding constants are large.

The results were independent of the excess of M^{2+} employed in the experiments. The final results for the stability constants of the M(H;ADP) and M(ADP)$^-$ complexes are the averages of at least ten independent pairs of titrations for each metal ion system.

The stability constants $K^M_{M(H;AMP)}$ and $K^M_{M(AMP)}$ for the M(H;AMP)$^+$ and M(AMP) complexes were determined under the same conditions as the acidity constants (see Section 4.3) where the conditions of the first set correspond to those used previously.[31] In all instances $NaNO_3$ was partly or fully replaced by $M(NO_3)_2$ (25 °C; $I = 0.1$M). For the second set of experiments with 0.6 mM AMP, the M^{2+}:AMP ratios used were approximately 56:1 (Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}), 28:1 (Mn^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}), 7:1 (Zn^{2+}), and 2.8:1 (Cu^{2+}).

The measurements of the Zn^{2+}/AMP system were hampered (as previously)[31] by precipitation which means that, depending on the conditions of the various titrations, in the maximum only formation degrees between 7 and 10% could be reached for the Zn(AMP) complex. In an effort to obtain a reliable result, in total 22 independent titration pairs were carried out. For all other M^{2+}/AMP systems at least 6 (usually 8) independent pairs of titrations were made.

The stability constants $K^M_{M(H;AMP)}$ and $K^M_{M(AMP)}$ were calculated as described above for the ADP systems. The values calculated individually for $\log K^M_{M(AMP)}$ showed no dependence on pH or on the excess amount of

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M^{2+} used in the experiments. The formation degree of the protonated species was low (about 2% for the alkaline earth ions and about 9–14% for Co^{2+}, Ni^{2+} or Cd^{2+}) and therefore the errors connected with these constants are relatively large.

Acknowledgement

The competent technical assistance of Mrs. Rita Baumbusch and Mrs. Astrid Sigel in the preparation of this manuscript, and research grants over several years from the Swiss National Science Foundation (H.S.), which made this study possible, are gratefully acknowledged.

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[56] In a family of isostructural complexes in the solid state[17, 18] with the general formula $M(NMP)(H_2O)_5$ the metal ion is directly N7 coordinated but it also interacts via hydrogen bonds involving coordinated water molecules with the phosphate group; this could well also hold for the protonated $(M \cdot AMP \cdot H)^+$ species. For the M(AMP) complexes in solution there is evidence that the interactions occur (mostly) innersphere; for a detailed discussion ref. [31] should be consulted.
[57] In an early study[32b] dealing with IMP the stability constant of $Ni(H;IMP)^+$ has been measured ($\log K^{Ni}_{Ni(H;IMP)} = 1.88$ as well as that of the Ni(inosine)$^{2+}$ complex, $\log K^{Ni}_{Ni(Ino)} = 1.15$; the stability difference

of 0.73 log unit is larger than expected for a pure charge effect (cf. the 0.40 ± 0.15 log unit)[54] and indicates that in the $(Ni \cdot IMP \cdot H)^+$ species macrochelate formation occurs. Since the extent of macrochelate formation is much higher for M(IMP) than for M(AMP) complexes[16, 31] we assume that in the present case the combination of the charge effect with the chelate effect is between the two extremes (0.40 versus 0.73) and amounts (to prevent an overestimation we add only 0.1 log unit) in total to 0.50 ± 0.20 log unit (with a generously estimated error limit), which means a chelate formation degree of ca. 20% in the $(M \cdot AMP \cdot H)^+$ species (for the calculation procedure see Section 2.7).

[58] The stability constant of $Cu(Ado)^{2+}$, $\log K^{Cu}_{Cu(Ado)} = 0.85 \pm 0.12$ (Table 3), is corrected for the different basicities of N1 in $H(AMP)^-$ and Ado [i.e., $\Delta pK_a = pK^H_{H_2(AMP)} - pK^H_{H(Ado)} = (3.84 \pm 0.02) - (3.61 \pm 0.03) = 0.23 \pm 0.04$] by applying the estimated slope $m = 0.394$ (see footnote [d] of Table 3) for the straight line of the $\log K$ versus pK_a plot and this leads to the "corrected" value $(0.85 \pm 0.12) + (0.09 \pm 0.05/\text{estimated error}) = 0.94 \pm 0.13$. This value needs to be further corrected for the charge effect which the $-P(O)_2(OH)^-$ group exerts on Cu^{2+} at the adenine residue [the effect of the same group on $(N1)H^+$ is taken care of via ΔpK_a] as well as for the chelate effect in the $(Cu \cdot AMP \cdot H)^+$ species which amount together[57] to 0.50 ± 0.20 log unit. Hence, one obtains $\log k^{Cu}_{Cu \cdot AMP \cdot H} = (0.94 \pm 0.13) + (0.50 \pm 0.20) = 1.44 \pm 0.24$.

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[63] UDP^{3-} has the properties of a simple diphosphate monoester,[26] i.e., the uridine residue does not affect complex formation and consequently the data for its $M(UDP)^-$ and M(H;UDP) complexes can be applied to estimate the stability decreasing effect resulting from protonation. For the term $\Delta \log K = \log K^M_{M(UDP)} - \log K^M_{M(H;UDP)}$ one obtains $3.68 - 2.0 = 1.68$ (Co^{2+}), $3.50 - 2.2 = 1.30$ (Ni^{2+}), $5.21 - 2.4 = 2.81$ (Cu^{2+}), $4.07 - 2.3 = 1.77$ (Zn^{2+}), and $4.22 - 2.5 = 1.72$ (Cd^{2+}) (always ± 0.3). Since M^{2+} with UDP coordinates to the α- and β-phosphate groups, the effect of the proton is expected to be somewhat larger in the $(AMP \cdot M \cdot H)^+$ complexes, where only a single phosphate group is available. The estimates given below for the stability constants of the $(AMP \cdot M \cdot H)^+$ species must therefore be considered as *upper* limits. In the following the first value in the equations refers to the stability of the open isomer, $M(AMP)_{op}$, in which M^{2+} is coordinated only to the phosphate group. These values can be calculated with $pK^H_{H(AMP)}$ (= 6.21; Table 1) and the straight-line equations ($\log K$ versus pK_a) for a simple phosph(on)ate-M^{2+} coordination determined previously (see also Section 2.6).[16, 22, 50] Hence, one obtains the following (upper limit) estimates: $\log k^M_{AMP \cdot M \cdot H} = \log K^M_{M(AMP)_{op}} - \Delta \log K = (1.94 \pm 0.06) - (1.68 \pm 0.3) = 0.26 \pm 0.31$ (Co^{2+}); $(1.94 \pm 0.05) - (1.30 \pm 0.3) = 0.64 \pm 0.30$ (Ni^{2+}); $(2.87 \pm 0.06) - (2.81 \pm 0.3) = 0.06 \pm 0.31$ (Cu^{2+}); $(2.13 \pm 0.06) - (1.77 \pm 0.3) = 0.36 \pm 0.31$ (Zn^{2+}); and $(2.44 \pm 0.05) - (1.72 \pm 0.3) = 0.72 \pm 0.30$ (Cd^{2+}). The given individual results are used for the calculations presented in the text. However, one may add that the given complexes are evidently of a low stability and that the interaction between M^{2+} and an $R\text{-}OP(O)_2(OH)^-$ or $R\text{-}OP(O)_3^-\text{-}R'$ unit occurs most likely to a large part in an outersphere manner and is on average quantified by $\log K \cong 0.4 \pm 0.3$ for the complexes formed with Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} or Cd^{2+}. This averaged value, in the absence of a measured constant, may be a helpful estimate.

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Received: July 23, 2002 [F4276]