Pure Appl. Chem., Vol. 76, No. 2, pp. 375–388, 2004.
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Adenosine 5′-triphosphate (ATP^{4-}): Aspects of the coordination chemistry of a multitalented biological substrate*

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Abstract: Firstly, the self-stacking properties of ATP^{4-} and the effects of metal ions and protons on these properties are described. Some examples involving macrochelate formation between phosphate-coordinated metal ions (M^{2+}) and N7 of the adenine residue in $M(ATP)^{2-}$ are discussed, and this is followed by considerations on mixed ligand complexes consisting of ATP^{4-}, M^{2+}, and amino acid anions with side chains that allow either aromatic-ring stacking or hydrophobic interactions with the adenine moiety; this gives rise to selectivity. Next, the properties of diphosphorylated 9-[2-(phosphonomethoxy)ethyl]adenine ($PMEA^{2-}$; *Adefovir*), i.e., of $PMEApp^{4-}$, are compared with those of (2′-deoxy)ATP^{4-} with regard to their metal ion-binding qualities, and in this way it can be explained why $PMEApp^{2-}$ is initially an excellent substrate for nucleic acid polymerases. Of course, after incorporation of the PMEA residue into the growing nucleic acid chain, this is terminated and this is how PMEA exerts its antiviral properties [its bis(pivaloyloxymethyl)ester, *Adefovir dipivoxil*, was recently approved for use in hepatitis B therapy]. Finally, the change in free energy connected with (macro)chelate formation or intramolecular stacking interactions and the effect of a reduced dielectric constant of the solvent on the stability of complexes and their structures in solution is considered.

INTRODUCTION

Adenosine 5′-triphoshate (ATP^{4-}) is an ubiquitous substrate for many biological reactions; it is a very versatile molecule that sits in the center of metabolism and is involved, either directly or indirectly through other nucleoside triphosphates, not only in biosynthesis, but also in the vast majority of cellular activities [1]. In fact, Boyer [2] has estimated that ATP and adenosine 5′-diphosphate (ADP^{3-}) 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.

In most reactions in which ATP^{4-} (Fig. 1 [3–5]) is involved also metal ions are participating, i.e., especially divalent metal ions (M^{2+}) [6–10], and among these Mg^{2+} is most prominent [9,11,12]. In the following, the interactions between ATP^{4-} and M^{2+} will be summarized and various isomeric forms of $M(ATP)^{2-}$ species will be discussed followed by an account on the effects which metal ions have on the reactivity of ATP as a biological substrate.

However, at first it is necessary to consider the self-association of ATP, which is an important property for the existence of certain cell organelles. The detailed understanding of this quality is also a precondition for studying the properties of monomeric metal ion complexes.

*Plenary lecture presented at the 2nd Santa María Workshop on Chemistry Devoted to Bioinorganic Chemistry, Santa María del Mar, Havana, Cuba, 7–11 July 2003. Other presentations are published in this issue, pp. 321–388.

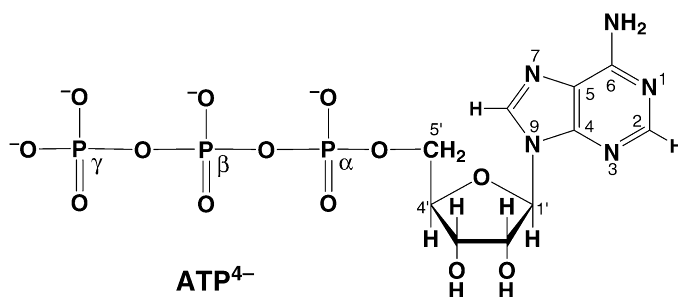


Fig. 1 Chemical structure of adenosine 5′-triphosphate (ATP^{4-}) in its dominating *anti* conformation [3–5]. Other abbreviations used are: Ado, adenosine; ADP^{3-}, adenosine 5′-diphosphate; AMP^{2-}, adenosine 5′-monophosphate; *I*, ionic strength; ITP^{4-}, inosine 5′-triphosphate; GTP^{4-}, guanosine 5′-triphosphate; L, general ligand; M^{2+}, general divalent metal ion; N, nucleoside or nucleotide; $PyNTP^{4-}$, pyrimidine-nucleoside 5′-triphosphate.

SELF-ASSOCIATION OF ATP

It was shown already more than 40 years ago that purines associate much better than pyrimidines [13]; regarding nucleotides, the situation was less clear for many years [14–16]. Today it is generally accepted that self-association of all these species occurs via nucleobase stacking [14,17,18], that it proceeds beyond the dimer stage and that oligomers are formed [14,16,18–24].

^{1}H NMR shift measurements are ideal to characterize the self-association of nucleosides and nucleotides (= N) in aqueous solution (D_2O) [14,15,22,23]. The upfield shifts of the resonances, especially of the nucleobase protons, e.g., H2 and H8 of adenines (Fig. 1), observed with increasing concentration of N, confirm that the association occurs via stacking of the aromatic moieties. In most instances, the experimental data can be explained best by application of the isodesmic model for an indefinite, noncooperative self-association [14–16,22,25]; i.e., all association constants are considered as equal [14,18,26,27]:

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

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

Some results for adenine derivatives are collected in Table 1 [22,23]. It is evident that self-stacking is most pronounced for adenosine (Ado). Its 5′-phosphorylation leads to adenosine 5′-monophosphate (AMP^{2-}), which has a much lower tendency to self-stack due to charge repulsion, and in fact, with increasing charge of the phosphate residue this tendency is further reduced, as demonstrated by ADP^{3-} and ATP^{4-} (entries 3 and 4 of Table 1). Consequently, neutralization of these negative charges facilitates self-stacking as is seen from the properties of $Mg(ATP)^{2-}$, which has the association constant $K = 4.0\ M^{-1}$ (entry 5). However, the fact that this constant is higher than the one due to AMP^{2-} ($K = 2.1\ M^{-1}$; entry 3) even though both species have the same charge of 2– indicates that the self-association of $Mg(ATP)^{2-}$ is somewhat facilitated, most likely by Mg^{2+} bridges between phosphate residues of neighboring nucleotides [16,28].

In any case, from the above example it is evident that coordination of metal ions clearly reduces the repulsing effect of the negatively charged phosphate groups on the self-association. However, for metal ions like Zn^{2+} or Cd^{2+}, which have a pronounced affinity toward hydrogen donors, the situation is more complicated [22,23]. In these instances, the self-association is much larger and there is evidence that relatively stable dimeric stacks, like $[Zn(ATP)]_2^{4-}$, are formed, which then may also further associate to larger aggregates [22,23]. The occurrence of these dimers is explained [22,23,28] by the formation of an *inter*molecular metal ion bridge by coordination of Zn^{2+} or Cd^{2+} to the phosphate moiety of *one* nucleotide and to N7 of the *other* [16,28].

Table 1 Equilibrium constants for self-stacking (eq. 1) of adenosine and its 5′-phosphates as well as of $M(ATP)^{2-}$ complexes as determined by ^1H NMR shift measurements [22,23] in D_2O (27 °C; I = 0.1 – ~2 M $NaNO_3$) (error limits: 2σ).

No.	System	K (M^{-1})
1	Ado	15 ± 2
2	AMP^{2-}	2.1 ± 0.4
3	ADP^{3-}	1.8 ± 0.5
4	ATP^{4-}	1.3 ± 0.2
5	$Mg(ATP)^{2-}$	4.0 ± 0.5
6	$Zn(ATP)^{2-}$	~11.1 ± 4.5
7	$Cd(ATP)^{2-}$	~17

Effect of protonation on the self-association

The interplay between charge neutralization and charge repulsion is also evident from the following two series: (i) For adenosine (Ado), the self-association constants according to equilibrium 1 decrease with increasing protonation at N1, i.e., Ado ($K = 15\ M^{-1}$) > $D(Ado)^+$/Ado = 1:1 ($6.0\ M^{-1}$) > $D(Ado)^+$ ($0.9\ M^{-1}$) [29]; clearly, the creation of a positive charge at the aromatic rings leads to repulsion and thus to reduced stacking. (ii) With AMP, a maximum for the self-association is observed in dependence on protonation, i.e., AMP^{2-} ($K = 2.1\ M^{-1}$) < $D(AMP)^-$ ($3.5\ M^{-1}$) < $D(AMP)^-/D_2(AMP)^{\pm}$ = 1:1 ($5.6\ M^{-1}$) >> $D_2(AMP)^{\pm}$ ($1\ M^{-1}$) > $D_3(AMP)^+$ ($K < 0.7\ M^{-1}$) [16,29].

It is evident that neutralization of one of the two negative charges of the $-PO_3^{2-}$ group by protonation gives $D(AMP)^-$, which experiences a reduced repulsion and thus a somewhat increased stacking tendency. However, self-stacking is most pronounced if 50 % of the adenine residues are protonated at N1, whereas complete nucleobase protonation reduces the stacking tendency drastically, i.e., $K = 1\ M^{-1}$ for $D_2(AMP)^{\pm}$. The final protonation of the $-PO_3H^-$ group leads to the overall positively charged $H_3(AMP)^+$ species with a further reduced self-association tendency.

The self-association of ATP in dependence on pH is considerably more complicated [25,30]: ATP^{4-} ($K = 1.3\ M^{-1}$) < $D(ATP)^{3-}$ ($2.1\ M^{-1}$) < $D(ATP)^{3-}/D_2(ATP)^{2-}$ = 1:1 ($6.0\ M^{-1}$) << $D_2(ATP)^{2-}$ (about $200\ M^{-1}$) >> $D_3(ATP)^-$ ($K < 10\ M^{-1}$ [16]). Evidently, the self-association tendency of ATP is most pronounced for the $D_2(ATP)^{2-}$ species and *not* for the 1:1 ratio of $D(ATP)^{3-}/D_2(ATP)^{2-}$, even though the association constant of the latter system with its 50 % protonation of N1 corresponds to that of the $D(AMP)^-/D_2(AMP)^{\pm}$ 1:1 system. The most pronounced stability is now due to a dimeric $[H_2(ATP)]_2^{4-}$ stack [31]. The dominance of this dimer rather than of a polymer is evident from the observed size of the upfield shifts [30]; *inter*molecular ion pairs and hydrogen bonds between the two ATPs compensate the positive charges at the adenine residues [25,30,31]. This interpretation agrees with the properties observed for $H_2(GTP)^{2-}$ and $H_2(ITP)^{2-}$ [25].

Matrix-assisted self-association and its significance for cell organelles with high ATP concentrations

The indicated results show that the extent of aggregation is much affected by external conditions such as pH or the presence of metal ions, the neutralization of the negative charges at the phosphate groups being an important factor for stack formation. This has prompted a study of the effect of poly-α,L-lysine, $p(Lys)_n$, on the self-stacking properties of ATP in D_2O [32,33]. At pD 8.4 all the ε-amino groups of the side chains of $p(Lys)_n$ are protonated and carry a positive charge; i.e., ATP^{4-} with its negative phosphate groups is expected to "line up" along the $p(H{\cdot}Lys)_n^{n+}$ matrix. Indeed, under the mentioned conditions with $[p\text{-}Lys]_{side\ chains} \simeq 0.4$ M and $[ATP^{4-}] \leq 0.25$ M, $K = 11.5 \pm 2.1\ M^{-1}$ based on the

isodesmic model (eq. 1) [33]. This value should be compared with $K = 1.3 \pm 0.2\ M^{-1}$ (Table 1) measured for ATP^{4-} in the absence of any promoter. That indeed the positively charged side chains of $p(H{\cdot}Lys)_n^{n+}$ are responsible for the increased stacking tendency is proven by the fact that at pD 12, where the side chains of $p(Lys)_n$ are largely deprotonated, the stack-promoting effect of $p(Lys)_n$ has largely disappeared [33].

The indicated self-association of ATP is important for certain cell organelles; for example, for the 5-hydroxytryptamine organelles of mammalian blood platelets it was estimated that among other solutes ATP is 0.5 M, ADP 0.1 M, Mg^{2+} 0.44 M, and Ca^{2+} 0.11 M [34,35]. Similar ATP concentrations appear to hold, e.g., for cholinergic, neurosecretory, or synaptic vesicles and granules (see refs. in [33]). Clearly, such vesicles should be osmotically unstable, yet based on the summarized results one may conclude that the high nucleotide concentrations in such vesicles can be handled by nature via self-association and aggregate formation. Indeed, e.g., the chromaffin granules of bovine adrenal medulla contain next to nucleotides (0.2 M; mainly ATP) and metal ions (0.03 M; mainly Mg^{2+} and Ca^{2+}) [36] also so-called chromogranines, i.e., low-molecular-weight proteins that might be used as a matrix for aggregate formation.

In fact, one is tempted to speculate further: Considering that extracellular ATP is universally employed in cell–cell communication, particularly in synaptic transmission [37], and if one recalls that electrons may migrate over long distances in DNA [38], one may propose that one way to achieve information transfer (and there are others) occurs in the following way. Assuming that, say, six ATPs are lined up to form a stack covering a distance of approximately 20 Å one could imagine that at one end of the stack a metal ion (Fe^{2+}, Mn^{2+}, Cu^{+}) is oxidized to a higher charged state (Fe^{3+}, Mn^{3+}, Cu^{2+}) by a redox reaction and that this oxidized ion triggers hydrolysis of the triphosphate residue and that the electron travels through the stack to an acceptor at its other end [33]. This is depicted in a simplified fashion in Fig. 2. That oxidation of a divalent metal ion (Mn^{2+}) to a trivalent one (Mn^{3+}) dramatically enhances the dephosphorylation rate of nucleoside 5′-triphosphates is known [8,39].

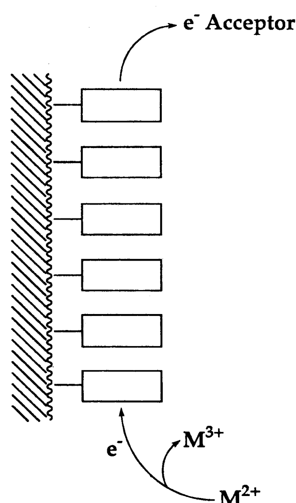


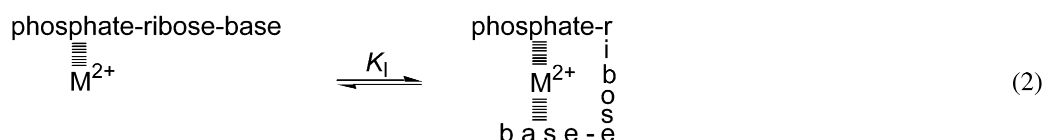
Fig. 2 Simplified picture of a matrix-assisted ATP stack indicating an electron and thus an information transfer through this stack from one end to the other.

PROPERTIES OF $M(ATP)^{2-}$ COMPLEXES: ISOMERIC EQUILIBRIA

With the information described in the preceding section at hand, great care was taken to study M^{2+}/ATP systems and the stabilities of the corresponding complexes under conditions which refer to monomeric

species. For example, if one applies the self-association constant $K = 15\ M^{-1}$ (which holds for adenosine; see Table 1), one calculates that in a 1 mM solution about 97 % of the species are present in their monomeric form [22,23,40]. In fact, most experiments with ATP were made at concentrations of 0.5 mM or below [41–44].

By ^{1}H NMR shift studies it has been proven more than 20 years ago that metal ions like Zn^{2+} or Cd^{2+} form in their $M(ATP)^{2-}$ complexes macrochelates through the interaction of the phosphate-coordinated metal ion with N7 of the adenine residue [22]. This means, the following intramolecular equilibrium 2



(2)

between an "open" isomer, $M(ATP)^{2-}_{op}$, and a chelated or "closed" isomer, $M(ATP)^{2-}_{cl}$, exists [43,45].

The formation of any kind of a chelated isomer must be reflected in an increased complex stability, compared to the stability of the open form [46]. This means, if we define in a general way for any ligand (L) the equilibrium constant for equilibrium 2, we obtain eq. 3 [47,48]:

$$K_I = [M(L)_{cl}]/[M(L)_{op}] \tag{3}$$

Values for K_I may be calculated [47–49] according to eq. 4,

$$K_I = \frac{K^M_{M(L)}}{K^M_{M(L)op}} - 1 \tag{4a}$$

$$= 10^{\log \Delta} - 1 \tag{4b}$$

where

$$\log \Delta = \log \Delta_{M/L} = \log K^M_{M(L)} - \log K^M_{M(L)op} \tag{5}$$

and

$$K^M_{M(L)} = \frac{[M(L)]}{[M][L]} = \frac{\{[M(L)_{op}] + [M(L)_{cl}]\}}{[M][L]} \tag{6}$$

$$K^M_{M(L)_{op}} = \frac{[M(L)_{op}]}{[M][L]} \tag{7}$$

Of course, knowledge of K_I allows us to calculate the formation degree of the closed species according to eq. 8:

$$\%\ M(L)_{cl} = 100 \cdot K_I/(1 + K_I) \tag{8}$$

The stability constants as defined by eq. 6 for the M(ATP) complexes of the biologically relevant metal ions Ca^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+}, and Zn^{2+}, as well as for the toxic ion Cd^{2+}, are listed in column 2 of Table 2 [41–44]; note, also monoprotonated $M(H;ATP)^{-}$ complexes are formed [42,44], but these are hardly of biological significance and therefore not considered in the present context. To be able to calculate K_I according to the above eq. 4, values for $K^M_{M(ATP)op}$ are needed. In the present case, these values are easily obtained since it has been shown by ^{1}H NMR shift experiments [22,23] and by other procedures as well that pyrimidine-nucleoside phosphates behave like simple phosphate ligands [50–52], i.e., equilibrium 2 is completely on its left side [43,45,53]. Hence, the stability constants for the com-

plexes of the mentioned metal ions and uridine 5′-triphosphate (UTP^{4-}), thymidine 5′-triphosphate ($dTTP^{4-}$), and cytidine 5′-triphosphate (CTP^{4-}) were measured [42,44]. For a given metal ion, the values were averaged for these pyrimidine-nucleoside 5′-triphosphates ($PyNTP^{4-}$), and these results are listed in column 3 of Table 2 [44]. The stability differences according to eq. 5 are seen in column 4 of Table 2 and it is evident that they are positive; in fact, in most instances they are clearly outside of the error limits (3σ, which corresponds to a 99.7 % confidence limit).

Table 2 Comparison according to eq. 5 of the stability constants of $M(ATP)^{2-}$ complexes (eq. 6) [42,43] with those for the corresponding $M(PyNTP)^{2-}$ species (eq. 7) [44], the latter having only a phosphate-M^{2+} coordination (aq. solution; 25 °C; I = 0.1 M, $NaNO_3$ or $NaClO_4$) (error limits: 3σ)[a,b].

M^{2+}	$\log K^{M}_{M(ATP)}$ [a]	$\log K^{M}_{M(PyNTP)}$ [b]	$\log \Delta_{M/ATP}$
Ca^{2+}	3.91 ± 0.03	3.84 ± 0.05	0.07 ± 0.06
Mg^{2+}	4.29 ± 0.03	4.21 ± 0.04	0.08 ± 0.05
Mn^{2+}	5.01 ± 0.07	4.93 ± 0.03	0.08 ± 0.08
Cu^{2+}	6.34 ± 0.03	5.86 ± 0.03	0.48 ± 0.04
Zn^{2+}	5.16 ± 0.06	5.02 ± 0.02	0.14 ± 0.06
Cd^{2+}	5.34 ± 0.03	5.07 ± 0.03	0.27 ± 0.04

[a] The acidity constants of $H_2(ATP)^{2-}$ are $pK^{H}_{H_2(ATP)}$ = 4.00 ± 0.01 and $pK^{H}_{H(ATP)}$ = 6.47 ± 0.01 [42]; the first value corresponds to the release of the proton from the $(N1)H^{+}$ site and the second one from the monoprotonated triphosphate residue, this proton being located at the γ group.
[b] Average of the values for $H(UTP)^{3-}$, $H(dTTP)^{3-}$, and $H(CTP)^{3-}$, i.e., for $H(PyNTP)^{3-}$: $pK^{H}_{H(PyNTP)}$ = 6.50 ± 0.05 [41,43]; this proton is released from the γ group.

Application of these values for $\log \Delta_{M/ATP}$ (Table 2, column 4) to eqs. 4 and 8 provides the results [44] summarized in Table 3, where for comparison also the corresponding data for the $M(ADP)^{-}$ and M(AMP) complexes are given [54]. It is astonishing to note that the formation degrees of the macrochelated species are rather similar for all three adenine-nucleotide complexes, this means, for M(AMP), $M(ADP)^{-}$, and $M(ATP)^{2-}$ and this despite the fact that the stability constants, which are largely determined by the coordinating properties of the phosphate residues [44,54] differ by several orders of magnitude between the complexes of the mono-, di-, and triphosphates [52].

Finally, it may be mentioned that there are (at least) two types of macrochelates, i.e., one, where the phosphate-coordinated metal ion interacts inner sphere with N7 and one, where this interaction occurs in an outer sphere manner with a water molecule between M^{2+} and N7. Evidence for this species has indirectly been provided by spectrophotometric [50] and ^{1}H NMR experiments [42]. Of course, the values provided in this review for the formation degrees of the closed species (Table 3) encompass both macrochelated species since potentiometric pH titrations cannot distinguish between them. For further information, refs. [42–45,53,54] may be consulted, where also stability constants for complexes of other M^{2+} are listed.

Table 3 Increased complex stabilities, log $\Delta_{M/AN}$ (eq. 5), for several adenine-nucleotide (AN) complexes, M(AN), and extent of macrochelate formation (eq. 2) in $M(ATP)^{2-}$ [44], $M(ADP)^{-}$ [54], and M(AMP) [54] species, as quantified by the dimensionless equilibrium constant K_I (eqs. 3 and 4) and the percentage of $M(AN)_{cl}$ (eq. 8) for aqueous solutions at 25 °C and I = 0.1 M ($NaNO_3$ or $NaClO_4$) (error limits: 3σ)[a].

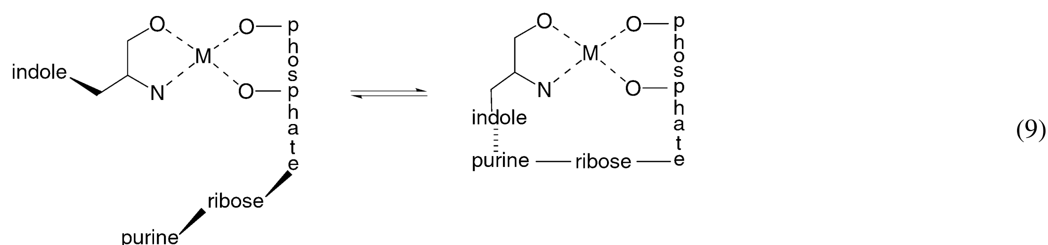
AN	M^{2+}	log $\Delta_{M/AN}$	K_I	% $M(AN)_{cl}$
ATP^{4-}	Ca^{2+}	0.07 ± 0.06	0.17 ± 0.16	15 ± 12
	Mg^{2+}	0.08 ± 0.05	0.20 ± 0.14	17 ± 10
	Mn^{2+}	0.08 ± 0.08	0.20 ± 0.22	17 ± 15
	Cu^{2+}	0.48 ± 0.04	2.02 ± 0.28	67 ± 3
	Zn^{2+}	0.14 ± 0.06	0.38 ± 0.19	28 ± 10
	Cd^{2+}	0.27 ± 0.04	0.86 ± 0.17	46 ± 5
ADP^{3-}	Ca^{2+}	0.04 ± 0.04	0.10 ± 0.09	9 ± 8
	Mg^{2+}	0.06 ± 0.04	0.15 ± 0.11	13 ± 9
	Mn^{2+}	0.10 ± 0.04	0.26 ± 0.10	21 ± 7
	Cu^{2+}	0.34 ± 0.05	1.19 ± 0.25	54 ± 5
	Zn^{2+}	0.16 ± 0.06	0.44 ± 0.19	31 ± 9
	Cd^{2+}	0.36 ± 0.05	1.29 ± 0.26	56 ± 5
AMP^{2-}	Ca^{2+}	0.03 ± 0.06	0.07 ± 0.14	7 ± 13
	Mg^{2+}	0.06 ± 0.05	0.15 ± 0.13	13 ± 10
	Mn^{2+}	0.07 ± 0.05	0.17 ± 0.15	15 ± 11
	Cu^{2+}	0.30 ± 0.06	1.00 ± 0.29	50 ± 7
	Zn^{2+}	0.25 ± 0.09	0.78 ± 0.38	44 ± 12
	Cd^{2+}	0.30 ± 0.07	1.00 ± 0.32	50 ± 8

[a]The acidity constants of $H_2(ATP)^{2-}$, $H_2(ADP)^{-}$ and $H_2(AMP)^{\pm}$ are $pK^H_{H_2(ATP)}$ = 4.01 ± 0.01 and $pK^H_{H(ATP)}$ = 6.47 ± 0.01 [42], $pK^H_{H_2(ADP)}$ = 3.92 ± 0.02 and $pK^H_{H(ADP)}$ = 6.40 ± 0.01 [54], and $pK^H_{H_2(AMP)}$ = 3.84 ± 0.02 and $pK^H_{H(AMP)}$ = 6.21 ± 0.01 [54], respectively. The first proton is released always from the $(N1)H^+$ site and the second one from the monoprotonated terminal phosphate group.

SOLUTION STRUCTURES OF MIXED LIGAND COMPLEXES CONTAINING ATP^{4-}

Considering that adenine nucleotides can self-stack, it is no surprise to find that the adenine residue can also interact with other aromatic moieties. Indeed, about 30 years ago intramolecular aromatic-ring stacking was described for the first time [55,56] between heteroaromatic N bases (Arm), i.e., 2,2′-bipyridine or 1,10-phenanthroline, and AMP^{2-}, ADP^{3-}, or ATP^{4-}, both ligands being bridged by a metal ion [57–60]. Indeed, these heteroaromatic amines and the adenine residue may form stacks themselves [56–58,61], but their interaction is tremendously facilitated by a metal ion bridge [58–62]. Originally, these interactions had been proven by UV spectrophotometry [55–58] and ^1H NMR shift measurements [61,62]. Later, complexes of the type M(Arm)(adenine nucleotide) were also isolated in the solid state and the intramolecular stacking interaction was proven by X-ray crystal structure studies [63–65].

The first mixed ligand complex containing ATP and an amino acid was one with tryptophanate, i.e., $Zn(ATP)(Trp)^{3-}$. By ^1H NMR shift experiments it was shown that an indole-adenine interaction takes place [66], which may be promoted by Zn^{2+}. Later, the position of the intramolecular equilibrium 9



(9)

was determined with Zn^{2+} as metal ion and it was concluded [67] that the stacked species occurs with a formation degree of approximately 75 %. Other metal ions were studied as well [67–70], and the occurrence of intramolecular stacks in $M(ATP)(Trp)^{3-}$ complexes was confirmed by several groups [71,72]. Related complexes containing phenyl and imidazole residues have also been investigated [73–75].

Moreover, it was shown that the isopropyl residue of leucinate is also able to undergo a hydrophobic interaction in $M(ATP)(Leu)^{3-}$ complexes; e.g., the formation degree of the "closed" species of the corresponding Mn^{2+} complex amounts to about 40 % [67]. The occurrence of such species with a hydrophobic interaction was again proven by ^1H NMR shift studies [67] and by comparisons of stability constants, which were mostly determined via potentiometric pH titrations [67].

Quantification of the formation of *intra*molecular stacks in mixed ligand complexes containing a heteroaromatic amine (Arm) allowed the conclusion [16] that the tendency of nucleobase residues to form stacking adducts increases in the series: orotate $\lesssim$ uracil $\lesssim$ cytosine $\lesssim$ thymine < hypoxanthine < guanine < adenine < 7-deazaadenine. Similarly, the recognition between the adenine residue and amino acid side chains in mixed ligand complexes of the type $M(ATP)(aa)^{3-}$, where aa = amino acetate derivative, decreases in the order (partly tentative): indole residue (tryptophan) > phenyl residue (phenylalanine) $\gtrsim$ isopropyl residue (leucine) $\gtrsim$ imidazole residue (histidine) > methyl residue (alanine).

NUCLEOTIDE ANALOGS AS ANTIVIRAL AGENTS

Since nucleotides are at the crossroad of many metabolic reactions [1], the attempts to obtain therapeutically useful compounds by varying either the nucleobase residue, the sugar moiety or the phosphate group (for refs., see [76]) are long-standing. One of the successful attempts has led to 9-[2-(phosphonomethoxy)ethyl]adenine ($PMEA^{2-}$), Ade(N9)-CH_2CH_2-O-CH_2-PO_3^{2-}, now also known as *Adefovir* ([77] and refs. therein). This compound was first mentioned in the literature in 1986 [78] and 16 years later, in 2002, its bis(pivaloyloxymethyl)ester (*Adefovir dipivoxil*) was approved by the U.S. Food and Drug Administration (FDA) for use against hepatitis B [79], which is caused by a DNA virus.

The mentioned diester was synthesized to facilitate the transport of the drug into the cell. Inside the cell, the ester is hydrolyzed and the resulting $PMEA^{2-}$ can be considered as an analog of adenosine 5′-monophosphate (AMP^{2-}) or 2′-deoxyadenosine 5′-monophosphate ($dAMP^{2-}$) [48,49]. Phosphonate derivatives of this kind are converted by cellular nucleotide kinases into their diphosphates and as such they inhibit viral and to a lesser extent cellular DNA synthesis of the host [80,81]. In other words, $PMEApp^{4-}$, which is an analog of 2′-deoxyATP^{4-} or of ATP^{4-}, is recognized by nucleic acid polymerases as a substrate and incorporated into the growing nucleic acid chain, which is terminated thereafter due to the lack of a 3′-hydroxy group [82] (and refs. in [83]).

In fact, for reverse transcriptase of the avian myeloblastosis virus is $PMEApp^{4-}$ initially a better substrate than $dATP^{4-}$ (by a factor of 20) [82]. This observation is astonishing, and the question arises why $PMEApp^{4-}$ is initially so effective.

Based on experiments devoted to the metal ion-promoted hydrolysis of various nucleoside 5′-triphosphates, we have realized already many years ago [84] that two metal ions need to be co-

ordinated to the triphosphate chain [8,85]. If the metal ion coordination is of the type $M(P_\alpha P_\beta)$-$M(P_\gamma)$ then transphosphorylation of the terminal γ-phosphate group occurs [8,85]. This binding mode has been confirmed in the meanwhile by an X-ray crystal structure study of *Escherichia coli* phosphoenolpyruvate carboxykinase [86]. Correspondingly, if the two metal ions are coordinated in a $M(P_\alpha)$-$M(P_\beta P_\gamma)$-type fashion then the break occurs between P_α and P_β [8,85] and a nucleotidyl group is transferred as it is catalyzed by nucleic acid polymerases; this has also been confirmed by X-ray crystal structure studies [87].

The reason why M_2(PMEApp) is formed in a facilitated manner compared to M_2(ATP) or M_2(dATP) is evident from the structures shown in Fig. 3. The ether oxygen facilitates metal ion binding to the α-phosphate group due to the formation of a 5-membered ring [83,88]. This together with the increased basicity of this group (phosphonates are more basic than phosphates) [89] leads to a higher concentration of the needed $M(P_\alpha)$-$M(P_\beta P_\gamma)$ binding mode of the metal ions making $PMEApp^{4-}$ a better substrate than $(d)ATP^{4-}$ [83,88].

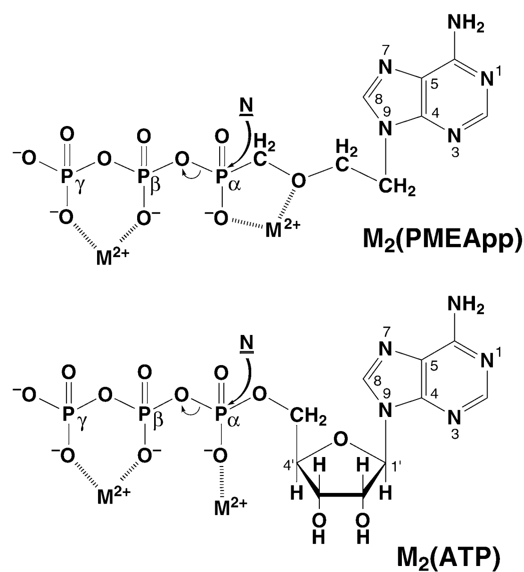
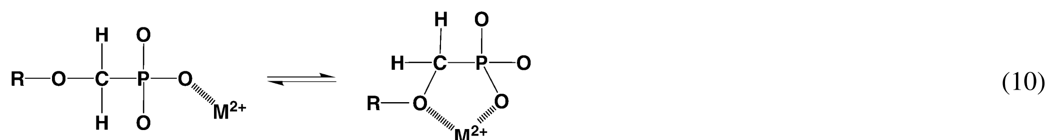


Fig. 3 Simplified structures of the M_2(PMEApp) and M_2(ATP) complexes. This $M(P_\alpha)$-$M(P_\beta P_\gamma)$ binding mode of the two metal ions is crucial for the transfer of a nucleotidyl unit by nucleic acid polymerases. Note, with M_2(PMEApp) this binding mode is favored due to the formation of the 5-membered chelate with the ether oxygen of the aliphatic chain [83,88]. Of course, one could think of situations where, e.g., M^{2+} at the β and γ groups is replaced by (a) monovalent metal ion(s) and/or ammonium residues.

Indeed, the formation degree of the chelated or closed (cl) species with the 5-membered ring in equilibrium 10



(10)

has been determined for a number of metal ions [90–92]. For example, $M(PMEA)_{cl}$ amounts for the metal ions Mg^{2+}, Ca^{2+}, Mn^{2+}, and Zn^{2+} to 31 ± 8, 22 ± 13, 38 ± 11, and 50 ± 12 % (3σ), respectively [48,83].

Finally, it may be emphasized that replacement of the ether oxygen in $PMEA^{2-}$ by a sulfur atom [93] or a CH_2 unit [80] as well as altering the position of the ether oxygen within the aliphatic chain [80,94] leads to compounds that have no useful biological activity. In addition, the coordination chemistry of these PMEA derivatives [95,96] also differs significantly from that of $PMEA^{2-}$ itself [48,83,95].

CONCLUDING REMARKS

Especially in the context of the last-mentioned section, it needs to be pointed out that the formation of chelates of the indicated kind is connected with very small changes in free energy (ΔG^0) [46,91]. For example, a stability enhancement of $\log \Delta = 0.3$ (see eq. 5) means that an intramolecular equilibrium is exactly in the middle ($K_I = 1.0$) and that 50 % of the closed isomer are formed; the connected change in free energy is –1.7 kJ/mol [91]. Moreover, a stability enhancement of only 0.1 log unit (= $\log \Delta$) corresponds already to a formation degree of the chelated species of 21 % and a change in free energy of only –0.6 kJ/mol [91]. Of course, the occurrence of 20 % of a substrate in the correct conformation is more than is needed by an enzyme to recognize this substrate. On the other hand, it needs to be emphasized that high formation degrees become quickly "expensive"; e.g., a formation degree of 90 % of the chelated species corresponds to $\log \Delta = 1.0$ and $\Delta G^0 = -5.7$ kJ/mol [91].

Another point that needs emphasis is the fact that all the results described herein refer to aqueous solutions. However, in an active site cavity of an enzyme, the intrinsic dielectric constants are significantly reduced compared to bulk water [97]. If one tries to mimic an "equivalent-solution" or "effective" dielectric constant of 35 in an active site cavity by a 50 % (v/v) 1,4-dioxane-water mixture, which also has a dielectric constant of 35 [97], one observes a stability increase for the Cu^{2+} complex of D-ribose 5-monophosphate by about 1.5 log units [98], i.e., by a factor of approximately 30. Generally speaking, interactions between metal ions and oxygen donor ligands increase drastically if the dielectric constant of the medium is reduced [98]. On the other hand, the interaction between metal ions and N donor sites is more complicated and passes through a minimum [99,100], i.e., the interaction is high in water and high again at a low dielectric constant. For example, the macrochelate formed in Cu(AMP) between the phosphate-coordinated Cu^{2+} and N7 reaches in water and in 50 % dioxane each a formation degree of about 50 %, whereas in 30 % (v/v) 1,4-dioxane-water $Cu(AMP)_{cl}$ occurs only with a formation degree of about 10 % [99].

It is evident that in an active site cavity [74,97], where the water molecules at the interface with the protein are largely structured, a small shift of a substrate, say of 2 Å, is enough to bring it from a rather hydrophilic to a hydrophobic region due to the presence of an aromatic or aliphatic amino acid side chain, i.e., from a high into a low dielectric medium, and this alone may significantly alter the stability as well as the structure of a complex. Clearly, in this respect more research is needed.

Other points of future research, with regard to adenine nucleotides, have to be, e.g., the evaluation of a systematic replacement of the oxygen atoms in a triphosphate chain by sulfur atoms on complex stabilities. In this respect, knowledge is at present rather scarce [76,101–103]. This kind of research is desirable, e.g., with regard to ribozymes [104] and the antisense strategy (see refs. in [103]), where phosphorothioates play a role. Another point is our inadequate understanding of the intrinsic acid-base properties of the N1, N3, and N7 sites of the adenine residue; the corresponding micro acidity constants regarding N1 and N7 have recently been derived [105] and the acidifying properties of M^{2+} coordinated to N7 on the $(N1)H^+$ site and vice versa were evaluated [106], but as far as N3 is concerned, no detailed study exists.

ACKNOWLEDGMENTS

The competent technical assistance in the preparation of this manuscript by Mrs. Rita Baumbusch and Mrs. Astrid Sigel and the support of the described research over many years by the Swiss National

Science Foundation and more recently by the Swiss Federal Office for Education & Science within the COST D8 and D20 programs are gratefully acknowledged.

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