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Self-Association, Protonation, and Metal-Coordination of 1,N^6-Ethenoadenine Derivatives in Comparison with Their Parent Compounds Adenosine, AMP and ATP**

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Since X-ray structure determinations of enzyme-substrate complexes are rare and not easily obtained, the use of so-called molecular probes has become popular. 1,N^6-ethenoadenine derivatives are often employed as such to substitute for adenine substrates due to their excellent fluorescent properties. By using these derivatives as an example, it is shown that a seemingly small alteration, namely the insertion of the 1,N^6-etheno-bridge into the adenine moiety, which may appear from an «organic» point of view as very satisfying, can drastically alter the metal ion-coordinating properties. As metal ions are essential to biological phosphoryl and nucleotidyl transfer, it is important to be aware of these changes; in fact, great care should be exercised in employing 1,N^6-ethenoadenine derivatives as probes for adenine substrates in the presence of metal ions: the stabilities and structures of the corresponding complexes in solution are often rather different.

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1. The Biological Relevance of Nucleotides

Nucleotides play a central role in the metabolism of living cells[1]. They serve as substrates for the enzyme-catalyzed transfers of nucleotidyl or phosphoryl groups – reactions which depend on the presence of divalent metal ions[2,3]. Nucleotides are also involved in the information storage via RNA and DNA or in energy-transfer processes[1]. Adenosine 5′-triphosphate (ATP) is an especially prominent member of this class of key-compounds: one-sixth of all known enzymes require ATP or a related adenine-containing cofactor[4]. It has been estimated[5] that a human being uses and resynthesizes his own body weight of ATP daily.

These examples nicely illustrate the importance of nucleotides, especially of ATP, and explain the efforts undertaken to probe the binding sites of nucleotides, e.g., to proteins. Indeed, the binding and recognition interactions[6,7] between nucleotides/nucleic acids and amino acids/proteins are an essential relationship between these two classes of key-compounds.

2. Altered Nucleotides as Probes: The Advent of 1,N^6-Ethenoadenine Derivatives

The use of structurally altered nucleotides as probes provides one way to study reactions of enzymes which involve nucleotides as substrates. Indeed, all three parts of nucleotides have been systematically modified: e.g., the ribose residue has been replaced by glucose[8]; in the phosphate moiety the bridging oxygen was substituted by a sulfur[9], an imido[10,11], a methylene[11,12], or a peroxo bridge[13]; and the purine base was enlarged as in *lin*-benzoadenine nucleotides[4,14].

The latter alteration leads to a so-called dimensional probe; such probes are related to natural cofactors by a defined dimensional change in the molecule[4], and they are widely utilized in solution studies. Other base modifications led to 1,N^6-ethenoadenosine (ε-adenosine) and 3,N^4-ethenocytidine[15]. ε-Adenosine[15] and its nucleotides[16,17] were first synthesized in 1972, based on the previously described reaction of chloroacetaldehyde with 9-methyladenine[18]. In this reaction the N-6 and N-1 atoms are linked by the 1,N^6-etheno bridge to a five-membered ring[19] (see Fig. 1)[20].

As ε-adenosine and its derivatives exhibit fluorescence properties[15–17], a great effort has been made to identify the species responsible for fluorescence[21–25]. These attempts are understandable, as the incorporation of the ε-adenosyl residue into biological macromolecules provides a powerful tool to gain structural information[25,26]:

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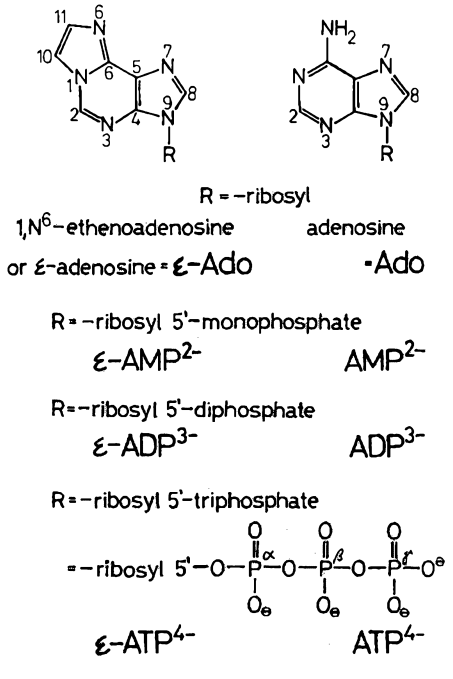


Fig. 1. Structural formulae of the 1,N⁶-ethenoadenine (= ε-adenine) derivatives and of their adenine-parent compounds considered in this account [20]. To facilitate comparisons between the ε-adenine and the adenine residues the conventional atom numbering for adenines is adapted, a procedure which is common [19].

ε-adenosine derivatives have been used, e.g., to probe the active sites of adenine nucleotide dependent enzyme systems [27], and the 1,N⁶-etheno analogues of flavin adenine dinucleotides [28] and adenosylcobalamin, i.e., the ε analogue of coenzyme B_{12} [29], were also synthesized and studied. ε-ATP [20], as a probe for ATP, is particularly widely utilized [26,30]; the reasons are (i) the mentioned excellent fluorescent properties [25,26,31] and (ii) the possibility to evaluate [32] the importance of the N-1 and 6-NH_2 positions of the purine residue for the specificity of binding to particular enzymes, due to the selective alteration of these positions by the etheno bridge (Fig. 1).

3. Aim of the Present Account

Being a coordination chemist, and considering that all ATP dependent enzyme systems are also metal ion dependent [1–3] and knowing that metal ions alter the structure of nucleotides in solution [33–35], one wonders about the influence of the 1,10-phenanthroline-like binding site in the 1,N⁶-ethenoadenine derivatives on the complex-forming properties of these derivatives in comparison with their parent ligands (cf. Fig. 1). In other words, questions are arising like: are ε-AMP or ε-ATP indeed useful probes for AMP or ATP in the *presence* of metal ions? Based on our recent studies with ε-adenosine [36] and some of its nucleotides [37–41] an effort is now made to evaluate this problem and to answer, at least partly, such questions.

One of the first points which have to be clarified before any metal-binding properties can be discussed is the characterization of the tendency for self-association of the ε-adenine derivatives. This is a property well-known for adenine derivatives [34,42], and it is also a property whose possible biological importance should not be underestimated: e.g., the concentration of ATP in the chromaffin granules of the adrenal medulla is about 0.1 M [43] and under these conditions, especially as also metal ions are present [43], considerable amounts of ATP have to exist in the form of dimers and oligomers [34].

4. Comparison of the Extent of Self-Stacking for Adenine and 1,N⁶-Ethenoadenine Derivatives

4.1. Self-Association of ε-Adenosine and of Its Parent Nucleoside

It is now well-known that self-association of adenosine and other purine derivatives occurs via stacking [34,35,44]. As the entire ε-adenine moiety is nearly planar with a maximum deviation of about 0.03 Å (=3 pm) among the ring atoms [19], self-stacking has also to be expected for ε-adenosine in aqueous solution. Indeed, the ^{1}H-NMR spectrum changes considerably when the concentration is increased from 5 to 81 mM (Fig. 1 in ref. [36]). Furthermore, the upfield shifts observed [36] for H-2, H-8, H-10, H-11, and H-1′ are much larger than would be expected for the shift due to a single adjacent molecule; i.e., these observations agree with those made at other purine derivatives and they preclude the assumption of only dimer formation [34,42]. The conclusion that polymers are formed agrees also with vapor-pressure osmometric data [45] of ε-adenosine.

In fact, it is now generally agreed [34,42,46] that self-association of purine derivatives [34,35,42,44–48] proceeds beyond the dimer stage, the distance between stacked molecules being in the order of 3.5 Å (= 0.35 nm) [19,49–51], and the experimental results [34–38,42] are best interpreted by the isodesmic model for indefinite non-cooperative self-stacking [47]. This model is based on the assumption that, e.g., for an adenine derivative (A), the equilibria (1) are all characterized by the same equilibrium constant (2):

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

$$K = [(A)_{n+1}]/[(A)_n]\,[A] \quad (2)$$

Adaption of this model to ^{1}H-NMR shift measurements was described in detail [34,42,52]; it results in a relationship between the observed chemical shift (δ_{obs}) and the total concentration [A]. Application to the experimental data gives a value for the association constant K as defined in Equation (2), and also values for the shift, δ_0, at infinite dilution (monomeric A) and for δ_∞, the shift of a molecule in an infinitely long stack.

The self-association constant as determined by ^{1}H-NMR shift experiments for adenosine, $K = 15 \pm 3$ M^{-1} [42,53], is apparently somewhat larger than that of ε-adenosine, $K = 9.4 \pm 1.2$ M^{-1} [36,54]; both values were determined under exactly the same conditions. The relatively large error limits are a consequence of the restricted solubility of these two nucleosides. It appears, that one has to conclude, that the self-stacking tendency of ε-adenosine, despite its larger planar system (Fig. 1), is rather somewhat smaller than that of adenosine (certainly it is *not* larger). This agrees with a conclusion [55] regarding the *intra*molecular association of the dinucleoside monophosphates 1,N⁶-ethenoadenylyl-(3′→5′)-1,N⁶-ethenoadenosine (εApεA) and adenylyl-(3′→5′)-adenosine (ApA), that stacking in ApA is more pronounced than in εApεA (pH 7.0) [56] *).

As expected, protonation of the base moieties of ε-adenosine and of adenosine leads to repulsion, and hence to a reduced stacking tendency: for H(ε-Ado)$^\oplus$ and H(Ado)$^\oplus$ $K \leqslant 0.4$ M^{-1} [36] and $K = 0.9$ M^{-1} [53], respectively. Like for the unprotonated base moieties, self-association appears again to be somewhat more pronounced for the adenine residue. That the presence of a positive charge at the base moiety leads to repulsion in the stacks is also evident from Zn(ε-adenosine)$^{2\oplus}$: the estimation [36] of the upper limit of the self-association constant gave $K \leqslant 1.2$ M^{-1}; a value to be compared with $K = 9.4$ M^{-1} for free ε-adenosine. These results are in line with related data [52,63]; e.g., for the 1,10-phenanthroline system it holds: Phen, $K = 31.1$ M^{-1} [60] > H(Phen)$^\oplus$, $K = 12.0$ M^{-1} [63] > Zn(Phen)$^{2\oplus}$, $K = 1.1$ M^{-1} [52].

In the solid state, forces other than stacking forces have a considerable influence, therefore no (simple) relation exists between results obtained in solution and

*) That seemingly small alterations in a molecule may influence self-stacking drastically, becomes evident, e.g., from the replacement of the amino group at C-6 in adenosine by a hydroxy group and isomerization of the enol to the more stable keto isomer to give inosine; this change reduces the association constant, $K = 15$ M^{-1}, by about one-fifth, to $K = 3.3$ M^{-1} [34]. However, the two-ring molecule inosine and the one-ring molecule uridine, which differ structurally mainly by the removal of the imidazole ring from inosine, differ in their self-association only by a factor of about one-third [34], i.e. $K = 3.3$ M^{-1} vs. 1.2 M^{-1}. The strictly coplanar three-ring system 1,10-phenanthroline ($K = 31.1$ M^{-1}) [60] and the two-ring system 2,2′-bipyridyl ($K = 7.4$ M^{-1}) [60] differ by a factor of about one-fourth, but ε-adenosine ($K = 9.4$ M^{-1}) which has about the same size as 1,10-phenanthroline, is in its self-association property more similar to 2,2′-bipyridyl.

The given examples indicate that factors other than the size of the aromatic system also influence the self-stacking tendency. These factors are probably mainly steric effects introduced by substituents, thus altering the orientation of the stacks, and the addition (or removal) of heteroatoms or polar groups that will influence the bonding or charge transfer in the stack [61]. It should be pointed out that the formation of such stacks is connected with a negative enthalpy (ΔH^0) contribution to ΔG^0 [45,62].

those in the solid state. For example, there is no base stacking in solid N^6-(2-chloroethyl)-substituted 1,N^6-ethylene(9-methyl)-adenine iodide[64], whereas there are infinite stacks in the related salt 10-ethyl-ε-adenosine hydrochloride[19]. There is also no stacking in crystalline 1,10-phenanthroline hydrate[65], despite the pronounced stacking tendency of phenanthroline in aqueous solution[52,60].

4.2. Self-Stacking in Adenine- and ε-Adenine-Nucleotide Systems

It is to be expected that the presence of the negatively charged phosphate groups in nucleotides influences the self-association tendency of the base residues, though it cannot be assumed (in contrast to earlier reasonings) that repulsion between the negative charges diminishes self-stacking completely. Indeed, the ^{1}H-NMR spectra of ε-$AMP^{2\ominus}$ and ε-$ATP^{4\ominus}$ change considerably as the concentration is increased from about 5 mM to 400 mM. The upfield shifts for the protons of the 1,N^6-ethenoadenine moiety and of H-1′ of the ribose residue are shown for ε-$AMP^{2\ominus}$ and ε-$ATP^{4\ominus}$ in Fig. 2.

Application of the isodesmic model for indefinite non-cooperative self-association (Equations (1) and (2)) to the experimental data results in the curves shown in Fig. 2. Computer-calculated least-squares fits for the variation of the upfield shifts for each of the protons with increasing concentration gave the same stability constant for each of the protons within experimental error for a given nucleotide. The association constants for self-stacking determined in this way and as defined by Equation (2) are summarized for several purine derivatives in Table 1.

Comparison of these equilibrium constants shows a decreasing tendency for self-stacking within the two series ε-adenosine ≫ ε-$AMP^{2\ominus}$ > ε-$ATP^{4\ominus}$ and adenosine ≫ $AMP^{2\ominus}$ > $ATP^{4\ominus}$ (Table 1). This order is expected due to the repulsion between the negatively charged phosphate group within the stacks. The data for $AMP^{2\ominus}$, $ADP^{3\ominus}$, and $ATP^{4\ominus}$ enable us to conclude that the value for K of ε-$ADP^{3\ominus}$ is within the limits given by the constants of ε-$AMP^{2\ominus}$ and ε-$ATP^{4\ominus}$, i.e. between 1.9 and 2.5 M^{-1}. The stacking tendency of ε-$AMP^{2\ominus}$ and ε-$ATP^{4\ominus}$ is similar to that of their parent nucleotides, though it is slightly more pronounced; this contrasts with the somewhat lower self-association tendency of ε-adenosine compared with that of adenosine. However, as indicated in Section 4.1, seemingly small alterations in a molecule may significantly influence the extent of self-stacking.

The size of the upfield shifts, $\Delta\delta = \delta_0 - \delta_{av}$ (see Section 4.1), especially for H-2, H-10, and H-11 of ε-adenosine[36], ε-$AMP^{2\ominus}$[37], and ε-$ATP^{4\ominus}$[38] confirms that stacking proceeds beyond the dimer stage; these upfield shifts are much higher than expected for a simple dimer. The same applies for the corresponding parent compounds[34]. The similarity of the $\Delta\delta$ values for a given proton of the ε-adenine derivatives suggests in addition, that the orientation of the stacks is also similar for all three substances.

A further interesting observation based on the chemical shifts is that the δ_0 values for H-8 and H-1′ differ somewhat for ε-adenosine and its nucleotides (see Table S1 in ref.[38]), whereas the δ_0 values for H-2, H-10, and H-11 are identical within experimental error. This indicates that, like the adenosine nucleotides[33], the ε-adenosine derivatives adopt in solution the *anti* conformation[66] with respect to the glycosyl bond (N-9/C-1′), and therefore the negatively charged phosphate groups are close to H-8 (and H-1′), but not to H-2, H-10, and H-11 (cf. Fig. 1). The corresponding observation has also been made for the δ_0 values of H-8 and H-1′ on the one hand and of H-2 on the other for adenosine and its nucleotides (see Table III in ref.[34]), confirming thus the *anti* conformation of these substances. These conclusions on the structure of these nucleotides in solution are important with regard to the possible coordination of a metal ion to both, the phosphate residue and the base moiety (see Sections 6.3 and 6.4).

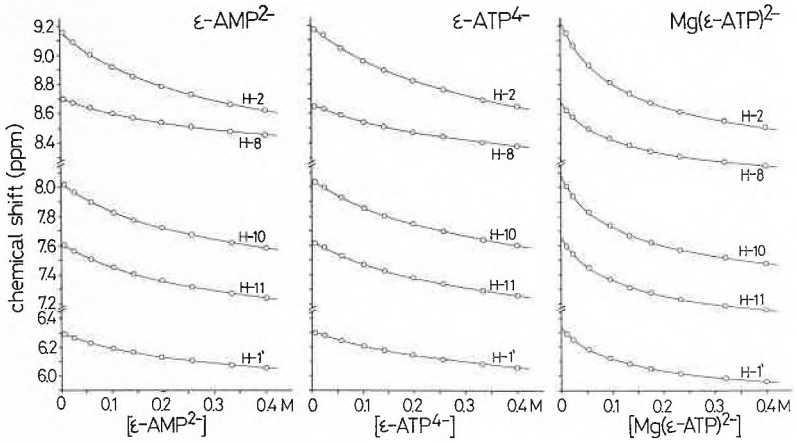


Fig. 2. Variation of the chemical shift for H-2, H-8; H-10, H-11, and H-1′ of the ε-adenosine moiety with varying concentrations of ε-$AMP^{2\ominus}$ (pD 8.9)[37], ε-$ATP^{4\ominus}$ (pD 8.4)[38], or Mg(ε-ATP)$^{2\ominus}$ (pD 7.3)[38]. The ^{1}H-NMR signals were assigned[37,38] according to a deuterium labeling study carried out with ε-adenosine[17]. The spectra were measured[37,38] on a Bruker FT 90 at 90.025 MHz (D_2O; 27°C; I = 0.1 to ≈ 1.2 for ε-AMP and 0.1 to ≈ 2 for the ε-ATP systems, $NaNO_3$), relative to internal $(CH_3)_4N^{\oplus}$ and converted to values relative to sodium 3-(trimethylsilyl)propanesulfonate by adding 3.188 ppm. The curves shown are the computer-calculated best fit of the experimental data (calculated with the K values of Table 1), using the indefinite non-cooperative stacking model[34,42] (Equations (1) and (2)).

Table 1. Comparison of the equilibrium constants K (Equation (2)) for self-stacking (equilibrium (1)) of several 1,N^6-ethenoadenine derivatives with the corresponding parent adenines and of some of their metal ion complexes as determined by ^{1}H-NMR shift measurements in D_2O (27°C)[a].

System[20]	pD[b]	I	K (M^{-1}) for	
			ε-adenine derivatives	adenine derivatives
Ns	7.0	0.1	9.4 ± 1.2[36]	15 ± 3[42,53]
$H(Ns)^{\oplus}$	3.1[36], 2.4[53]	0.1	≤ 0.4[36]	0.9 ± 0.2[53]
$Zn(Ns)^{2\oplus}$	6.1	1.5	≤ 1.2[36]	
$NMP^{2\ominus}$	8.9	0.1–≈ 1.2	2.5 ± 0.3[37]	2.1 ± 0.4[34]
$NDP^{3\ominus}$	8.9	0.1–≈ 1.7		1.8 ± 0.5[35]
$NTP^{4\ominus}$	8.4	0.1–≈ 2	1.9 ± 0.2[38]	1.3 ± 0.2[34]
$Mg(NTP)^{2\ominus}$	7.3[38], 7.4[34]	0.1–≈ 2	7.6 ± 0.5[38]	4.0 ± 0.5[34]
$Zn(NTP)^{2\ominus}$	7.2	0.1–≈ 2	≈ 4.4 ± 2.4[38][c,d]	≈ 11.1 ± 4.5[34][c]
			≈ 3.3 ± 1.2[38][c,e]	≈ 9.6 ± 0.8[34][c,f]

[a] The ionic strength was adjusted to 0.1 M by adding $NaNO_3$ if necessary. The given range of error is *twice* the standard error.
[b] pD of the solutions used for the measurements.
[c] Regarding the validity of these values see text in Section 4.4[34,38].
[d] Weighted mean of the individual values of K obtained from the chemical shifts of H-2, H-8, H-10, H-11, and H-1′; see text in Section 4.4[38].
[e] Weighted mean of the individual values of K obtained from the chemical shifts of only H-2, H-10, and H-1′; see text in Section 4.4[38].
[f] See footnote 71 in ref.[34] (weighted mean of the individual values of K obtained from the chemical shifts of H-2 and H-1′ under omission of a single experimental value, i.e. the one measured of $[Zn^{2\oplus}/ATP^{4\ominus}] = 0.0061$ M).

4.3. The Promoted Self-Association of Mg(ε-ATP)$^{2\ominus}$ and Mg(ATP)$^{2\ominus}$

A comparison of the experimental data shown in Fig. 2 for Mg(ε-ATP)$^{2\ominus}$ and ε-$ATP^{4\ominus}$ reveals immediately, i.e., without

any calculations, that self-stacking of the complex is more pronounced than of the free nucleotide. The experimental data can again be well explained by the isodesmic model for indefinite non-cooperative self-association and the resulting association constant (Table 1) confirms the promotion of self-stacking by $Mg^{2\oplus}$: K is increased by a factor of about 4 (7.6 M^{-1} vs. 1.9 M^{-1}), a result which corresponds approximately to the situation with $ATP^{4\ominus}$ and $Mg(ATP)^{2\ominus}$, where K increases by a factor of about 3.

This promotion of self-stacking, which again proceeds beyond the dimer stage[34,38], can be attributed to a partial neutralization of the negative charge at the phosphate moiety by formation of a $Mg(NTP)^{2\ominus}$ complex[20]. Indeed, the association constants for $Mg(\varepsilon\text{-ATP})^{2\ominus}$ and $Mg(ATP)^{2\ominus}$ are between those for their nucleotides and nucleosides (Table 1). However, the values of the twofold negatively charged $Mg(\varepsilon\text{-ATP})^{2\ominus}$ ($K = 7.6$ M^{-1}) and $Mg(ATP)^{2\ominus}$ ($K = 4.0$ M^{-1}) complexes considerably exceed those of the also twofold negative $\varepsilon\text{-AMP}^{2\ominus}$ ($K = 2.5$ M^{-1}) and $AMP^{2\ominus}$ ($K = 2.1$ M^{-1}) species. Hence, it is evident that $Mg^{2\oplus}$ affects the stability of the NTP-stacks beyond a pure charge neutralization, and this is then an indication for an intermolecular bridging of the stacks by $Mg^{2\oplus}$. As the values of δ_∞[34,38] give no indication for a selective $Mg^{2\oplus}$/base interaction in the stacks, this suggests a bridging by $Mg^{2\oplus}$ via the phosphate chains – an assumption that appears reasonable from the viewpoint of coordination chemistry.

At first sight the difference in self-association between $\varepsilon\text{-ATP}^{4\ominus}$ and $ATP^{4\ominus}$ in the presence of $Mg^{2\oplus}$ may appear as marginal; but this conclusion is not really accurate, especially not for experiments dealing with kinetics, as here a species occurring only in a relatively low concentration may be the one responsible for the reactivity of a system. Thus, if one requests that 99% of $Mg(NTP)^{2\ominus}$ are present in the monomeric form one may work with a 1 mM solution of $Mg^{2\oplus}$/ATP, but only with a 0.6 mM solution of $Mg^{2\oplus}/\varepsilon$-ATP. To mediate further a realistic feeling of how the proportions of the various oligomers vary with changing concentration, the calculations summarized in Fig. 3 were carried out[34,38]: it is evident that the percentage of $Mg(NTP)^{2\ominus}$ present in the monomeric form decreases rapidly with the increasing total concentration of $Mg(\varepsilon\text{-ATP})^{2\ominus}$ or $Mg(ATP)^{2\ominus}$. However, it is also evident that the situation in the $Mg(NTP)^{2\ominus}$ systems differs considerably: e.g., in $5 \cdot 10^{-2}$ M solutions of $Mg(ATP)^{2\ominus}$ about 73% are present as the monomer, 21% as the dimer, 5% as the trimer, and 1% as the tetramer; the corresponding numbers for $Mg(\varepsilon\text{-ATP})^{2\ominus}$ are 60%, 27%, 9%, and 3% plus an additional 1% in form of higher oligomers.

To conclude: it appears that $Mg(\varepsilon\text{-ATP})^{2\ominus}$ may be employed as a probe for $Mg(ATP)^{2\ominus}$, provided the situation as described in the preceding paragraph is kept in mind. It seems that the mode of coordination in $Mg(ATP)^{2\ominus}$ and $Mg(\varepsilon\text{-ATP})^{2\ominus}$ is about the same (see also Sections 6.3 and 6.4) and that the differences are mainly a matter of concentration of the occurring stacks. The somewhat larger self-stacking tendency of $Mg(\varepsilon\text{-ATP})^{2\ominus}$, compared with that of $Mg(ATP)^{2\ominus}$, is probably due to the steric restrictions imposed by the $Mg^{2\oplus}$-phosphate bridges; the larger three-ring ε-adenine residue is probably more adaptable to the resulting restricted orientation of the base moieties than the smaller two-ring adenine unit.

4.4. Evidence for a Base–Phosphate-Bridging via $Zn^{2\oplus}$ in Dimeric Units Occurring in the Self-Stacks of $Zn(\varepsilon\text{-ATP})^{2\ominus}$ and $Zn(ATP)^{2\ominus}$

By ^{1}H-NMR shift measurements the self-association of $Zn(ATP)^{2\ominus}$ and $Zn(\varepsilon\text{-ATP})^{2\ominus}$ is easily demonstrated, but the calculations show[34,38] that the simple isodesmic model for an indefinite non-cooperative self-association (Equations (1) and (2)) is inadequate to explain the experimental data: the data-fit for the shifts of H-8 (and H-11) is poor, and the calculated association constants according to Equation (2) differ for the individual protons. Hence, the values given in Table 1 for the $Zn(NTP)^{2\ominus}$ systems are for principal reasons only approximately quantifying the extent of stacking; the second value given for $Zn(\varepsilon\text{-ATP})^{2\ominus}$ in Table 1 characterizes the situation probably somewhat better[38] as in the calculation of the weighted mean the results for the protons neighboring N-6 and N-7 (i.e., the metal ion binding sites; see below) were not considered.

Taking into account the well-known affinity of $Zn^{2\oplus}$ towards nitrogen donor sites, and the fact that the fit with the simple model was especially poor for the shifts of H-8 (and H-11), i.e. the proton(s) which neighbor(s) N-7 (and N-6) (see Fig. 1), the following model was developed[34,35,38]. It is assumed that an *inter*molecular bridge by a metal ion coordinated to the phosphate residue of one nucleotide and to the base moiety of the neighbor favors especially the formation of dimers:

$$2\,M(NTP)^{2\ominus} \rightleftharpoons M_2(NTP)_2^{4\ominus} \qquad (3)$$

$$K^*_{Di} = [M_2(NTP)_2^{4\ominus}]/[M(NTP)^{2\ominus}]^2 \qquad (4)$$

The *inter*molecularly bridged dimers of equilibrium (3) may stack with each other, as well as with monomeric $M(NTP)^{2\ominus}$; the association constants for such non-bridged stacks are again expected to be equal. This is expressed in a general form by equilibrium (5) and Equation (6):

$$M_2(NTP)_2^{4\ominus} + M_n(NTP)_n^{2n\ominus} \rightleftharpoons M_{2+n}(NTP)_{2+n}^{(4+2n)\ominus} \qquad (5)$$

$$K_{St} = \frac{[M_{2+n}(NTP)_{2+n}^{(4+2n)\ominus}]}{[M_2(NTP)_2^{4\ominus}]\,[M_n(NTP)_n^{2n\ominus}]} \qquad (6)$$

Due to the increased number of variable parameters (K^*_{Di}, K_{St}, δ_0, δ^*_{Di}, and δ_∞) it proved impossible to perform a least-squares fit of the experimental data using *all* parameters. Nevertheless, this model is able to explain at least qualitatively the shift of all protons[34,38]. It should be pointed out that the self-association in the $Zn(ATP)^{2\ominus}$ and $Zn(\varepsilon\text{-ATP})^{2\ominus}$ systems may be more complicated than described by this model through the equilibria (3) and (5), but it cannot be simpler; the experimental data can of course also be explained with more variables, but not with less.

Several least-squares calculations with the experimental data indicated for $Zn(\varepsilon\text{-ATP})^{2\ominus}$ an equilibrium constant, K_{St}, between 5 and 10 M^{-1} and a dimerization constant, K^*_{Di}, within the range 10 to 30 M^{-1}[38]. For $Zn(ATP)^{2\ominus}$ the situation is similar[34]: K_{St} is about 4 to 5 M^{-1} and K^*_{Di} must be within the range 10 to 50 M^{-1}. Again:

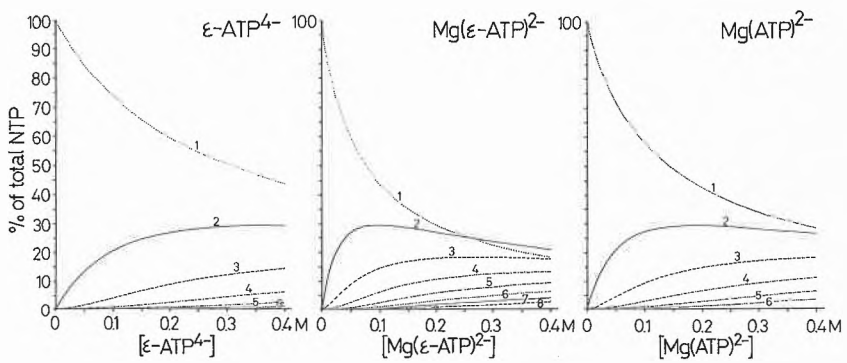


Fig. 3. Variation of the proportions of NTP present in the monomer (1), dimer (2), trimer (3), ..., and octamer (8) in D_2O solutions as a function of the total concentration of $\varepsilon\text{-ATP}^{4\ominus}$ ($K = 1.9$ M^{-1})[38], $Mg(\varepsilon\text{-ATP})^{2\ominus}$ ($K = 7.6$ M^{-1})[38], and $Mg(ATP)^{2\ominus}$ ($K = 4.0$ M^{-1})[34] at 27 °C and $I = 0.1$ to ≈ 2 ($NaNO_3$). The above figure is a combination of figure 2 from ref.[38] and the middle part of figure 7 from ref.[34].

with these constants a fit of the measured chemical shifts is possible[34,38].

The above assumption on the participation of a $Zn^{2\oplus}$/base interaction in the stacking process of $Zn(\varepsilon\text{-ATP})^{2\ominus}$ is confirmed by the chemical shifts. It is well-known that coordination of a diamagnetic metal ion to a binding site deshields neighboring protons and therefore the resonance signals for such protons shift *down*field. Although there is no simple relation for aromatic systems between the distance of the site of metalation and the *size* of the downfield shifts for the observed protons[67] the effect is clear. Hence, if $Zn^{2\oplus}$ coordinates in the dimeric stacks of $Zn(\varepsilon\text{-ATP})^{2\ominus}$ to the N-6/N-7 site forming an *inter*molecular metal ion bridge, this should affect the values of δ_∞. Indeed, the differences $\Delta\delta_\infty = \delta_{\infty/Zn(\varepsilon\text{-ATP})} - \delta_{\infty/Mg(\varepsilon\text{-ATP})}$ are *down*field (by about 0.35 ppm); it should be pointed out, that the values of δ_∞ for $Mg(\varepsilon\text{-ATP})^{2\ominus}$ are close to the δ_∞ values of ε-adenosine (and $\varepsilon\text{-ATP}^{4\ominus}$)[38]. The $Zn^{2\oplus}$/N-7 interaction in the stacks of the $Zn(ATP)^{2\ominus}$ system is correspondingly confirmed by considering the shifts of H-8[34].

There is one further aspect to be mentioned; coordination of a metal ion to the N-6/N-7 site in ε-ATP does not necessarily favor stacking: ε-adenosine self-stacks very well, while the coordination of $Zn^{2\oplus}$ to the $1,N^6$-ethenoadenine moiety strongly suppresses this property (Table 1) due to the repulsion occurring in stacks composed of $Zn(\varepsilon\text{-Ado})^{2\oplus}$ (Section 4.1). Indeed, $Mg(\varepsilon\text{-ATP})^{2\ominus}$ without a $Mg^{2\oplus}$/base interaction self-stacks better than $Zn(\varepsilon\text{-ATP})^{2\ominus}$ (Table 1)[68a]. This *decrease* in the stacking tendency of $Zn(\varepsilon\text{-ATP})^{2\ominus}$ contrasts with the situation for $Mg(ATP)^{2\ominus}$ and $Zn(ATP)^{2\ominus}$; the latter complex self-stacks considerably better than the other two. These observations are clearly the result of the relatively large affinity of the $1,N^6$-ethenoadenine moiety towards $Zn^{2\oplus}$ ($\lg K^{Zn}_{Zn(\varepsilon\text{-Ado})} = 1.5$; Sections 6.1 and 6.2)[36]: this high affinity causes coordination of $Zn^{2\oplus}$ in a $Zn^{2\oplus}/\varepsilon\text{-ATP}^{4\ominus}$ system not only to the phosphate chains but also to most of the base moieties (see also Sections 6.3 and 6.4). For comparison, the adenine moiety is by a factor of about 1/60 less effective and interacts only weakly with $Zn^{2\oplus}$ ($\lg K^{Zn}_{Zn(Ado)} = -0.3$)[34].

From a general point of view it is easy to see that (i) in a dimeric $M_2(NTP)_2^{4\ominus}$ stack bridging of the two nucleotides by one metal ion via phosphate coordination to one nucleotide and base coordination to the other will favor the stack, while (ii) bridging by *both* metal ions will somewhat disfavor it due to charge repulsion favoring thus the formation of monomers with an *intra*molecular bridge (Sections 6.3 and 6.4). Furthermore, if stacking proceeds beyond the dimer stage, in the first case only about each second purine system will carry a positive charge due to the metal ion interaction and this will hardly interfer with the formation of a polymer, while in the second case nearly each purine residue will carry such a positive charge giving rise to repulsion. To conclude, it seems that $Zn(ATP)^{2\ominus}$ is an example for category (i) with $K \approx 10\ M^{-1}$ (Table 1), while $Zn(\varepsilon\text{-ATP})^{2\ominus}$ is one for category (ii) with $K \approx 3.3\ M^{-1}$[38]. It is apparent that these differences should be considered if $Zn(\varepsilon\text{-ATP})^{2\ominus}$ is employed as a probe for $Zn(ATP)^{2\ominus}$, especially if higher concentrations of the complexes ($> 10^{-3}$ M) are used!

4.5. Conclusions Regarding the Self-Association of ε-Adenine Derivatives in the Presence of Metal Ions

Adenosine and ε-adenosine differ somewhat in their self-stacking tendency (Table 1; Section 4.1), but it appears possible to use ε-adenosine as a probe for adenosine, as long as no steric restrictions due to its larger size occur (Fig. 1). However, this «favorable» conclusion is not correct anymore for studies in the presence of metal ions with a pronounced affinity towards nitrogen donor sites, like $Zn^{2\oplus}$; these metal ions coordinate to the 1,10-phenanthroline-like binding site of ε-adenosine (Section 6.2) and reduce thus, due to charge repulsion, the self-stacking tendency considerably (Table 1). In this respect a corresponding concentration of metal ions will have practically no effect on adenosine, because the metal ion affinity of its base residue is much lower (Sections 6.1 and 6.2).

The tendency for self-stacking is relatively similar for the ε- and the corresponding parent nucleotides (Table 1). However, in the presence of metal ions the situation may again change drastically: $Zn^{2\oplus}$ promotes the self-association of $ATP^{4\ominus}$ much more strongly than $Mg^{2\oplus}$, but its promotion on $\varepsilon\text{-ATP}^{4\ominus}$ is even less than that of $Mg^{2\oplus}$ (Table 1). $Mg^{2\oplus}$ affects the stability of the stacks beyond a pure charge neutralization and the additional promotion (most probably) occurs via phosphate-bridging (Section 4.3). Evidently, another metal ion which may act in the same way is $Ca^{2\oplus}$; in general all metal ions which have a small affinity for heteroaromatic nitrogen bases but a pronounced one for negatively charged oxygen groups will act in this way and must therefore be expected to facilitate the self-association of $\varepsilon\text{-ATP}^{4\ominus}$ more than that of $ATP^{4\ominus}$. Metal ions, like $Zn^{2\oplus}$ (or $Cd^{2\oplus}$), which have a significant affinity for heteroaromatic nitrogen bases (Section 6.4) can also promote stacking beyond a simple charge neutralization, but here the effects on self-stacking cannot easily be generalized (see Section 4.4); in this case the additional promotion involves (beside phosphate binding) also an intermolecular coordination to the base moieties, which especially favors dimers (Section 4.4).

A feeling of how the proportions of the various oligomers vary with changing concentration is provided by the results shown in Fig. 3 for the two representative examples, $Mg(\varepsilon\text{-ATP})^{2\ominus}$ and $Mg(ATP)^{2\ominus}$. In addition, one may note that a solution containing 97% of the monomeric form may be as much as 8 mM in $\varepsilon\text{-ATP}^{4\ominus}$ but only 2 mM in $Mg(\varepsilon\text{-ATP})^{2\ominus}$; for $ATP^{4\ominus}$ and $ADP^{3\ominus}$ the corresponding concentrations are 12 mM and 9 mM, respectively[68b].

In conclusion, based on the results summarized in Sections 4.1 to 4.4, it is not to be expected that in any of the metal ion containing $\varepsilon\text{-ATP}^{4\ominus}$ systems self-association would increase beyond that of ε-adenosine, which exists in a 10^{-3} M solution to about 98% in the monomeric form[36]. Hence, it is recommended that any studies aiming for properties of monomeric $M^{2\oplus}/\varepsilon$-ATP (or ATP) systems are carried out in solutions with [ε-ATP] < 1 mM, or better (to be quite on the safe side) with [ε-ATP] < 0.5 mM.

5. Acidity Constants of Adenine and ε-Adenine Derivatives, and Sites of Protonation

The nucleotides presently considered are composed of a base moiety and a phosphate residue which are linked together by a ribose group. This «inserted» sugar linkage leads to a relatively large distance between the base and the phosphate residues and therefore the corresponding acidity constants of the protonated groups are reflected in those of the independent molecules. For example, the acidity constant of protonated adenosine ($pK^H_{H(Ado)} = 3.61$)[69] is similar to the first deprotonation constant of $H_2(AMP)^{\oplus\ominus}$ ($pK_{a/1} = pK^H_{H_2(AMP)} = 3.84$)[69], while the second one for $H(AMP)^{\ominus}$ ($pK_{a/2} = pK^H_{H(AMP)} = 6.22$)[69] is reflected in the deprotonation of H(D-ribose 5′-monophosphate)$^{\ominus}$ ($pK^H_{H(RibP)} = 6.26$)[69]. The equilibria considered here for protonated nucleosides, $H(Ns)^{\oplus}$, and twofold protonated nucleotides[20], $H_2(NP)^{\oplus\ominus}$ or $H_2NP^{2\ominus}$, are given by Equations (7), (9), and (11) and the corresponding acidity constants are defined by Equations (8), (10), and (12):

$$H(Ns)^{\oplus} \rightleftharpoons H^{\oplus} + Ns \qquad (7)$$

$$K^H_{H(Ns)} = [H^{\oplus}]\,[Ns]/[H(Ns)^{\oplus}] \qquad (8)$$

$$H_2(NP)^{\oplus\ominus/2\ominus} \rightleftharpoons H^{\oplus} + H(NP)^{\ominus/3\ominus} \qquad (9)$$

$$K^H_{H_2(NP)} = [H^{\oplus}]\,[H(NP)]/[H_2(NP)] \qquad (10)$$

$$H(NP)^{\ominus/3\ominus} \rightleftharpoons H^{\oplus} + NP^{2\ominus/4\ominus} \qquad (11)$$

$$K^H_{H(NP)} = [H^{\oplus}]\,[NP]/[H(NP)] \qquad (12)$$

One would expect that the protonation/deprotonation reaction alters the chemical shifts for the protons of ε-adenosine. This is indeed the case and ^{1}H-NMR shift experiments yielded for D_2O as solvent $pK^D_{D(\varepsilon\text{-Ado})} = 4.64$ ($I = 0.1$, $NaNO_3$; 27° C)[36]. From this value the corresponding constant for $H(\varepsilon\text{-Ado})^{\oplus}$ in H_2O may be estimated using a published transformation procedure[70]; the result $pK^H_{H(\varepsilon\text{-Ado})} = 4.13$ is in fair agreement with the constant ob-

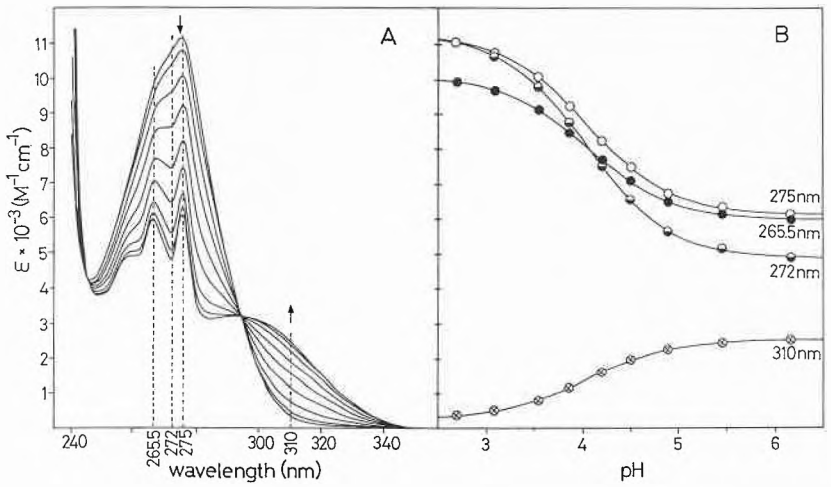


Fig. 4. Dependence of the UV spectrum of ε-adenosine on pH in aqueous solution at I = 0.1 ($NaClO_4$) and 25°C (measured in 1 cm cells with [ε-Ado] = 1.8 × 10^{-4} M): (A) change of the UV spectrum resulting from the increase of pH from 2.70 to 6.16 (via 3.05, 3.54, 3.86, 4.20, 4.50, 4.89, and 5.46), the direction of the spectral change is indicated by arrows; (B) evaluation of the spectra shown in (A) by plotting the extinction coefficient ε ($M^{-1}cm^{-1}$) versus pH. The solid curves represent the computer-calculated best fits of the experimental data with $pK^H_{H(\varepsilon\text{-Ado})} = 4.05$. Reproduced from ref. [36].

tained in aqueous solution from the spectrophotometric titration shown in Fig. 4: $pK^H_{H(\varepsilon\text{-Ado})} = 4.05 \pm 0.01$ ($I = 0.1$, $NaClO_4$; 25°C) [36]. This latter value is identical with the one determined by potentiometric pH titrations; it is listed in Table 2 together with those of other ε-adenine and adenine derivatives. It may be emphasized that all acidity constants given in Table 2 were determined [36,37,39,60,69,71–74] under conditions where self-association is negligible. In addition, the UV absorbance spectrum of ε-ATP also shows a pronounced dependence on pH in the range from 2 to 7, the spectra being very similar to those shown in Fig. 4 for ε-adenosine, confirming thus that the resulting acidity constant, $pK^H_{H2(\varepsilon\text{-ATP})} = 4.41 \pm 0.04$ ($I = 0.1$, $NaClO_4$; 25°C) [39]; is due to the release of the proton from the base moiety.

It is evident that the acidity constants, $pK_a \approx 4$ (Table 2), are connected with the deprotonation of the protonated ε-adenine residue. Hence, the question arises: where is the proton located? Is it at N-6 or at N-7? From the fact that the downfield shift upon protonation of ε-adenosine is largest for H-11 (see $\Delta\delta^*_H$ in Table IV of ref. [36]), one cannot unambiguously conclude that protonation occurs at the neighboring N-6 (see Fig. 1), because in aromatic systems shifts induced by protonation do not necessarily decrease with increasing distance from the protonated site [67a]. However, a detailed study [23] comparing H(ε-adenosine)$^{\oplus}$ with the corresponding compounds methylated at N-6 or N-7 shows that the predominant site of protonation is indeed N-6; a conclusion in agreement with the crystal structure analysis of 10-ethyl-ε-adenosine hydrochloride [19]. Fig. 5 illustrates the deprotonation equilibrium together with the two most likely resonance structures [19] of protonated 1,N^6-ethenoadenosine and its derivatives.

Table 2. Comparison of the negative logarithms of the acidity constants of several protonated 1,N^6-ethenoadenine derivatives with the corresponding values of their parent compounds. All constants were determined by potentiometric pH titrations of aqueous solutions ($I = 0.1$, $NaNO_3$; 25°C)[a].

Constants (Equations (8), (10), (12))	ε-adenine derivatives	adenine derivatives[b]
$pK^H_{H(Ns)}$	4.05 ± 0.01 [36]	3.61 ± 0.03 [69]
$pK^H_{H_2(NMP)}$	4.23 ± 0.02 [37]	3.84 ± 0.02 [69]
$pK^H_{H(NMP)}$	6.23 ± 0.01 [37]	6.22 ± 0.01 [69] (6.14 [71][c])
$pK^H_{H_2(NTP)}$	4.45 ± 0.02 [39]	4.01 ± 0.01 [60]
$pK^H_{H(NTP)}$	6.50 ± 0.01 [39]	6.49 ± 0.01 [60] (6.42 [72][c])

[a] For the sites of protonation see text in Section 5. The range of error given with the constants is *three times* the standard error of the mean value or the sum of the probable systematic errors, whichever is larger.

[b] The values for $H_2(ADP)^{\ominus}$ at $I = 0.1$ (KNO_3) are $pK^H_{H_2(ADP)} = 3.93$ (25°C) [73], 4.05 (15°C) [74] and $pK^H_{H(ADP)} = 6.44$ (25°C) [73], 6.41 (15°C) [74].

[c] Background electrolyte in this case: $NaClO_4$.

To summarize, protonation occurs at pH about 4 at N-6 in the ε-adenine residue of ε-adenosine and its nucleotides (Fig. 5). In adenosine and its nucleotides the site of protonation at the base moiety (see Fig. 1) is N-1 [33,69,75]. Protonation at the phosphate group occurs in nucleoside 5′-monophosphates [33,69,75] at pH about 6.2, and in nucleoside 5′-triphosphates [33,60] at the terminal γ-phosphate group at pH about 6.5.

From the constants listed in Table 2 follows that introduction of the 1,N^6-etheno bridge into adenosine and its nucleotides results in an increased basicity of about 0.4 log unit for the base moieties. The decreasing acidity within the series H(ε-Ado)$^{\oplus}$ > H_2(ε-AMP)$^{\oplus\ominus}$ > H_2(ε-ATP)$^{2\ominus}$ is expected due to the variation of the charge of these species, and paralleled by the corresponding series H(Ado)$^{\oplus}$ > H_2(AMP)$^{\oplus\ominus}$ > H_2(ATP)$^{2\ominus}$. The data given in Table 2 indicate in addition that the basicity of the phosphate groups of the nucleotides is not remarkably affected by the modification of the base moieties. Hence, under this aspect for H(AMP)$^{\ominus}$, H(ADP)$^{2\ominus}$, and H(ATP)$^{3\ominus}$ the corresponding ε-adenine nucleotides are usable probes in the neutral pH region.

6. Comparison of Binary Metal Ion Complexes of 1,N^6-Ethenoadenosine and Its Phosphates with Related Nucleoside and Nucleotide Complexes

6.1. Stability of Metal Ion Complexes of ε-Adenosine, ε-AMP$^{2\ominus}$, ε-ADP$^{3\ominus}$, ε-ATP$^{4\ominus}$, and Some Related Ligands

The stability constants listed in Table 3 [36,37,39,40,76–83] were mainly determined by potentiometric pH titrations and under conditions were the self-association of the nucleosides and their derivatives is negligible. The experimental data [36] of the metal ion systems with ε-adenosine and adenosine can easily be accounted for by using the acidity constants (Equation (8)) of these nucleosides (Ns) and by considering the following equilibrium:

$$M^{2\oplus} + Ns \rightleftharpoons M(Ns)^{2\oplus} \quad (13)$$

$$K^M_{M(Ns)} = [M(Ns)^{2\oplus}]/[M^{2\oplus}]\,[Ns] \quad (14)$$

The experimental data [37,39] of the metal ion/nucleotide (NP) systems request for their explanation, aside from the acidity

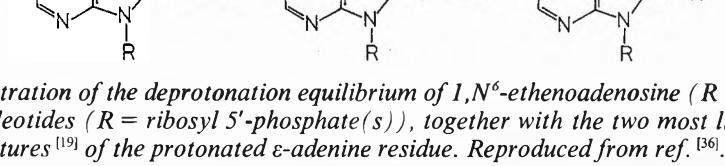


Fig. 5. Illustration of the deprotonation equilibrium of 1,N^6-ethenoadenosine (R = ribosyl) and its nucleotides (R = ribosyl 5′-phosphate(s)), together with the two most likely resonance structures [19] of the protonated ε-adenine residue. Reproduced from ref. [36].

Table 3. Logarithms of the stability constants (Equations (14) and (18)) for several metal ion complexes of ε-adenosine, ε-AMP$^{2\ominus}$, and ε-ATP$^{4\ominus}$. The corresponding data for adenosine, AMP$^{2\ominus}$, and ATP$^{4\ominus}$, as well as for UMP$^{2\ominus}$ and PNTP$^{4\ominus}$ are given for comparison[20]. If nothing else is mentioned the constants were determined by potentiometric pH titrations of aqueous solutions with $I = 0.1$ ($NaNO_3$) and 25°C[a].

$M^{2\oplus}$	$\lg K^{M}_{M(Ns)}$		$\lg K^{M}_{M(NMP)}$			$\lg K^{M}_{M(NTP)}$		
	ε-Ado[36]	Ado[76][c]	ε-AMP$^{2\ominus}$[37]	AMP$^{2\ominus}$[g]	UMP$^{2\ominus}$[37]	ε-ATP$^{4\ominus}$[39]	ATP$^{4\ominus}$[i]	PNTP$^{4\ominus}$[j]
$Ca^{2\oplus}$		≤ −0.8		1.39		≈ 3.9[h]	3.88	3.94[k]
$Mg^{2\oplus}$	≤ 0.3	≤ −0.8	1.61 ± 0.02	1.63	1.5	4.24 ± 0.03	4.24	4.27[k]
$Mn^{2\oplus}$	0.72 ± 0.15	−0.82	2.59 ± 0.04	2.14	2.01	5.10 ± 0.15	4.81	4.63
$Co^{2\oplus}$	≈ 2.2[b]	−0.30	≈ 3.5[f]	2.19		≈ 5.1[h]	4.86	4.53
$Ni^{2\oplus}$	≈ 2.2[b]	−0.17, 0.3[d]	≈ 4[f]	2.62		≈ 5.8[h]	4.85	4.29
$Cu^{2\oplus}$	2.81 ± 0.09	0.84, 0.70[80]	5.87 ± 0.02	3.04	2.80	9 ± 1[40]	6.32[60]	5.81[k]
$Zn^{2\oplus}$	1.51 ± 0.03	−0.28, −0.3[e]	3.18 ± 0.04	2.23	2.03	5.44 ± 0.13	5.16	4.77
$Cd^{2\oplus}$	≈ 1.8	−0.11[e]		2.68[81]			5.31[82]	4.99[l]

[a] The range of error given for the stability constants of the complexes of the ε-adenine derivatives is *three times* the standard error of the mean value or the sum of the probable systematic errors, whichever is larger.
[b] These values should be viewed with care; they were determined[24] in the presence of buffers by fluorescence quenching at circa 22°C. Especially the value given for Co(ε-Ado)$^{2\oplus}$ is probably too large; an estimate based on the data listed in table V of ref.[36] gives $\lg K^{Co}_{Co(\varepsilon\text{-Ado})} \simeq 1.7$.
[c] Most values are from ref.[77]; they were determined by UV spectrophotometry at natural ionic strength and 20°C.
[d] From ref.[78,79]; by spectrophotometry.
[e] From ref.[34]; by ^{1}H-NMR shift measurements in D_2O at $I = 0.1$–5 ($NaNO_3$) and 27°C.
[f] Estimates[37] based on values determined[22,24] in the presence of buffers by fluorescence quenching at about 22°C[24] and 25°C[22]. The reliability of these values is discussed in ref.[37].
[g] $I = 0.1$, $NaClO_4$; 25°C[71].
[h] Estimates based on values determined[22,24] in the presence of buffers by fluorescence quenching at about 22°C[24] and 25°C[22]. Most of the constants given in ref.[22,24] are not very reliable; the reasons are indicated in ref.[39] (and ref.[37]).
[i] $I = 0.1$, $NaClO_4$; 25°C[72]. For $Mn^{2\oplus}$ and $Zn^{2\oplus}$ the average of the values given in ref.[72] is listed above.
[j] $I = 0.1$, $NaClO_4$; 25°C. Values[39] for complexes of pyrimidine-nucleoside 5′-triphosphates (PNTP$^{4\ominus}$) (Fig. 7); these are the averages[39] of the constants given in ref.[72].
[k] Values for UTP$^{4\ominus}$ complexes; those for $Ca^{2\oplus}$ and $Mg^{2\oplus}$ are from ref.[83], the constant for Cu(UTP)$^{2\ominus}$ is from ref.[60].
[l] Value for the Cd(CTP)$^{2\ominus}$ complex from ref.[82].

constants of the ligands (Equations (10) and (12)), the following equilibria (charges are omitted for clarity):

$$M + H(NP) \rightleftharpoons M(H \cdot NP) \quad (15)$$

$$K^{M}_{M(H \cdot NP)} = [M(H \cdot NP)]/[M]\,[H(NP)] \quad (16)$$

$$M + NP \rightleftharpoons M(NP) \quad (17)$$

$$K^{M}_{M(NP)} = [M(NP)]/[M]\,[NP] \quad (18)$$

In case of the $Cu^{2\oplus}$/ε-ATP system the situation is more complicated and the dimerization equilibrium (19) has to be taken into account in addition[40]:

$$Cu(H \cdot \varepsilon\text{-ATP})^{\ominus} + Cu(\varepsilon\text{-ATP})^{2\ominus} \rightleftharpoons Cu_2H(\varepsilon\text{-ATP})_2^{3\ominus} \quad (19)$$

Of course, the complex M(H·NP) may release a proton according to equilibrium (20),

$$M(H \cdot NP) \rightleftharpoons M(NP) + H \quad (20)$$

and the corresponding acidity constant,

$$K^{H}_{M(H \cdot NP)} = [M(NP)]\,[H]/[M(H \cdot NP)] \quad (21)$$

can be calculated with Equation (22):

$$pK^{H}_{M(H \cdot NP)} = pK^{H}_{H(NP)} + \lg K^{M}_{M(H \cdot NP)} - \lg K^{M}_{M(NP)} \quad (22)$$

The complexes M(H·AMP)$^{\oplus}$ are usually not observed[73,74], though evidence for their existence has been reported[78,84]; in other words, their stability is too low to allow formation of significant amounts. The Mg(H·ε-AMP)$^{\oplus}$ complex could also not be discovered[37], but the M(H·ε-AMP)$^{\oplus}$ complexes with $Mn^{2\oplus}$, $Cu^{2\oplus}$, and $Zn^{2\oplus}$ are known, and the values for their acidity constants, $pK^{H}_{M(H \cdot \varepsilon\text{-AMP})}$, are between 4.0 and 5.1[37]. Similarly, M(H·ε-ATP)$^{\ominus}$ complexes exist; their acidity constants, $pK^{H}_{M(H \cdot \varepsilon\text{-ATP})}$, are between 4.3 and 4.8[39,40]. Furthermore, the corresponding constants for M(H·ADP) and M(H·ATP)$^{\ominus}$ are between 3.2–5.5[85] and 3.7–5.1[86], respectively. Hence, none of these protonated M(H·NP) complexes is extending its existence significantly into the neutral pH range (see also Fig. 6), despite the fact that their concentration in the lower pH range may be considerable. As the focus in this essay is on those complexes which dominate under physiological conditions these protonated species are not considered further and their stability constants[37,39,40,73,83,87] are not listed in Table 3. On the other hand, the existence of these protonated species cannot simply be ignored[83]; in kinetic experiments and enzymatic tests such a complex occurring in only low concentration may well be the reactive one: then ref.[37] and[39] should directly be consulted regarding M(H·ε-AMP)$^{\oplus}$ and M(H·ε-ATP)$^{\ominus}$, respectively*).

The stability constants given in Table 3 for the metal ion complexes of ε-AMP$^{2\ominus}$ and ε-ATP$^{4\ominus}$ are quite reliable, especially for the biological important metal ions $Mg^{2\oplus}$, $Mn^{2\oplus}$, and $Zn^{2\oplus}$. The partly rather large error limits are mainly due to the scarcity of these ligands, which allowed only a limited number of experiments[36–40].

At present unfortunately no stability constants of M(ε-ADP)$^{\ominus}$ complexes are available, which were determined by potentiometric pH titrations. The available constants were obtained by fluorescence quenching in the presence of buffers (which may also form metal ion complexes[89]) and without the explicit consideration of equilibrium (15)[22,24]; hence, in view of the experience made with the complexes of ε-AMP[37] and ε-ATP[39] and the corresponding results from this method[22,24], the data for the M(ε-ADP)$^{\ominus}$ complexes must also be viewed with scepticism. However, to provide those researchers who are in need for stability constants of M(ε-ADP)$^{\ominus}$ complexes with some information, the constants assembled in Table 4 have been estimated, based on (i) my experience, (ii) the results of Table 3, and (ii) the data given in references[22,24].

6.2. Structural Considerations on the Complexes of ε-Adenosine and Adenosine

It may be emphasized that the stability constants determined by potentiometric pH titration and given in Table 3 for the complexes of ε-adenosine with $Mn^{2\oplus}$, $Cu^{2\oplus}$, and $Zn^{2\oplus}$, have also been confirmed by independent methods[36]. In this connection ^{1}H-NMR shift experiments had been carried out with the $Zn^{2\oplus}$/ε-adenosine system[36], and these results provide also a first

*) Very recently a further attempt has been made[83] to provide a comprehensive set of stability constants obtained under the same conditions and with clearly defined error limits for the complexes formed between $Mg^{2\oplus}$, $Ca^{2\oplus}$, $Mn^{2\oplus}$, $Co^{2\oplus}$, $Ni^{2\oplus}$, $Cu^{2\oplus}$, $Zn^{2\oplus}$ or $Cd^{2\oplus}$ and ATP or the PNTPs (i.e., CTP, UTP, and TTP)[20]. In this study[83] also a detailed analysis of the isomeric equilibria occurring with M(H·NTP)$^{\ominus}$ complexes is provided; furthermore, estimates for the formation degrees of the isomers carrying the proton at the nucleic base residue or at the γ-phosphate group are given by taking into account also macrochelate formation where appropriate. A short review on the «isomeric equilibria in complexes of ATP with divalent metal ions» and on related problems will soon also be available[88].

Table 4. Estimates for the logarithms of the stability constants (Equation (18)) for several metal ion complexes of ε-ADP$^{3\ominus}$ [a]. The corresponding data for complexes of ADP$^{3\ominus}$ and pyrimidine-nucleoside 5'-diphosphates (PNDP$^{3\ominus}$) are given for comparison. The constants refer to aqueous solutions and conditions close to $I = 0.1$ ($Na^{\oplus}$, $K^{\oplus}/NO_3^{\ominus}$) and 25°C.

$M^{2\oplus}$	$\lg K^{M}_{M(\varepsilon\text{-ADP})}$ [a]				$\lg K^{M}_{M(ADP)}$ [e]	$\lg K^{M}_{M(PNDP)}$ [f]
	from ref. [22] [b]	corrected values [c]	from ref. [24]	best estimate		
$Ca^{2\oplus}$	2.81	2.87		2.9	2.86	2.9
$Mg^{2\oplus}$	3.11	3.18		3.2	3.19	3.2
$Mn^{2\oplus}$	4.57	4.63	2.87 [d]	≈ 4.5	4.16	3.8
$Co^{2\oplus}$	4.04	4.10	4.46	≈ 4.5	4.20	3.8
$Ni^{2\oplus}$			4.73	≈ 5	4.3	3.6
$Cu^{2\oplus}$	5.51	5.57		≈ 8	5.90	4.7
$Zn^{2\oplus}$	3.72	3.78		≈ 5	4.28	3.8

[a] See text in Section 6.1. Estimations for the acidity constants (based on Table 2) for $H_2(\varepsilon\text{-ADP})^{\ominus}$ are $pK^{H}_{H2(\varepsilon\text{-ADP})} = 4.4$ and $pK^{H}_{H(\varepsilon\text{-ADP})} = 6.4$. For the calculation of species-distribution curves (like those of Fig. 6) it will be necessary to estimate also the stability constants for $M(H\cdot\varepsilon\text{-ADP})^{\ominus}$; this may be done by using the known values of $M(H\cdot\varepsilon\text{-AMP})^{\oplus}$ [37] and $M(H\cdot\varepsilon\text{-ATP})^{\ominus}$ [39] and by taking also into account the acidity constants $pK^{H}_{M(H\cdot NP)}$ (Equations (21) and (22)) [37, 39].

[b] These are apparent stability constants valid at pH 7.2.

[c] Calculated from the apparent constants [b] by adding $\lg (1 + [H^{\oplus}]/K^{H}_{H(\varepsilon\text{-ADP})})$; see ref. [90].

[d] Maybe there is a printing error in table II of ref. [24] and this value should actually be read as 4.87.

[e] These constants are from ref. [73], except those of $Mg(ADP)^{\ominus}$ (3.17 [73], 3.21 [74]) and $Ni(ADP)^{\ominus}$ (4.50 [73], 4.18 [74]) which are averages. For the acidity constants it holds $pK^{H}_{H2(ADP)} = 4.0$ and $pK^{H}_{H(ADP)} = 6.4$; see footnote [b] of Table 2.

[f] These values are based on constants published for $M(CDP)^{\ominus}$ and $M(H\cdot P_2O_7)^{\ominus}$ complexes; the above estimates are taken from table VI of ref. [35]. The estimate for the acidity constant of $H(PNDP)^{2\ominus}$ is $pK^{H}_{H(PNDP)} = 6.4$.

indication on the structure of the M(ε-adenosine)$^{2\oplus}$ complexes, despite the uncertainty for aromatic systems [67], about the distance of the site of metalation and the size of the downfield shifts: the downfield shifts, $\Delta\delta^{*}_{Zn}$, observed for H-8 and H-11 point to a complexation of $Zn^{2\oplus}$ to N-6 *and* N-7 of ε-adenosine (see Fig. 1).

Indeed, *chelate* formation with ε-adenosine is confirmed by a comparison of the stability constants of the M(ε-adenosine)$^{2\oplus}$ complexes with those of the corresponding M(adenosine)$^{2\oplus}$ complexes, in which the metal ion is coordinated only in a *unidentate* way to N-1 *or* N-7 [75, 76]. The results listed in Table 3 show that the ε-adenosine complexes are more stable by about 2 orders of magnitude. The fact that ε-adenosine is about 3 times more basic than adenosine (see Section 5; Table 2) can definitely not account for the high stability of the M(ε-adenosine)$^{2\oplus}$ complexes. Therefore it is concluded that ε-adenosine acts as a bidentate ligand, the donor atoms being N-6 and N-7.

This conclusion is confirmed by a comparison of the stability constants of M(ε-adenosine)$^{2\oplus}$ with those for the complexes of the unidentate pyridine (see [36]). Pyridine is about 15 times more basic than ε-adenosine, but the pyridine complexes are by a factor of about ⅓ *less* stable than the corresponding M(ε-adenosine)$^{2\oplus}$ complexes. Hence, again a «1,10-phenanthroline-like» structure is born out for the M(ε-adenosine)$^{2\oplus}$ complexes.

The fact that the M(ε-adenosine)$^{2\oplus}$ complexes (Table 3) are not as stable as the corresponding $M^{2\oplus}$ complexes with 1,10-phenanthroline (Phen), e.g., $\lg K^{Mg}_{Mg(Phen)} = 1.45$ and $\lg K^{Zn}_{Zn(Phen)} = 6.55$ [91, 92], is most probably mainly due to the larger distance between N-6 and N-7 in ε-adenosine compared with N-1 and N-10 in 1,10-phenanthroline. This larger distance is already evident from the angles N-6, C-6, C-5 and C-6, C-5, N-7 in ε-adenosine (see Fig. 1), which are 135.6° and 131.6° [19], while the corresponding angles in phenanthroline are close to 120°, i.e. between 116.5° and 119.7° [93]. Indeed, an estimate of the distance gives for N-6 ... N-7 of ε-adenosine 3.3 Å and for N-1 ... N-10 of phenanthroline 2.7 Å. Hence, the coordinated metal ion is probably somewhat «shuttling» between N-6 and N-7 in ε-adenosine.

6.3. Metal Ion/Base Interactions in M(NP) Complexes of Adenine- and 1,N^6-Ethenoadenine-Nucleotides

From the results discussed in the preceding section it is evident that the metal ion-affinity of the ε-adenine moiety is quite pronounced. Indeed, by spectrophotometric measurements a base–metal ion interaction can also be confirmed for the monomeric $Zn(\varepsilon\text{-ATP})^{2\ominus}$ and $Cu(\varepsilon\text{-ATP})^{2\ominus}$ complexes [39, 40]. Moreover, a comparison of the stability constants determined by potentiometric pH titrations for the M(NP) [20] complexes shows for the divalent transition metal ions and $Zn^{2\oplus}$ unequivocally that complex stability increases within the series of ligands: $UMP^{2\ominus} < AMP^{2\ominus} < \varepsilon\text{-AMP}^{2\ominus}$ (Table 3); pyrimidine-nucleoside 5'-diphosphates ($PNDP^{3\ominus}$) < $ADP^{3\ominus}$ < $\varepsilon\text{-ADP}^{3\ominus}$ (Table 4); pyrimidine-nucleoside 5'-triphosphates ($PNTP^{4\ominus}$) < $ATP^{4\ominus}$ < $\varepsilon\text{-ATP}^{4\ominus}$ (Table 3).

It is interesting to note that these differences in complex stability, despite their significance, are hardly reflected at first sight if the formation degree of the complexes in dependence on pH is viewed: as an illustration for this, the four systems of Fig. 6 have been collected [41, 94, 95]. This result is understandable, as the overall stability of these complexes is mainly governed by the number of phosphate groups present in the nucleotide. However, the *structural* differences between complexes containing the same number of phosphate groups can be very pronounced: in the complexes with $Zn^{2\oplus}$ and the related divalent transition metal ions the neutral pyrimidine moieties do not participate in complex formation of the pyrimidine-nucleotides [34, 35, 60, 79] (Fig. 7; in the N-3 ionized ligands this is different [34, 95a]); in other words, the stability of these nucleotide complexes is solely determined by the affinity of the phosphate residues. Consequently it has recently been concluded [37, 39] that the only source of the increased stability of the 1,N^6-etheno-adenine-nucleotides can be the ε-adenine moiety with the N-6/N-7 binding site. The

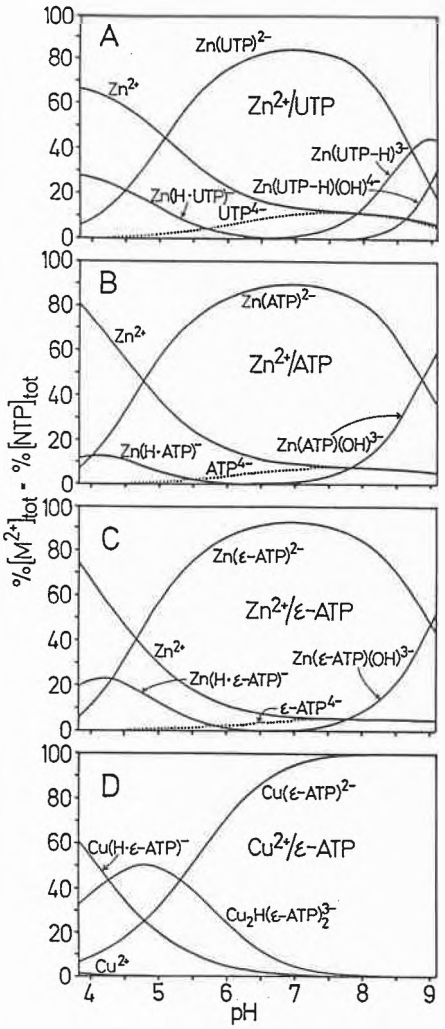
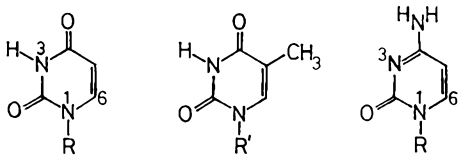


Fig. 6. Comparison of the effect of pH on the concentration of the species present in an aqueous solution of (A) $Zn^{2\oplus}$/UTP [95b], (B) $Zn^{2\oplus}$/ATP [82], (C) $Zn^{2\oplus}$/ε-ATP [39, 41], or (D) $Cu^{2\oplus}$/ε-ATP [40, 41]: [ε-ATP$^{4\ominus}$] < 1%; $pK^{H}_{Cu(\varepsilon\text{-ATP})(H_2O)} > 8.5$ [41]. The results are given as the percentage of the total $M^{2\oplus}$ present (= total NTP); they were computed with the potentiometrically determined constants [39-41, 82, 95] for concentrations of 10^{-3} M for each reactant at $I = 0.1$ and 25°C.



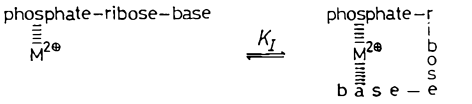
R = –ribosyl 5'–(mono–/di–/or tri–)phosphate

R' = –2'–deoxyribosyl 5'–(mono–/di–/or tri–)phosphate

UMP^{2-}	TMP^{2-}	CMP^{2-}
UDP^{3-}	TDP^{3-}	CDP^{3-}
UTP^{4-}	TTP^{4-}	CTP^{4-}

Fig. 7. Structural formulae of the pyrimidine-nucleotides considered in this account [20].

participation of this site in complex formation does lead to *intra*molecular macrochelates; such macrochelates involving the phosphate groups and N-7 of the adenine moiety are already well-known [33–35,60,77,79,82] for adenine-nucleotide complexes with the mentioned metal ions:



(23)

The next point to be emphasized is the equality of the $Mg^{2\oplus}$ or $Ca^{2\oplus}$ complexes within a nucleotide-series: $UMP^{2\ominus} \approx AMP^{2\ominus} \approx \varepsilon\text{-}AMP^{2\ominus} < PNDP^{3\ominus} \approx ADP^{3\ominus} \approx \varepsilon\text{-}ADP^{3\ominus} < PNTP^{4\ominus} \approx ATP^{4\ominus} \approx \varepsilon\text{-}ATP^{4\ominus}$ (Tables 3 and 4). This means, the stability of these alkaline earth ion complexes is dependent on the number of phosphate groups present in the nucleotides but hardly on the kind of base moiety; hence, equilibrium (23) is on its left side for these complexes. This conclusion is confirmed by ^{1}H-NMR shift experiments with the $Mg^{2\oplus}$ complexes of $ADP^{3\ominus}$ [35], $ATP^{4\ominus}$ [34], and $\varepsilon\text{-}ATP$ [39]: there is no indication of any direct metal ion/base interaction [96]. In contrast to this, ^{1}H-NMR experiments show clear downfield shifts of H-8 for the $Zn^{2\oplus}$ and $Cd^{2\oplus}$ complexes of $ADP^{3\ominus}$ [35] and $ATP^{4\ominus}$ [34,82] confirming thus the macrochelate-formation via N-7 and phosphate coordination.

Similarly, such an 1,N^6-ethenoadenine interaction in the $Zn(\varepsilon\text{-}ATP)^{2\ominus}$ complex should also be reflected in the ^{1}H-NMR shift positions. This is indeed the case [39]: the experiments carried out to study the self-association (see Section 4.4) allow extrapolation of the shifts of the protons neighboring the potential N-6/N-7 binding site, i.e. of H-8 and H-11 (see Fig. 1), to infinite dilution (δ_0). In this way the shift positions for these protons in the monomeric $Zn(\varepsilon\text{-}ATP)^{2\ominus}$ complex are obtained, and these are clearly downfield compared with the corresponding shifts for $\varepsilon\text{-}ATP^{4\ominus}$ and $Mg(\varepsilon\text{-}ATP)^{2\ominus}$ (the latter being similar). Furthermore, by comparing the extent of the downfield shifts, $\Delta\delta_0$, for H-8 and H-11 of $Zn(\varepsilon\text{-}ATP)^{2\ominus}$ with the corresponding shift values obtained for complete complexation of $Zn^{2\oplus}$ at the N-6/N-7 site of ε-adenosine, the percentage of the macrochelated isomer in equilibrium (23) can be estimated [39]: the resulting 85% are in excellent accord with the 76% calculated from the stability data (see below). It should be emphasized that the crucial result at this point is not so much the estimate of the percentage, but the clear proof through the measured downfield shifts that the macrochelated form of $Zn(\varepsilon\text{-}ATP)^{2\ominus}$ does indeed exist.

6.4. Comparison of the Extent of Macrochelate Formation in Monomeric M(NP) Complexes of Adenine and ε-Adenine-Nucleotides

It is evident that a general estimation of the position of equilibrium (23) for M(NP) complexes from stability data is desirable. To be able to quantify the extent of the metal ion/base interaction, the «open» isomer is designated as $M(NP)_{op}$ and the «closed» species as $M(NP)_{cl}$. This allows now the definition of the intramolecular and therefore dimensionless equilibrium constant K_I by Equation (24) and its calculation via Equation (25) [34,37,39,79,97]:

$$K_I = [M(NP)_{cl}]/[M(NP)_{op}] \quad (24)$$

$$K_I = (K^M_{M(NP)}/K^M_{M(NP)_{op}}) - 1 \quad (25)$$

$K^M_{M(NP)}$ is the experimentally accessible (overall) stability constant (Tables 3 and 4), while $K^M_{M(NP)_{op}}$ applies to the «open» isomer $M(NP)_{op}$. This latter constant is not directly accessible by experimental determinations, but in the present cases it is well represented by the stability constants of the pyrimidine-nucleotide complexes [34,35,37,39] because these exist only in the «open» form (see Section 6.3). Hence the crucial difference

$$\lg \Delta = \lg K^M_{M(NP)} - \lg K^M_{M(NP)_{op}} \quad (26)$$

may be calculated, and thus K_I and the percentage of the «closed» isomer $M(NP)_{cl}$ can also be obtained [98]. On this basis, the results assembled in Table 5 were calculated by using essentially the stability constants listed in Tables 3 and 4.

The results of Table 5 confirm the conclusions of the preceding Section 6.3: there is no (or only a very weak) metal ion/base interaction in any of the Mg(NP) or Ca(NP) complexes [96], but this interaction is quite significant in the M(NP) complexes containing $Zn^{2\oplus}$ (or $Cd^{2\oplus}$) or one of the

Table 5. Estimates for the extent of intramolecular macrochelate formation in aqueous solution for the metal ion complexes of adenosine- and ε-adenosine-phosphates as represented by the percentage of the «closed» isomer $M(NP)_{cl}$ (equilibrium (23) and Equation (24)) (I = 0.1; 25°C) [a]

$M^{2\oplus}$	% $[M(NP)_{cl}]$ for the ε-Ado-phosphates			% $[M(NP)_{cl}]$ for the Ado-phosphates		
	ε-$AMP^{2\ominus}$ [b]	ε-$ADP^{3\ominus}$ [c]	ε-$ATP^{4\ominus}$ [d]	$AMP^{2\ominus}$ [b]	$ADP^{3\ominus}$ [e]	$ATP^{4\ominus}$ [f]
$Ca^{2\oplus}$	0	0	0	0	0	0
$Mg^{2\oplus}$	0	0	0	0	0	0 [g]
$Mn^{2\oplus}$	69	≈ 80 (60)	62, 32	28	55	38, 17 ± 10 [83]
$Co^{2\oplus}$	≈ 97	≈ 80 (60)	≈ 70, ≈ 55	34	60	57, 38 ± 9 [83]
$Ni^{2\oplus}$	≈ 99	≈ 96 (92)	≈ 97, ≈ 95	79, 68 [81]	80	74, 64
$Cu^{2\oplus}$	99.9	≈ 99.9 (99.9)	> 99.5 [d]	44, 41 [81]	94	76, 68 ± 4 [60]
$Zn^{2\oplus}$	92	≈ 94 (87)	76, 62	38	67	62, 28 ± 7 [83]
$Cd^{2\oplus}$						52 [82]

[a] The values given for the $Ca^{2\oplus}$ and $Mg^{2\oplus}$ complexes are based on Tables 3 and 4; those of the other metal ions are essentially also based on these data, but in footnotes [b–f] the direct sources are given.

[b] From ref. [37]. The 0% given for Ca(ε-AMP) and Ca(AMP) is an assumption based on the other results.

[c] Calculated with Equation (25) and the estimated constants listed in Table 4. To provide a feeling for the influence of errors the values of lg Δ (Equation (26)) were artificially reduced by 0.3 log units; this results in the percentages given in parenthesis.

[d] From ref. [39]. The second values given for $Mn^{2\oplus}$, $Co^{2\oplus}$, $Ni^{2\oplus}$, and $Zn^{2\oplus}$ were calculated using as basis the recently [83] determined stability constants for $M(PNTP)^{2\ominus}$ complexes (for $Cu^{2\oplus}$ again the above value is obtained). If a comparison with $M(ATP)^{2\ominus}$ is made, these newly calculated values *must* be compared with those percentages for $M(ATP)^{2\ominus}_{cl}$ which are from ref. [83]. It is evident that all conclusions given in the text are unaffected, but these additional values indicate the difficulties in establishing exact values for the concentrations of the isomers (see also ref. [83]), especially if the difference lg Δ in Equation (26) is becoming small.

[e] From ref. [35].

[f] From ref. [34], except where another source is given. The second value provided for $Ni(ATP)^{2\ominus}_{cl}$ is from footnote «f» in table VII of ref. [34].

[g] There might be traces of an outer-sphere macrochelate present in which a water molecule is between N-7 and the metal ion (see also footnote 95 in ref. [34]); this problem has very recently been further investigated (see ref. [83,88,96]).

divalent transition elements and an adenine- or ε-adenine-nucleotide, i.e., the macrochelated species of equilibrium (23) is for these systems very important. For a given nucleotide, the percentage of $M(NP)_{cl}$ usually reflects quite well the general affinity of these metal ions for nitrogen binding sites; the common order is $Ca^{2\oplus} \approx Mg^{2\oplus} < Mn^{2\oplus} < Co^{2\oplus} < Ni^{2\oplus} < Cu^{2\oplus} > Zn^{2\oplus}$.

Most important, in agreement with the larger metal ion affinity of ε-adenosine, compared with adenosine (Section 6.2), the extent of macrochelate formation is always significantly larger in the ε-adenosine phosphate complexes, compared with the corresponding parent adenosine phosphate complexes. For the latter the extent of macrochelate formation apparently also depends for all studied metal ions on the number of phosphate groups[37], i.e., % $M(AMP)_{cl}$ < % $M(ADP)^{\ominus}_{cl}$ > % $M(ATP)^{2\ominus}_{cl}$ (Table 5). For ε-adenine nucleotides (= ε-adenosine phosphates) no final conclusion can in this respect yet be drawn (Table 4), but the situation may well be similar.

6.5. Conclusions Regarding Monomeric ε-Adenosine Phosphate Complexes as Probes for Adenosine Phosphate Complexes

Solutions with concentrations below 1mM of metal ions and ε-adenosine phosphates will self-associate only to a negligible amount (Section 4.5). However, in such solutions the formation degree of complexes, especially with ε-$ADP^{3\ominus}$ (or $ADP^{3\ominus}$) and ε-$ATP^{4\ominus}$ (or $ATP^{4\ominus}$), will still be high (cf. Fig. 6). Therefore, the question is justified: to what extent may, e.g., M(ε-$ATP)^{2\ominus}$ be employed as a probe for $M(ATP)^{2\ominus}$? From the available information it appears that the $Mg^{2\oplus}$ and $Ca^{2\oplus}$ complexes of ε-adenine nucleotides may be used as probes. For the complexes of $Zn^{2\oplus}$ and the divalent transition elements considered here, great care is advised in using them as probes due to the delicacy of the intramolecular equilibrium (23): there are structural differences between the macrochelated isomers (Section 6.2), in addition to the much higher formation degree of these isomers with the ε-adenine nucleotides (Table 5). It is to be expected that in an enzymic system, e.g. in the presence of $Mn^{2\oplus}$ and/or $Zn^{2\oplus}$, a sensitive balance exists regarding the formation of macrochelates between the complexes of $AMP^{2\ominus}$, $ADP^{3\ominus}$, and $ATP^{4\ominus}$; it is most probable that the ratios of the isomers will change if the 1,N^6-etheno derivatives are employed.

The situation with $Cu^{2\oplus}$ may appear as an extreme case, but it is mentioned here, because there are indications[99] that $Cu(ATP)^{2\ominus}$ might be a natural active form of $Cu^{2\oplus}$. The stabilities and structures of the complexes in the $Cu^{2\oplus}$/ε-ATP system differ so much from those of $Cu^{2\oplus}$/ATP (Tables 3 and 5)[40], that ε-ATP should never be employed as a probe for ATP in the presence of $Cu^{2\oplus}$. Indeed, these differences in the coordinating properties are reflected already in the «simple» $Cu^{2\oplus}$-promoted hydrolysis of these two nucleotides (Section 8)[41]. The same conclusion must also be drawn for Cu(ε-AMP) and Cu(ε-$ADP)^{\ominus}$; both complexes are certainly no reliable probes.

There is one further point, which may easily be overlooked: e.g., the difference between about 38% $Mn(ATP)^{2\ominus}_{cl}$ and 62% Mn(ε-$ATP)^{2\ominus}_{cl}$ may at first sight not seem very significant and might even appear as rather comforting; the problem is that this difference corresponds already to 1.2 kJ/mol. If the extent of macrochelation is more pronounced the energy differences become even more significant: 90% versus 99% macrochelated isomers means a difference in ΔG^0 of 5.7 kJ/mol – and this difference might well mean inhibition of an enzymic reaction.

7. Monomeric Metal Ion Complexes Containing Two Different Ligands, One Bearing an Adenine or 1,N^6-Ethenoadenine Residue

7.1. Stability and Structure of Ternary Complexes Containing 2,2'-Bipyridyl and ε-Adenosine or Its Parent Nucleoside

To find out whether the steric restrictions resulting from the bidentate coordination of ε-adenosine (Section 6.2) still allow the formation of a stable ternary complex with another bidentate ligand the stability of the ternary Cu(Bpy)(ε-Ado)$^{2\oplus}$ complex had been determined by potentiometric pH titration[36]. In general, the stability of a mixed ligand complex, like M(Bpy)(A), where A = adenine derivative, is best quantified by a comparison with the stability of the binary complexes[100–102]. This is expressed by the stability difference, Δ lg K (Equation (27)), which also quantifies the position of equilibrium (28) (charges are omitted for clarity):

$$\Delta \lg K = \lg K^{M(Bpy)}_{M(Bpy)(A)} - \lg K^{M}_{M(A)} \quad (27)$$

$$M(Bpy) + M(A) \rightleftharpoons M(Bpy)(A) + M \quad (28)$$

The stability of the ternary Cu(Bpy)-(ε-Ado)$^{2\oplus}$ complex is indeed quite significant: lg $K^{Cu(Bpy)}_{Cu(Bpy)(\varepsilon\text{-Ado})} = 2.46 \pm 0.03$ ($I = 0.1$, $NaNO_3$; 25 °C)[36]; this together with the value of the binary Cu(ε-Ado)$^{2\oplus}$ complex, lg $K^{Cu}_{Cu(\varepsilon\text{-Ado})} = 2.81$ (Equation (14); Table 3), results in only a slightly negative value for Δ lg K (= −0.35) implying that ε-adenosine coordinates also in the ternary complex in a bidentate fashion.

This observation may be generalized twofold: (i) It is to be expected that ε-adenosine forms mixed ligand complexes also with other metal ions and with ligands other than 2,2'-bipyridyl. (ii) Based on previous experience with mixed ligand complexes[103–105], the stability of other M(Bpy)(ε-Ado)$^{2\oplus}$ complexes may be estimated by subtracting 0.4 log unit from the stability constants of the binary M(ε-Ado)$^{2\oplus}$ complexes listed in Table 3; the resulting estimate is expected to be correct within ±0.3 log unit.

How is the situation in ternary systems containing the unidentate adenosine (Section 6.2)? Again, based on previous experience *negative* values for Δ lg K are expected[36] for ternary $M^{2\oplus}$/Bpy/adenosine systems; hence, for the metal ions listed in Table 3 for the *coordination* of adenosine to M(2,2'-bipyridyl)$^{2\oplus}$ it holds lg $K^{M(Bpy)}_{M(Bpy)(Ado)} < 0.8$. This value should be compared with the stability constants of the *stacking* adducts formed between adenosine and 2,2'-bipyridyl (lg $K^{(Bpy)}_{(Bpy)(Ado)} = 1.36 \pm 0.06$[106]) or M(2,2'-bipyridyl)$^{2\oplus}$ (lg $K^{[M(Bpy)]}_{[M(Bpy)](Ado)} \simeq 1.2 \pm 0.2$[107]). The comparison shows clearly that the stacking interaction between the aromatic ring systems is of a larger stability than the metal ion–adenosine interaction in these ternary systems. Consequently, the ternary complexes all have about the stability of the *binary* (Bpy)(Ado) stacking adduct[106,107], the metal ion being *only* coordinated to the bipyridyl unit.

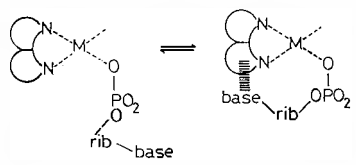
The stability of the stacking adduct between 1,N^6-ethenoadenosine and 2,2'-bipyridyl is expected (Sections 4.1, 4.2, and also 7.3) to be of the same order as the corresponding adduct between adenosine and bipyridyl (i.e., lg $K^{(Bpy)}_{(Bpy)(\varepsilon\text{-Ado})} \simeq 1.4$). By considering also the above conclusions about the stability of the ternary M(Bpy)(ε-Ado)$^{2\oplus}$ complexes it becomes evident that the structure of these complexes with $Co^{2\oplus}$, $Ni^{2\oplus}$, or $Cu^{2\oplus}$ is governed by the coordination tendency of these metal ions toward the N-6/N-7 unit of ε-adenosine, which is larger than the stacking tendency in the binary (Bpy)(ε-Ado) adduct. This means that in these ternary complexes the metal ions are coordinated to ε-adenosine *and* to bipyridyl, thereby preventing a direct intramolecular ligand–ligand interaction within the ternary complex due to the geometry of their coordination spheres. This contrasts with the corresponding ternary systems containing $Mg^{2\oplus}$ or $Mn^{2\oplus}$, where the structure will certainly be governed by the stacking interaction between the two ligands, while in the ternary system with $Zn^{2\oplus}$, most probably an isomeric mixture of ternary complexes will exist: in some complexes $Zn^{2\oplus}$ will be bridging the two ligands, whereas in others a stack between the ligands will exist, with $Zn^{2\oplus}$ being coordinated only to bipyridyl.

7.2. Intramolecular Stacking in Mixed Ligand Complexes Containing 2,2'-Bipyridyl and Nucleotides

The stability constants needed to characterize the position of equilibrium (28) have been measured[37,39,60,72,82] for several

ternary $M^{2\oplus}$/2,2′-bipyridyl/nucleotide systems (e.g., Table 6): usually the equilibrium is displaced to its right side as is indicated by positive values for Δ lg K (Equation (27)). This observation is due to two cooperative effects[60,97,100]: (i) π-accepting heteroaromatic nitrogen bases like 2,2′-bipyridyl favor, if coordinated to a transition metal ion, the coordination of oxygen donor ligands, like phosphates[103]; and (ii) intramolecular aromatic-ring stacking also enhances the stability of ternary complexes[108].

It is well-known[60,82,91,109,110] that the pyrimidine (Fig. 7) and adenine moieties (see also Section 7.1) may undergo stacking interactions with the aromatic rings of 2,2′-bipyridyl. Hence, for ternary complexes of the type M(Bpy)(NP) the question arises about the position of the intramolecular equilibrium (29), which is formulated below for M(Bpy)(NMP) complexes:



(29)

The occurrence of an intramolecular stack in Cu(Bpy)(AMP) and Cu(Bpy)-$(ATP)^{2\ominus}$ systems, i.e. of $Cu(Bpy)(NP)_{st}$ corresponding to the isomer at the right side in equilibrium (29), was proven already in 1974 by studying the charge-transfer absorption connected with the formation of the aromatic-ring adduct[106]. For related $Zn^{2\oplus}$ complexes this was confirmed by ^{1}H-NMR shift measurements[110]. The occurence of intramolecular stacks between the purine residue of AMP or ATP, the pyrimidine residue of UMP, and pyridyl ring systems has in the meanwhile also been shown for the solid state by X-ray crystal studies of $[Cu(HAMP)(Bpy)(H_2O)]_2(NO_3)_2 \cdot 6\,H_2O$[50], $[Cd(UMP)(2,2'\text{-dipyridylamine})(H_2O)_2]_n \cdot (5\,H_2O)_n$[51], $[Zn(H_2ATP)(Bpy)]_2 \cdot 4\,H_2O$[111], and $[Cu(H_2ATP)(Phen)]_2 \cdot 7\,H_2O$[112,113].

As purines stack much better than pyrimidines[109,110] a larger percentage of the stacked isomer is expected for M(Bpy)(NP) complexes containing an adenine residue, despite the connected release[110] of N-7 from the coordination sphere of the metal ion (Section 6.4) by the formation of the ternary complex. In fact, this is clearly born out by the results listed in Table 6: the intramolecular stack is more favored in M(Bpy)(AMP) or $M(Bpy)(ATP)^{2\ominus}$ than in M(Bpy)(UMP) or $M(Bpy)(UTP)^{2\ominus}$, though the effect is not as pronounced as one might expect by comparing the stabilities of the binary $[(UTP)(Bpy)]^{2\ominus}$ and $[(ATP)(Bpy)]^{2\ominus}$ stacks (see lower part of Table 6). This outcome agrees with the general observation that by an intramolecular linkage especially weak interactions are favored[60,108].

Table 6. Quantification of the stability of some mixed ligand M(Bpy)(NP) complexes in aqueous solution by the values of Δ lg K (Equation (27)), together with the estimated percentage[a] of the isomer, $M(Bpy)(NP)_{st}$, with an intramolecular stack (equilibrium (29) and Fig. 8) for the corresponding systems (I = 0.1; 25 °C). For comparison the stability constants of two binary stacks are also given (D_2O; I = 0.1; 27 °C).

ref.	M(Bpy)(Np)	Δ lg K	% $[M(Bpy)(NP)_{st}]$[a]
[37]	Cu(Bpy)(UMP)	0.21	≈ 30
[37]	Cu(Bpy)(AMP)	0.5	≈ 80
[37]	Cu(Bpy)(ε-AMP)	≈ −2.2	≈ 80
[60]	$Cu(Bpy)(UTP)^{2\ominus}$	0.35	≈ 55
[60]	$Cu(Bpy)(ATP)^{2\ominus}$	0.33	86(±3)
[39]	$Cu(Bpy)(\varepsilon\text{-ATP})^{2\ominus}$	≈ −2.2	≈ 90
[72,82]	$Zn(Bpy)(UTP)^{2\ominus}$	0.15	≈ 65; by ^{1}H-NMR: ≈ 40[110]
[72,82]	$Zn(Bpy)(ATP)^{2\ominus}$	0.10	≈ 70; by ^{1}H-NMR: ≈ 55[110]
[109]	$UTP^{4\ominus} + Bpy \rightleftharpoons [(UTP)(Bpy)]^{4\ominus}$		$K \approx 1$ M^{-1} (by ^{1}H-NMR)
[91]	$ATP^{4\ominus} + Bpy \rightleftharpoons [(ATP)(Bpy)]^{4\ominus}$		K = 8.1 ± 1.8 M^{-1} (by ^{1}H-NMR)

[a] If nothing else is mentioned, these estimated percentages are based on stability constants measured by potentiometric pH titrations.

In contrast to the so far discussed Cu(Bpy)(NP) complexes the ternary Cu(Bpy)(ε-AMP) and $Cu(Bpy)(\varepsilon\text{-ATP})^{2\ominus}$ complexes are considerably less stable than their binary parent complexes Cu(ε-AMP) and $Cu(\varepsilon\text{-ATP})^{2\ominus}$: i.e., Δ lg $K \approx -2$ (Equation (27); Table 6). This is due to the high affinity of the ε-adenine moiety toward $Cu^{2\oplus}$ in Cu(ε-AMP) and $Cu(\varepsilon\text{-ATP})^{2\ominus}$ (Section 6.2), but this does not mean that no stacking interaction in Cu(Bpy)(ε-AMP) and $Cu(Bpy)(\varepsilon\text{-ATP})^{2\ominus}$ occurs. Indeed, release[114] of the base moieties from the tetragonal[115,116] coordination sphere of $Cu^{2\oplus}$ leads to comparable stability constants for Cu(Bpy)(AMP) and Cu(Bpy)(ε-AMP) (cf.[37]) or $Cu(Bpy)(ATP)^{2\ominus}$ and $Cu(Bpy)(\varepsilon\text{-ATP})^{2\ominus}$ (cf.[39]), and evaluation[37,39] of the stability data gives estimates of 80% and 90% for the stacked isomers of Cu(Bpy)(ε-AMP) and $Cu(Bpy)(\varepsilon\text{-ATP})^{2\ominus}$, respectively. These percentages are close to the estimates for Cu(Bpy)(AMP) and $Cu(Bpy)(ATP)^{2\ominus}$ (Table 6) and in accordance with the general stacking properties observed for adenine and ε-adenine derivatives (Sections 4.1 and 4.2). A simplified structure for the stacked species of $Cu(Bpy)(\varepsilon\text{-ATP})^{2\ominus}$ is shown in Fig. 8.

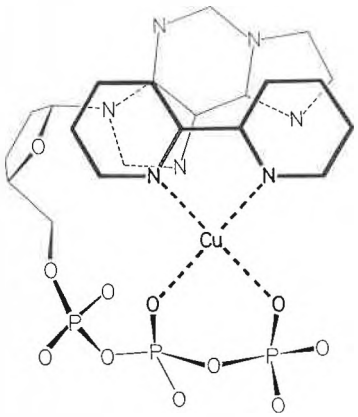


Fig. 8. Probable (schematic) structure for the isomer with an intramolecular stack of $Cu(Bpy)(\varepsilon\text{-ATP})^{2\ominus}$ in solution.

7.3. Intramolecular Stacks in Ternary Complexes Composed of ε-$ATP^{4\ominus}$ or $ATP^{4\ominus}$/L-Tryptophanate/$Mg^{2\oplus}$ or $Zn^{2\oplus}$

The similarity of the stacking properties of the adenine and the ε-adenine moieties became evident again in the last paragraph, and they are also born out by the following stability data concerning binary stacks involving tryptophan (by ^{1}H-NMR in D_2O; I = 0.1; 27 °C):

ε-Ado	+ Trp	⇌	(ε-Ado)(Trp)	K = 6.0 ± 1.1 M^{-1} [36]
$AMP^{2\ominus}$	+ Trp	⇌	$[(AMP)(Trp)]^{2\ominus}$	K = 6.8 ± 1.6 M^{-1} [117]
$ATP^{4\ominus}$	+ Trp	⇌	$[(ATP)(Trp)]^{4\ominus}$	K = 6.2 ± 1.2 M^{-1} [110]
$\varepsilon\text{-ATP}^{4\ominus}$	+ Trp	⇌	$[(\varepsilon\text{-ATP})(Trp)]^{4\ominus}$	K = 7.5 ± 0.8 M^{-1} [39]

As the recognition interactions[6,7] between nucleotides/nucleic acids and amino acids/proteins are an essential relationship between these two classes of important ligands such observations deserve some notice. The amino acid tryptophan is of further interest here, because there are indications for stacking between the purine residue of ATP and the tryptophanyl indole group of myosin and it appears in addition that this interaction is promoted by $Mg^{2\oplus}$[118]; there are in fact many such purine–indole interactions expected[119] to occur in nature.

Indeed, for several ternary M(ATP)-$(Trp)^{3\ominus}$ complexes the formation of intramolecular purine–indole stacks has been proven directly by spectrophotometric and ^{1}H-NMR shift measurements and indirectly by the enhanced stability of the complexes as determined by potentiometric pH titrations[120,121], and these results have been repeatedly confirmed by independent studies[110,122,123]. It is immediately obvious

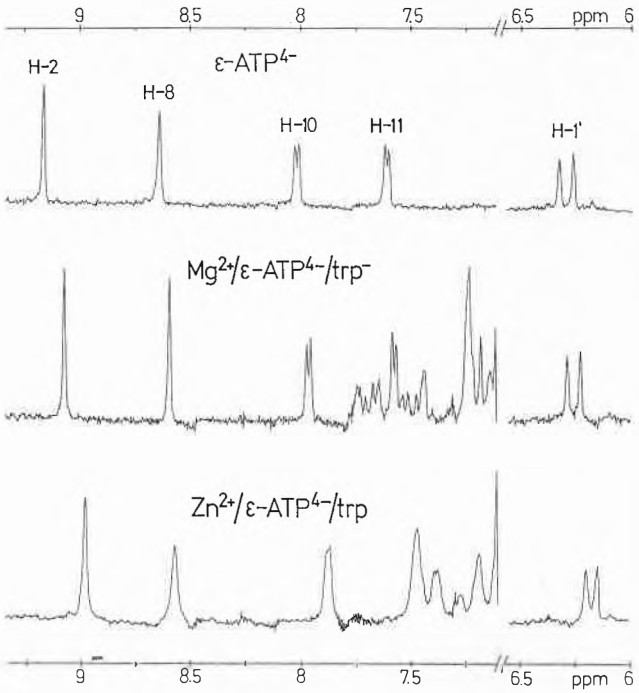
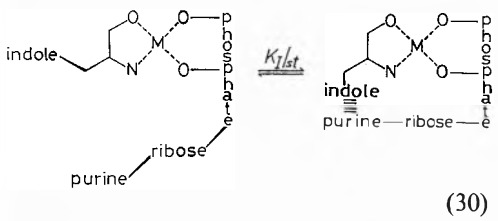


Fig. 9. ^{1}H-NMR spectra of L-*tryptophan/ε-ATP systems: ε-ATP$^{4\ominus}$ (0.01* M*) at pD 8.3; Mg$^{2\oplus}$, ε-ATP$^{4\ominus}$, and Trp (each 0.01* M*) at pD 10.5; Zn$^{2\oplus}$, ε-ATP$^{4\ominus}$, and Trp (each 0.01* M*) at pD 9.1. The spectra were measured relative to $(CH_3)_4N^{\oplus}NO_3^{\ominus}$ (0.002* M*) and converted to values relative to sodium 3-(trimethylsilyl)propanesulfonate by adding 3.188 ppm (90.025 MHz; 27°C; I = 0.1, $NaNO_3$; D_2O). Adapted from figure 3 of ref.* [39].

from the ^{1}H-NMR spectra shown in Fig. 9 that stacking also occurs between the indole residue of tryptophan and the ε-adenine moiety of ε-ATP: the upfield shift of the proton signals, especially for H-2, is nicely seen [39]. From these observed upfield shifts and the computed formation degree of the corresponding Mg(ε-ATP)(Trp)$^{3\ominus}$ and Zn(ε-ATP)(Trp)$^{3\ominus}$ complexes, a calculation of the shifts due to the resonances of H-2, H-8, H-10 and H-1′ in these ternary complexes is possible: the upfield shifts in the complexes are between 0.13 and 0.53 ppm. These values may be compared with those determined for the binary (ε-adenosine)(Trp) stack, which are between 0.38 and 1.30 ppm, and then it becomes clear that again an intramolecular equilibrium must exist in the ternary complexes between an «open» and a «stacked» form, i.e. between the two isomers indicated in equilibrium (30):



(30)

If the two isomers in equilibrium (30) are designated as M(NTP)(Trp)$^{3\ominus}_{op}$ and M(NTP)(Trp)$^{3\ominus}_{st}$, the constant of this intramolecular and therefore concentration-independent equilibrium is defined by Equation (31):

$$K_{I/st} = \frac{[M(NTP)(Trp)^{3\ominus}_{st}]}{[M(NTP)(Trp)^{3\ominus}_{op}]} \quad (31)$$

A comparison of the relative shifts calculated for the several protons in the ternary complexes with those in the corresponding binary adducts allows an estimation of the percentages of the stacked isomers, and hence also of a value for $K_{I/st}$. The corresponding results are listed in Table 7: for all four ternary complexes, independent of the bridging metal ion, be it Mg$^{2\oplus}$ or Zn$^{2\oplus}$, and also independent of the 1,N^6-etheno substituent, the percentage of the closed isomer (equilibrium (30)) is around 40%. Consequently, in these ternary complexes the stacking tendencies of the adenine and the ε-adenine moieties, as well as the coordination spheres of Mg$^{2\oplus}$ and Zn$^{2\oplus}$ in their respective complexes, are quite alike. It may be added that mixed ligand complexes containing ATP$^{4\ominus}$ and L-leucinate, M(ATP)(Leu)$^{3\ominus}$, are also known [121], and that in a certain percentage of these ternary complexes an intramolecular hydrophobic interaction between the isopropyl residue of the amino acid and the purine moiety of ATP occurs. The same kind of interaction may also be surmised for M(ε-ATP)(Leu)$^{3\ominus}$ complexes.

Table 7. Estimates for the intramolecular equilibrium constants, $K_{I/st}$ (Equation (31)), and approximate percentages of the stacked isomers, M(NTP)(Trp)$^{3\ominus}_{st}$ (equilibrium (30)), in ternary complexes consisting of Mg$^{2\oplus}$ or Zn$^{2\oplus}$, ε-ATP$^{4\ominus}$ or ATP$^{4\ominus}$, and L-tryptophanate, as determined by ^{1}H-NMR shift measurements in D_2O (I = 0.1, $NaNO_3$; 27°C)[39].

M(NTP)(Trp)$^{3\ominus}$	$K_{I/st}$	% [M(NTP)(Trp)$^{3\ominus}_{st}$][a]
Mg(ε-ATP)(Trp)$^{3\ominus}$	1.0	51 ± 11
Zn(ε-ATP)(Trp)$^{3\ominus}$	0.45	31 ± 6
Mg(ATP)(Trp)$^{3\ominus}$	0.8	44 ± 19
Zn(ATP)(Trp)$^{3\ominus}$	0.7	40 ± 15

[a] The error limits are estimated.

7.4. Concluding Considerations on Mixed Ligand Complexes Involving the ε-Adenosine Residue

From the results discussed in Sections 7.2 and 7.3 it is evident that the extent of intramolecular stacking in ternary complexes containing either a nucleotide with an adenine or an ε-adenine moiety is comparable, indicating that in this respect the two types of nucleotides appear as interchangeable. Of course, this is only true as long as the larger size of the ε-adenine moiety does not give rise to steric restrictions regarding space and orientation, e.g., at the surface of a protein.

Another point one has to be aware of is the availability of the N-6/N-7 site for metal ion coordination in stacked complexes (see, e.g., Fig. 8). To give just a single example on what this could mean: The affinity of the N-6/N-7 site of the ε-adenine moiety toward Zn$^{2\oplus}$ in the physiological pH region is larger than that of a carboxylate group; i.e. lg $K^{Zn}_{Zn(\varepsilon\text{-Ado})} = 1.51$ (Table 3) > lg$K^{Zn}_{Zn(R\text{-}COO)} \simeq 1.0$ [124]. As there is no reason to assume that in a ternary stacked complex the affinity of the free N-6/N-7 site will be strongly reduced due to the presence of the metal ion coordinated to the phosphate groups, this means, that Zn$^{2\oplus}$ might change its place of coordination. It is evident that this could considerably disturb the reactivity of a system. Clearly, there is no such problem with the adenine residue (lg $K^{Zn}_{Zn(Ado)} \simeq -0.3$, Table 3; Section 6.2), and this despite the similarity of both base residues in the affinity toward the proton (Section 5).

A related problem is the formation of ternary complexes in which the metal ion is coordinated only to one ligand while both ligands are kept together by stacking; such examples are well-known [101, 109, 125] and several more have been discussed in Section 7.1. We have seen that if the stacking tendency is comparable to the affinity of a certain metal ion toward the N-6/N-7 site this may give rise to two isomeric M(Bpy)(ε-adenosine)$^{2\oplus}$ complexes: one in which the metal ion bridges the two ligands, and another one which is kept together solely by stacking. Similar situations can be imagined with ε-AMP complexes, especially under conditions where the mental ion affinity of the phosphate group is reduced, e.g., via hydrogen bonding or an ionic interaction; in an enzymic system this appears as quite feasible.

8. Caveat by Considering the Different Properties of ATP and ε-ATP in a Metal-Promoted Reaction

Metal ions are essential to biological phosphoryl and nucleotidyl transfer [2, 3].

Hence, it is not surprising that the metal ion promoted dephosphorylation of nucleoside 5'-triphosphates in aqueous solution to the corresponding diphosphates and orthophosphate has long been recognized[126,127]. Indeed, studies of the transfer of a phosphoryl group from nucleotides to a water molecule appears likely to provide some insight into transphosphorylations[128–130].

In Fig. 10 some recent results[41] about the $Zn^{2\oplus}$ and $Cu^{2\oplus}$ promoted dephosphorylation of CTP (Fig. 7), ATP and ε-ATP (Fig. 1) in 1:1 systems are summarized. The important points to note are: (i) differences in the dephosphorylation properties of these nucleotides become apparent only in the presence of metal ions: this means, the different base/metal ion affinities (Section 6) lead to structurally different complexes in solution and these are responsible for the differences in reactivity. (ii) When the rates in the neutral pH range are compared, the orders are: with $Cu^{2\oplus}$, ATP > ε-ATP > CTP; and with $Zn^{2\oplus}$, ε-ATP > ATP > CTP. (iii) The effectiveness of the metal ions is $Cu^{2\oplus}$ > $Zn^{2\oplus}$ for ATP and CTP, but very surprisingly $Zn^{2\oplus}$ > $Cu^{2\oplus}$ for ε-ATP.

It is known[95b] that for $M^{2\oplus}$/CTP and other triphosphates with a non-coordinating terminal organic residue the monomeric $M_2(NTP)(OH)^{\ominus}$ is the most reactive species, both metal ions being coordinated to the triphosphate chain. Therefore CTP is always the final link in the above orders, and $Cu^{2\oplus}$ is more active than $Zn^{2\oplus}$, because its complexes are the more stable ones and it has also a higher tendency to form hydroxo species[130].

Hence, we are left with the problem: why is in the presence of $Cu^{2\oplus}$ in the neutral pH range the dephosphorylation rate of ATP faster than that of ε-ATP, and why is the reverse order[131] observed with $Zn^{2\oplus}$? In short: in these four systems metal ion/base interactions are important and detailed kinetic measurements[41,130] reveal that (i) for all four systems the most reactive species contains $M^{2\oplus}$/NTP in the ratio 2:1[132], and (ii) for $Cu^{2\oplus}$/ε-ATP the reaction proceeds via a monomeric complex, while for $Cu^{2\oplus}$/ATP and both $Zn^{2\oplus}$ systems the intermediate is of a dimeric stacked nature. The proposed structures of the reactive complexes are shown in Fig. 11.

What can we learn from these observations regarding the evaluation in how far ε-ATP may be employed as probe for ATP? The answer appears as rather discouraging: the somewhat *reduced* tendency for self-association of $Zn(\varepsilon\text{-ATP})^{2\ominus}$, compared with that of $Zn(ATP)^{2\ominus}$ (Section 4.4), and the *enhanced* $Zn^{2\oplus}$/base interaction with $\varepsilon\text{-ATP}^{4\ominus}$, compared with that of $ATP^{4\ominus}$ (Sections 6.2 and 6.4), combines in the effect to an approximately 6-fold increase in the rate of the dephosphorylation reaction of $Zn^{2\oplus}$/ε-ATP over that of $Zn^{2\oplus}$/ATP in the neutral pH range and in 1:1 systems (Fig. 10). However, there re-

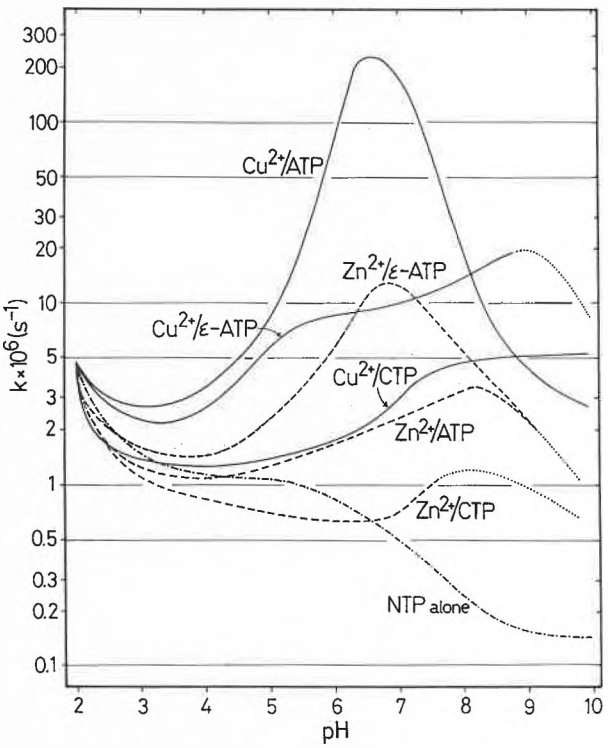


Fig. 10. Comparison of the $Cu^{2\oplus}$ (—) and $Zn^{2\oplus}$ (- - -) promoted dephosphorylation of ε-ATP, ATP, and CTP (always in the ratio 1:1) as a function of pH, characterized as the first-order rate constants k (s^{-1}). For further comparison the rate in the nucleoside 5'-triphosphate systems alone, NTP = ε-ATP, ATP, and CTP (–·–·), is also given. The dotted line portions indicate uncertainty due to precipitation. The concentration of each reactant (when present) was always 10^{-3} M; I = 0.1, $NaClO_4$; 50 °C. The detailed experimental data from which this figure is composed are given in figures 2 and 3 of ref.[41], and in the references cited there.

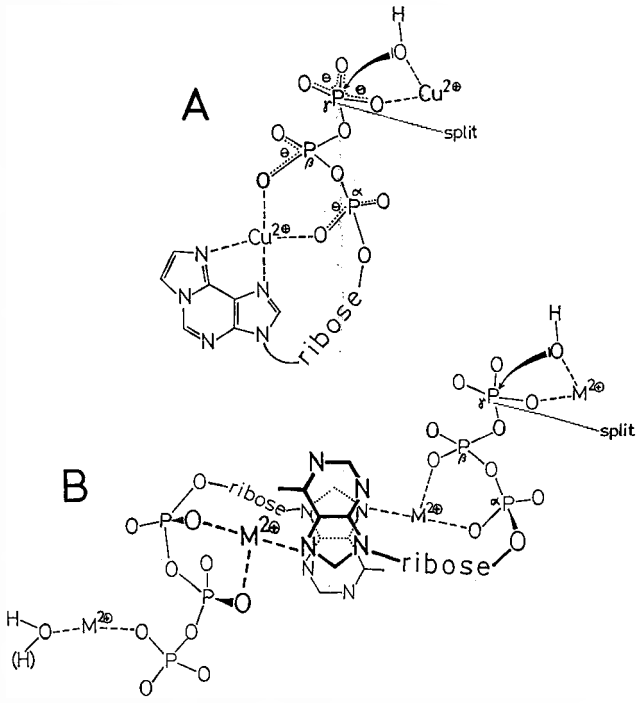


Fig. 11. A) Probable structure of the reactive $Cu_2(\varepsilon\text{-ATP})(OH)^{\ominus}$ complex formed in the $Cu^{2\oplus}$ promoted dephosphorylation of ε-ATP; the α,β-coordination of one $Cu^{2\oplus}$ is facilitated due to its base back-binding[41]. – B) Proposed structure for the reactive $[M_2(ATP)]_2(OH)^{\ominus}$ dimer, which occurs in low concentrations in the $Zn^{2\oplus}$ and $Cu^{2\oplus}$ promoted dephosphorylation of ATP[130], as well as in the $Zn^{2\oplus}$ promoted dephosphorylation of ε-ATP[41] (for the latter case, in the schematic structure shown above the adenine moiety has to be replaced by an ε-adenine moiety with $M^{2\oplus}$ coordinated to the N-6/N-7 site; see Section 6.2). The intramolecular attack of $OH^{\ominus}$ is indicated on the right side of the above structure, while the left side is ready to transfer also into the reactive state by deprotonation of the coordinated water molecule or to undergo an intermolecular water attack. The above figure is a combination of chart II from ref.[41] and figure 7 from ref.[130].

mains in this case at least one comfort: the pathway of the reaction[41] is the same for the $Zn^{2\oplus}$ promoted dephosphorylation of $ATP^{4\ominus}$, as well as for ε-$ATP^{4\ominus}$ (Fig. 11B). In case of $Cu^{2\oplus}$, the increase in complex stability and in the extent of base backbinding is so different for ε-ATP and ATP (Sections 6.1 and 6.4), that different pathways are observed (see Fig. 11A and 11B, respectively); and this fact then leads to the peculiar situation[41] that in 1:1 systems and at pH 7 in 10^{-5} M solutions $Cu^{2\oplus}$/ε-ATP is more reactive by a factor of about 5, while in 10^{-2} M solutions $Cu^{2\oplus}$/ATP is 200 times more reactive than $Cu^{2\oplus}$/ε-ATP. This example is self-explanatory.

9. Perspectives

From the Conclusion-Sections 4.5, 6.5, 7.4 and the preceding Section 8 it is clear that great care must be exercized in employing 1,N^6-ethenoadenine derivatives in the presence of metal ions as probes for adenine derivatives. Even in the few cases where an application appears as possible, like with alkaline earth ions in dilute solutions, one has to be aware that hidden pitfalls may exist[96], especially in studies involving kinetic experiments.

Because of the absence of highly resolved X-ray structure determinations of crystalline enzyme–ATP and similar complexes[4], molecular probes of the described kind are, *a priori*, legitimate tools to study, for example, in solution the binding sites for ATP in proteins if the appropriate care is taken to prevent misinterpretations. It is evident that one is facing in this connection one further problem which is only slowly becoming more clear: namely that of the properties of low dielectric cavities in proteins – and in these the active sites are usually located. The equivalent solution (or effective) dielectric constants for the active-site cavities of bovine carbonic anhydrase and carboxypeptidase A have recently been estimated as being in the order of 35 and 70, respectively[133]. Values of a similar order have also been derived for some hemoproteins[134]. There is now increasing evidence that properties of complexes may change under these conditions in unexpected ways: e.g., intramolecular ligand-ligand interactions involving stacks in mixed ligand complexes may to a certain extent be favored[60,135]. This is unexpected, because addition of an organic solvent like ethanol or dioxane to an aqueous solution containing binary stacks destroys these.

These indicated problems are to be attacked by model studies. This is the only way to develop a feeling and finally to provide a solid basis about the influence of a reduced effective dielectric constant on properties of metal ion complexes; already now there is no doubt that in this way specificity and selectivity can be promoted[60,136]. Here a wide field is open for inorganic or coordination chemists with an interest toward biochemistry or biology[137]. It is obvious that model studies will usually suffer due to incompleteness in one way or in another. However, to an open mind there is always a gain! A nice example for this is provided by the $Cu^{2\oplus}$/ε-ATP system described in this account: it is clear ε-ATP is in the presence of $Cu^{2\oplus}$ a poor probe for ATP (Sections 6.5 and 8). However, the search for a monomeric complex in which the α,β-coordination of a metal ion at a triphosphate is facilitated by the presence of additional binding sites in a sterically favorable position is a long standing goal. The search for such a species had been initiated in 1956 by the hypothesis of *Szent-Györgyi*[138] that macrochelate formation in nucleotide complexes is of importance for biological systems. Consequently, such a reactive complex has been sought in model studies[2,139] and in NTP dephosphorylations[127,140]; in model studies no significant rate enhancement was observed[2] and for NTP systems other reactive complexes were identified[128,130]. Now 30 years later, it is shown for the ATP-derivative, 1,N^6-ethenoadenosine 5'-triphosphate, that the $Cu^{2\oplus}$ promoted dephosphorylation proceeds via such a complex as depictured in Fig. 11A. Needless to say, that this result is also improving our understanding of transphosphorylations and the connected structural tuning by metal ion coordination.

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Expressions like adenine nucleotides and adenosine phosphates are interchangeable
The phosphate groups in NTP are labeled as α, β, and γ where the latter refers to the terminal phosphate group (see Fig 1)
The term «dephosphorylation» is used for the transfer of a phosphate group from NTP to a water molecule, and the term «hydrolysis» for the formation of hydroxo complexes of metal ions.
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