workers reported 93% inversion and 7% retention at the α-phosphate of ADP.[13] At least some of this scrambling of label probably occurred at the start of the reaction before the pH dropped. By starting at pH 3, we have reduced the degree of label scrambling so that no ^{18}O was found in the β-phosphate of ADP by ^{31}P NMR after the reaction. Mass spectrometric analysis showed less than 3% ^{18}O in the β-phosphate, which suggests that the reaction went with ~97% inversion. With ATPβS we also found ^{18}O only in the β-phosphate and none in the α- or γ-phosphates. The ^{18}O shifts reported in Table II show that this reaction also went with inversion. Here again, the initial low pH keeps the γ-phosphate protonated so that it is too poor a nucleophile to compete with water and produce cyclic triesters whose hydrolysis would scramble the label. Thus, desulfurization by Br_2 with the initial pH kept at 3 or below is a rapid and efficient method for preparing chiral ^{18}O-labeled nucleotides. The low pH results in some hydrolysis, but the yields could probably be increased above those reported here by careful adjustment of temperature, time of incubation, pH during the reaction, and level of Br_2 present.

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Comparison of the Properties of Binary and Ternary Metal Ion Complexes of 1,N^6-Ethenoadenosine 5′-Triphosphate (ε-ATP) and Adenosine 5′-Triphosphate (ATP), Including Macrochelate and Purine–Indole Stack Formation

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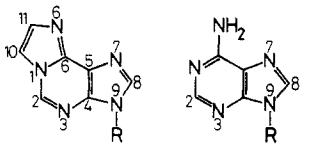
Abstract: As ε-ATP can undergo self-association, the experimental conditions for the potentiometric pH titrations, used to determine the acidity constants of $H_2(\epsilon\text{-ATP})^{2-}$ (the first proton is released from the base residue as spectrophotometric measurements reveal) and the stability constants of several metal ion (M^{2+} = Mg^{2+}, Mn^{2+}, Zn^{2+}) complexes of $H(\epsilon\text{-ATP})^{3-}$ and $\epsilon\text{-ATP}^{4-}$, were chosen such that the ligand was present in its monomeric form. The stabilities of the Mg^{2+} complexes of $\epsilon\text{-ATP}^{4-}$ and ATP^{4-} are quite similar, but the stabilities of $Mn(\epsilon\text{-ATP})^{2-}$ and $Zn(\epsilon\text{-ATP})^{2-}$ are clearly larger than those of the corresponding $M(ATP)^{2-}$ complexes. On the basis of the stabilities of complexes formed with pyrimidine nucleoside 5′-triphosphates (PNTP), in which the base moiety does not participate in complex formation, the extent of macrochelate formation (i.e., the simultaneous coordination of a metal ion to the phosphate and the base residues) is evaluated for monomeric $M(\epsilon\text{-ATP})^{2-}$ complexes and compared with the $M(ATP)^{2-}$ species; the percentage of the macrochelated isomer is always larger for $M(\epsilon\text{-ATP})^{2-}$. When the chemical shifts of H-8 and H-11 at infinite dilution (δ_0) for $Mg(\epsilon\text{-ATP})^{2-}$ and $Zn(\epsilon\text{-ATP})^{2-}$ are compared, the macrochelate formation in the Zn^{2+} complex is confirmed. Intramolecular aromatic-ring stacking occurs in the ternary 2,2′-bipyridyl complexes $Cu(bpy)(PNTP)^{2-}$, $Cu(bpy)(ATP)^{2-}$, and $Cu(bpy)(\epsilon\text{-ATP})^{2-}$; the percentage of the stacked isomer increases within this series from about 50 to 90%. ^1H NMR shift measurements of the ternary complexes formed between Mg^{2+} or Zn^{2+}, $\epsilon\text{-ATP}^{4-}$ or ATP^{4-}, and L-tryptophanate prove that in all four complexes intramolecular purine–indole stacks are formed; an estimate gives about 40% of the stacked isomer in all cases, thus giving evidence that in these ternary complexes the stacking tendencies of the adenine and the ε-adenine moieties, as well as the coordination spheres of Mg^{2+} and Zn^{2+}, are quite alike. Several points are outlined that should be considered if ε-ATP is employed as a probe for ATP in enzymic systems.

Metal ion activated processes of nucleotide-depending enzyme systems are playing a prime role in biology,[1] and, consequently, nucleotide–metal ion as well as nucleic acid–metal ion interactions are intensively studied.[2-5] Among the nucleotides, ATP[6] is especially prominent: One-sixth of all known enzymes requires ATP or a related adenine-containing cofactor.[7] It has been estimated[8]

Chart I



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(6) Abbreviations and definitions: A, adenine derivative; Ado, adenosine; ε-Ado, ε-adenosine = 1,N^6-ethenoadenosine; AMP, ADP, and ATP, adenosine 5′-mono-, di-, and triphosphate; ε-AMP, ε-ADP, and ε-ATP, ε-adenosine 5′-mono-, di-, and triphosphate; bpy, 2,2′-bipyridyl; CTP, UTP, and TTP, cytidine, uridine, and thymidine 5′-triphosphate; M^{2+}, bivalent metal ion; NP, nucleotide; Ns, nucleoside; NTP, nucleoside 5′-triphosphate; phen, 1,10-phenanthroline; PNTP, pyrimidine nucleoside 5′-triphosphate; Trp, L-tryptophan. The phosphate groups in NTP are labeled as α, β, and γ, where the latter refers to the terminal phosphate group.

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that a human being uses and resynthesizes his own body weight of ATP daily. This nicely illustrates the importance of ATP and explains the efforts undertaken to probe the ATP binding sites of proteins; these binding and recognition interactions[9] are essential for the relationships between nucleic and amino acids.

For solution studies of coenzymes, so-called dimensional probes have become popular; these probes are related to natural cofactors by a defined dimensional change in the molecule.[7,10] As a probe for ATP, 1,N^6-ethenoadenosine 5'-triphosphate (ε-ATP) is particularly widely utilized.[12-14] This is due to the excellent fluorescent properties of this derivative,[13-17] and the possibility[12e] of evaluating the importance of the N-1 and 6-NH_2 positions of the purine residue, which are selectively altered by the etheno bridge (see Chart I), for the specificity of binding to particular enzymes.

Considering that all ATP-dependent enzyme systems are also metal ion dependent,[1] it is surprising that no detailed studies of the metal ion binding properties of ε-ATP exist. So far, only the fluorescent properties have been studied in the presence of metal ions,[18,19] but the equilibrium constants derived have to be viewed with care because buffers have been employed in these experiments and buffers are known to form ternary M^{2+}(ATP)(buffer) complexes.[20] The extent of macrochelate formation, i.e., the simultaneous coordination of a metal ion to the phosphate and the base residues, in M(ε-ATP)$^{2-}$ complexes has so far not been considered, nor have any mixed-ligand complexes been studied; corresponding results will be presented now.

After the evaluation of the properties of ε-adenosine[11] and ε-AMP[21] in the presence of metal ions and after the study of the influence of Mg^{2+} and Zn^{2+} on the self-association of ε-ATP^{4-},[22] the necessary background information was available to handle also the considerably more complicated ε-ATP/metal ion systems. The results obtained for the monomeric complexes by employing UV spectrophotometry, ^{1}H NMR, and potentiometric pH titrations are compared with the corresponding data obtained earlier[23-25] for the parent nucleotide, ATP (Chart I).

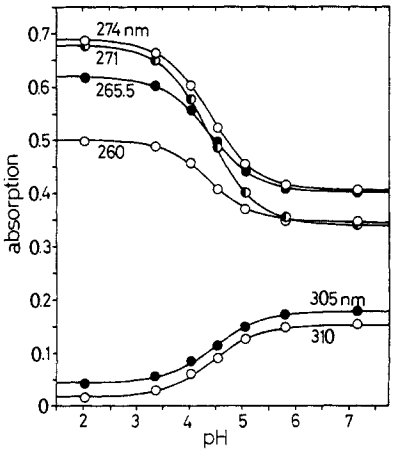

Figure 1. Evaluation of the dependence of the UV absorption of ε-ATP on pH in aqueous solution (measured in 2-mm cells with [ε-ATP] ≃ 3.36 × 10^{-4} M; I = 0.1, $NaClO_4$; 25 °C) by plotting the absorption vs. pH. The solid curves represent the computer-calculated best fits of the experimental data; the individual results for $pK^H_{H_2(\epsilon\text{-ATP})}$ are 4.383 at 260 nm, 4.396 at 265.5 nm, 4.400 at 271 nm, 4.380 at 274 nm, 4.459 at 305 nm, and 4.432 at 310 nm; average result, $pK^H_{H_2(\epsilon\text{-ATP})}$ = 4.41 ± 0.04.

Experimental Section

Materials. The sodium salt of 1,N^6-ethenoadenosine 5'-triphosphate was purchased from Sigma Chemical Co. and zinc perchlorate from K&K Laboratories, Inc. All the other chemicals were the same as given in ref 11.

The titer of the NaOH used for the titrations was determined with potassium hydrogen phthalate; the exact concentrations of the ε-ATP solutions used in the titrations with metal ions (titrated in the presence of an excess of HNO_3; see next sections) were measured by titrations with NaOH. The concentrations of the stock solutions of the divalent metal ions were determined with EDTA.

All experiments with ε-ATP were done in such a way that dephosphorylation of ε-ATP, which is metal ion promoted[26] like that of other nucleoside 5'-triphosphates,[27] was kept to a minimum.

^{1}H NMR and UV Spectroscopy. The experiments were carried out and evaluated as described in ref 11 and 21.

The center peak of the tetramethylammonium ion triplet was used as internal reference in the NMR experiments (in D_2O);[23] all chemical shifts were converted to a 3-(trimethylsilyl)propanesulfonate reference by adding 3.188 ppm. In case of duplets, as they occur for H-10, H-11, and H-1' of ε-ATP (Figure 3), the center was evaluated. The experimental details are given in the legend to Figure 3. The pD of the D_2O solutions was obtained by adding 0.40 to the pH meter reading.[28]

Potentiometric pH Titrations. The pH titrations were carried out with a Metrohm potentiograph E 536 and a macro EA 121 glass electrode. The buffers (pH 4.64, 7.00, and 9.00) used for calibration were also from Metrohm AG. The direct pH meter readings were used in the calculations for the acidity constants.

The acidity constants $K^H_{H_2(\epsilon\text{-ATP})}$ and $K^H_{H(\epsilon\text{-ATP})}$ for H_2(ε-ATP)$^{2-}$ were determined by titrating 50 mL of aqueous 0.72 mM HNO_3 and $NaNO_3$ (I = 0.1; 25 °C) in the presence and absence of 0.334 or 0.459 mM ε-ATP^{4-} under N_2 with 1 mL of 0.04 M NaOH. These constants were calculated from six independent titration pairs within the range from about 25% neutralization for the equilibrium H_2(ε-ATP)$^{2-}$/H(ε-ATP)$^{3-}$ (lower values are not reached under the given conditions) to 98% for the equilibrium H(ε-ATP)$^{3-}$/ε-ATP^{4-}.

The same conditions were employed for the determination of the stability constants $K^M_{M(H\cdot\epsilon\text{-ATP})}$ and $K^M_{M(\epsilon\text{-ATP})}$ in the binary ε-ATP systems with Mg^{2+}, Mn^{2+}, and Zn^{2+}, the [M^{2+}]:[ε-ATP] ratios being always 1:1. These constants were computed from three independent titrations for each system with a curve-fitting procedure[29] that became satisfactory by taking into account the species H^+, H_2(ε-ATP)$^{2-}$, H(ε-ATP)$^{3-}$, ε-ATP^{4-}, M^{2+}, M(H·ε-ATP)$^-$, and M(ε-ATP)$^{2-}$. In the Zn^{2+}/ε-ATP system a value for Zn(ε-ATP)$(OH)^{3-}$ was also estimated.

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Table I. Logarithms of the Stability Constants (eq 3 and 4) for Several Metal Ion Complexes with ε-ATP (I = 0.1, $NaNO_3$; 25 °C) [Corresponding Data for ATP and Pyrimidine Nucleoside 5′-Triphosphates (PNTP) Given for Comparison][a]

M^{2+}	log $K^M_{M(H\cdot\epsilon\text{-ATP})}$	$pK^H_{M(H\cdot\epsilon\text{-ATP})}$[b]	log $K^M_{M(\epsilon\text{-ATP})}$ pH titrn	log $K^M_{M(\epsilon\text{-ATP})}$ fluor quen[c]	log $K^M_{M(ATP)}$[d]	$pK^H_{M(H\cdot ATP)}$[e]	log $K^M_{M(PNTP)}$[f]
Ca^{2+}				3.74[i]	3.88	4.7	3.68
Mg^{2+}	2.3 ± 0.3	4.6	4.24 ± 0.03	4.05[i]	4.24	4.4	4.05
Mn^{2+}	3.26 ± 0.15	4.7	5.10 ± 0.15	5.16[i]/4.90[j]	4.81	4.0	4.63
Co^{2+}				5.05[i]/4.61[j]	4.86	3.9	4.53
Ni^{2+}				5.8[j]	4.85	4.3	4.29
Cu^{2+}			>8[g]	5.69[i]	6.32[k]	3.74[k]	5.62
Zn^{2+}	3.26 ± 0.19	4.3	5.44 ± 0.13[h]	4.31[i]	5.16	3.9	4.77
$Cu(bpy)^{2+}$	4.58 ± 0.07	4.25	6.83 ± 0.06		~6.5		6.0

[a] The acidity constants for $H_2(\epsilon\text{-ATP})^{2-}$ used in the calculations of the above complexes are given in section 1. The range of error for all newly determined constants is *3 times* the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. The partly rather large errors mainly result from the relatively low formation degrees of the species $M(H\cdot\epsilon\text{-ATP})^-$ (see the example in the lower part of Figure 1). [b] Calculated with eq 6 from the other data listed above and in section 1. [c] Constants determined by fluorescence quenching. [d] From ref 24; for Mn^{2+} and Zn^{2+} the average of the values given in ref 24 is listed above (I = 0.1, $NaClO_4$; 25 °C). For Cu^{2+} see footnote *k*. [e] Calculated with eq 6, $pK^H_{H(ATP)}$ = 6.42,[d,24] the values of log $K^M_{M(H\cdot ATP)}$ given in ref 36 (for Cu^{2+} see ref 34), and the values listed above. [f] The pyrimidine residue of nucleotides does not participate in complex formation;[23,32,37] hence, the stability of these $PNTP^{4-}$ complexes is solely determined by the affinity of the phosphate groups. The constants given in ref 24 (I = 0.1, $NaClO_4$; 25 °C) for CTP ($pK^H_{H(CTP)}$ = 6.51), UTP ($pK^H_{H(UTP)}$ = 6.45), and TTP ($pK^H_{H(TTP)}$ = 6.50) were "normalized" to pK_a = 6.45 by subtracting from the log stability constants of $M(CTP)^{2-}$ and $M(TTP)^{2-}$ 0.06 and 0.05 log unit, respectively; the resulting values were averaged and listed above (see also ref 23). [g] See ref 31. [h] $pK^H_{Zn(\epsilon\text{-ATP})(H_2O)} \simeq 9.0$. [i] Calculated from apparent stability constants taken from ref 18 and valid at pH 7.2 (determined in 0.1 M Tris or HEPES buffer at 25 °C) by adding log $(1 + [H^+]/K^H_{H(\epsilon\text{-ATP})})$; see ref 38. [j] Taken from ref 19; these values were determined in Tris or HEPES buffer at ~22 °C. [k] From ref 34.

The ternary Cu^{2+}/2,2′-bipyridyl/ε-ATP system was titrated four times in the ratio 1:1:1 with $[Cu^{2+}]$ = 0.288, 0.336, and 0.460 mM. In the curve-fitting procedure[29] H^+, $H_2(\epsilon\text{-ATP})^{2-}$, $H(\epsilon\text{-ATP})^{3-}$, $\epsilon\text{-ATP}^{4-}$, $H_2(bpy)^{2+}$, $H(bpy)^+$, bpy, Cu^{2+}, $Cu(H\cdot\epsilon\text{-ATP})^-$, $Cu(\epsilon\text{-ATP})^{2-}$, $Cu(bpy)^{2+}$, $Cu(bpy)_2^{2+}$, $Cu(bpy)(H\cdot\epsilon\text{-ATP})^-$, and $Cu(bpy)(\epsilon\text{-ATP})^{2-}$ were taken into account. The constants for the binary Cu^{2+}/bpy system were taken from the work of Anderegg.[30] $Cu(H\cdot\epsilon\text{-ATP})^-$ and $Cu(\epsilon\text{-ATP})^{2-}$ are only formed in small amounts due to the high stability of $Cu(bpy)^{2+}$. Consequently, the results obtained for the stability of the ternary complexes are rather independent from the stability constants used for $Cu(H\cdot\epsilon\text{-ATP})^-$ and $Cu(\epsilon\text{-ATP})^{2-}$;[31] in the corresponding examinations the values for log $K^{Cu}_{Cu(H\cdot\epsilon\text{-ATP})}$ and log $K^{Cu}_{Cu(\epsilon\text{-ATP})}$ were varied between 5.4–7.3 and 7.1–9.0, respectively.

Results and Discussion

Adenosine and its derivatives show a considerable tendency for self-association in aqueous solution;[23,32] the same has been observed for ε-adenosine,[11] $\epsilon\text{-AMP}^{2-}$,[21] and ε-ATP.[22] As the following studies by UV spectrophotometry and by potentiometric pH titrations (sections 1 and 2) are all aimed to learn something about the properties of monomeric ε-ATP systems, the experimental conditions for these studies have been chosen with the following reasoning: On the basis of recent results[22] it is not to be expected that in any of the metal ion containing $\epsilon\text{-ATP}^{4-}$ systems self-association would increase beyond that of ε-adenosine. For the latter compound in a 10^{-3} M solution about 98% of ε-adenosine exists in the monomeric form;[11] hence, to be on the safe side all experiments with ε-ATP/M^{2+} systems were carried out in solutions with [ε-ATP] < 0.5 mM.

(1) Acidity Constants of $H_2(\epsilon\text{-ATP})^{2-}$ and Sites of Protonation. The ultraviolet absorbance spectra of ε-ATP show a pronounced dependence on pH in the range from 2 to 7, the spectra being very similar to those obtained with ε-adenosine (see Figure 2 in ref 11). Figure 1 depicts the dependence of the UV absorption of ε-ATP at six different wavelengths on pH in the range from 2 to 7.2. A computer-calculated best fit of these experimental data gives the acidity constant, $pK^H_{H_2(\epsilon\text{-ATP})}$ = 4.41 ± 0.04 (I = 0.1, $NaClO_4$; 25 °C).

Potentiometric titrations (I = 0.1, $NaNO_3$; 25 °C) in the pH range 3.5–8.5 reveal two equilibria as shown in eq 1 and 2. The

$$H_2(\epsilon\text{-ATP})^{2-} \rightleftharpoons H(\epsilon\text{-ATP})^{3-} + H^+ \quad (1a)$$

$$K^H_{H_2(\epsilon\text{-ATP})} = [H(\epsilon\text{-ATP})^{3-}][H^+]/[H_2(\epsilon\text{-ATP})^{2-}] \quad (1b)$$

$$H(\epsilon\text{-ATP})^{3-} \rightleftharpoons \epsilon\text{-ATP}^{4-} + H^+ \quad (2a)$$

$$K^H_{H(\epsilon\text{-ATP})} = [\epsilon\text{-ATP}^{4-}][H^+]/[H(\epsilon\text{-ATP})^{3-}] \quad (2b)$$

corresponding acidity constants are $pK^H_{H_2(\epsilon\text{-ATP})}$ = 4.45 ± 0.02 and $pK^H_{H(\epsilon\text{-ATP})}$ = 6.50 ± 0.01 (I = 0.1, $NaNO_3$; 25 °C). The constant determined for the first buffer region at pH about 4.5 is in excellent agreement with the value from the spectrophotometric experiments. It is evident that $K^H_{H_2(\epsilon\text{-ATP})}$ is due to a deprotonation of the base moiety, while $K^H_{H(\epsilon\text{-ATP})}$ reflects the removal of a proton from the terminal γ-phosphate group of $H(\epsilon\text{-ATP})^{3-}$.

In a detailed study[17] it was shown by comparing H(ε-adenosine)$^+$ with the corresponding compounds methylated at N-6 or N-7 (see Chart I) that the predominant site of protonation is N-6, a conclusion in agreement with the crystal structure analysis of 10-ethyl-ε-adenosine hydrochloride.[33]

The 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 ($pK^H_{H_2(ATP)}$ = 4.01 ± 0.01).[34] The decreasing acidity within the series $H(\epsilon\text{-Ado})^+$ (see ref 11) > $H_2(\epsilon\text{-AMP})^{\pm}$ (see ref 21) > $H_2(\epsilon\text{-ATP})^{2-}$ is expected due to the variation of the charge of these species and paralleled by the corresponding series $H(Ado)^+ > H_2(AMP)^{\pm} > H_2(ATP)^{2-}$. Comparison with $pK^H_{H(ATP)}$ = 6.49 ± 0.01 (I = 0.1, $NaNO_3$; 25 °C)[34] indicates in addition that the basicity of the γ-phosphate group of ATP^{4-} is hardly affected by the modification of the base moiety.

(2) Stability Constants of ε-ATP and Some Related Nucleoside 5′-Triphosphate Complexes. Due to the scarcity of ε-ATP this study was restricted to some metal ions of biological interest. The experimental data of the potentiometric pH titrations for the ε-ATP systems with Mg^{2+}, Mn^{2+}, or Zn^{2+} show that the two complex equilibria shown in eq 3 and 4 occur.[35] The acidity

$$M^{2+} + H(\epsilon\text{-ATP})^{3-} \rightleftharpoons M(H\cdot\epsilon\text{-ATP})^- \quad (3a)$$

$$K^M_{M(H\cdot\epsilon\text{-ATP})} = [M(H\cdot\epsilon\text{-ATP})^-]/([M^{2+}][H(\epsilon\text{-ATP})^{3-}]) \quad (3b)$$

$$M^{2+} + \epsilon\text{-ATP}^{4-} \rightleftharpoons M(\epsilon\text{-ATP})^{2-} \quad (4a)$$

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

constant of the connected equilibrium 5 can be calculated with

$$M(H\cdot\epsilon\text{-ATP})^- \rightleftharpoons M(\epsilon\text{-ATP})^{2-} + H^+ \quad (5a)$$

$$K^H_{M(H\cdot\epsilon\text{-ATP})} = [M(\epsilon\text{-ATP})^{2-}][H^+]/[M(H\cdot\epsilon\text{-ATP})^-] \quad (5b)$$

eq 6. The constants for equilibria 3–5 are listed in Table I

(30) Anderegg, G. *Helv. Chim. Acta* **1963**, *46*, 2397–2410.
(31) Kaden, T. A., Scheller, K. H., Sigel, H. *Inorg. Chem.* **1986**, *25*, 1313.
(32) Scheller, K. H.; Sigel, H. *J. Am. Chem. Soc.* **1983**, *105*, 5891–5900.
(33) Wang, A. H.-J.; Dammann, L. G.; Barrio, J. R.; Paul, I. C. *J. Am. Chem. Soc.* **1974**, *96*, 1205–1213.
(34) Tribolet, R.; Malini-Balakrishnan, R.; Sigel, H. *J. Chem. Soc., Dalton Trans.* **1985**, 2291–2303.
(35) The situation with Cu^{2+}/ε-ATP is considerably more complicated; this system is therefore treated separately; see ref 31.

$$pK^{H}_{M(H\cdot\epsilon\text{-ATP})} = pK^{H}_{H(\epsilon\text{-ATP})} + \log K^{M}_{M(H\cdot\epsilon\text{-ATP})} - \log K^{M}_{M(\epsilon\text{-ATP})} \quad (6)$$

together with some related data.[36-38]

The stability constants determined by potentiometric pH titrations for the $M(NTP)^{2-}$ complexes with the divalent transition-metal ions and Zn^{2+} unequivocally increase for the series of ligands, pyrimidine nucleoside 5'-triphosphates ($PNTP^{4-}$) < ATP^{4-} < ϵ-ATP^{4-}. The stabilities of the corresponding Mg^{2+} (and Ca^{2+}) complexes are much more similar, and it is not certain that the indicated small differences are real.[39] However, the stability constants (see Table I) determined earlier[18] for $Zn(\epsilon\text{-ATP})^{2-}$ and $Cu(\epsilon\text{-ATP})^{2-}$ are by far too small and not reliable; this may be due to the presence of buffers in the corresponding experiments, the method used (fluorescence quenching), or both. Similarly, the one value given earlier[19] for $Co(\epsilon\text{-ATP})^{2-}$ is also certainly too low while the other[18] might be of the correct order, as are apparently the constants for the complexes[18] with Ca^{2+}, Mg^{2+}, and possibly also[19] Ni^{2+}. The stability constants determined for $Mn(\epsilon\text{-ATP})^{2-}$ agree within experimental error for all three studies. In any case, the present data determined by potentiometric pH titrations are certainly reliable.

In the lower pH range monoprotonated $M(H\cdot\epsilon\text{-ATP})^{-}$ complexes are also formed. Their stability (eq 3) and acidity constants (eq 5) are also given in Table I. A comparison of the values for $pK^{H}_{M(H\cdot\epsilon\text{-ATP})}$ with $pK^{H}_{H_2(\epsilon\text{-ATP})} = 4.45$ (section 1) indicates for the complexes with Mg^{2+} and Mn^{2+} that the proton must mainly be located at the γ-phosphate group, because these two complexes are less acidic than the proton at N-6 in uncomplexed $H_2(\epsilon\text{-ATP})^{2-}$.

For the Zn^{2+}/ϵ-ATP system UV spectra were recorded at various pH. In the upper part of Figure 2 the dependence of the change in absorption on pH is compared at three wavelengths with the alterations in the metal ion free ϵ-ATP system (dotted lines); the lower part of Figure 2 gives the distribution of complex species in dependence on pH under the same conditions. These distribution curves were calculated with the constants determined by potentiometric pH titration. Both parts of Figure 2 are well compatible with each other, but it is evident that $Zn(H\cdot\epsilon\text{-ATP})^{-}$ is only formed in low concentration. A careful comparison at pH 4.3 reveals that the percentage-wise difference in absorption between the free and complexed system (e.g., at 305 nm) closely corresponds to the degree of formation of $Zn(H\cdot\epsilon\text{-ATP})^{-}$, indicating again that the proton is mainly located at the terminal γ group. For this species a considerable percentage of ring back-binding must be expected because in the deprotonated $1,N^6$-ethenoadenine moiety there exists a significant affinity toward Zn^{2+} (see sections 3 and 4). Of course, this does not mean that the $Zn(H\cdot\epsilon\text{-ATP})^{-}$ isomer with the proton at N-6 does not exist at all; it only implies that the γ-protonated isomer dominates.

The acidity constants, $pK^{H}_{M(H\cdot ATP)}$, of the $M(H\cdot ATP)^{-}$ complexes are on the average about 0.4 log unit below the values determined for $M(H\cdot\epsilon\text{-ATP})^{-}$. This difference in acidity closely corresponds to the difference observed between $H_2(ATP)^{2-}$ and $H_2(\epsilon\text{-ATP})^{2-}$ (section 1), indicating that for the $M(H\cdot ATP)^{-}$ complexes[34] the isomer with the proton at the γ-phosphate group is also important.

(3) NMR Evidence for an Intramolecular Zn^{2+}/ϵ-Adenine Residue Interaction in Monomeric $Zn(\epsilon\text{-ATP})^{2-}$ Complexes. The results of section 2 show that the $M(\epsilon\text{-ATP})^{2-}$ complexes for Mn^{2+}, Cu^{2+}, and Zn^{2+} are more stable than expected from the coordination tendency of the triphosphate chain alone (Table I). The only source of this increased stability can be the ϵ-adenine moiety with the N-6/N-7 binding site. Its participation in complex formation will lead to *intra*molecular macrochelates. Such macrochelates involving the phosphate groups and the adenine moiety are already known for several adenine–nucleotide complexes,[21,32] including $M(ATP)^{2-}$.[23,25,34,37]

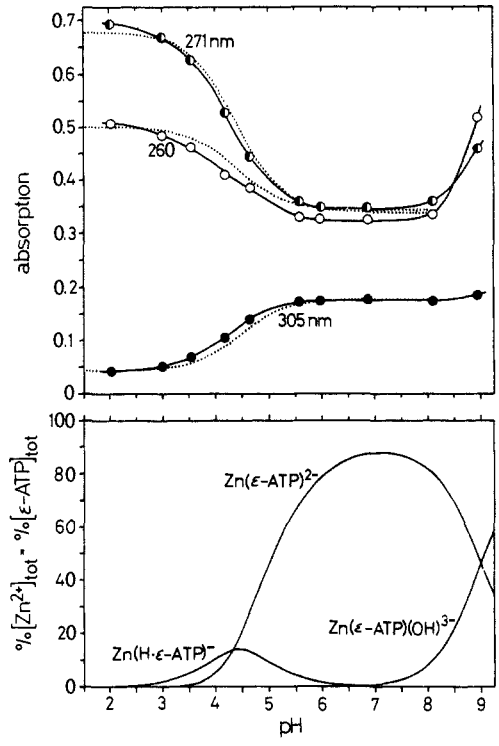
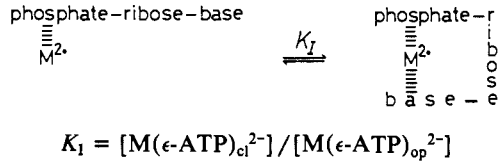


Figure 2. Comparison of the UV absorption of the Zn^{2+}/ϵ-ATP system with the formation of complex species in the same system. Upper part: Dependence of the UV absorption of Zn^{2+}/ϵ-ATP on pH in aqueous solution (measured in 2-mm cells with $[\epsilon\text{-ATP}] \simeq [Zn(ClO_4)_2] = 3.36 \times 10^{-4}$ M; $I = 0.1$ $NaClO_4$; 25 °C). The dotted curves represent the alteration of the absorption of ϵ-ATP alone in dependence on pH; these curves are taken from Figure 1. Lower part: Effect of pH on the concentration of the complexes present in an aqueous solution of $[Zn^{2+}] = [\epsilon\text{-ATP}] = 3.36 \times 10^{-4}$ M. The results were computed with the potentiometrically determined constants of section 1 and Table I; they are given as the percentage of the total Zn^{2+} (=total ϵ-ATP) present. In the lower pH range the formation of the diprotonated complex $Zn(H_2\cdot\epsilon\text{-ATP})$ with a proton each at N-6 and at the terminal γ-phosphate group seems possible, but its stability is not known.

Such an $1,N^6$-ethenoadenine interaction in $Zn(\epsilon\text{-ATP})^{2-}$ should also be reflected in the ^1H NMR shift positions. We have therefore used our recent NMR experiments on the Zn^{2+}/ϵ-ATP system[22] to extrapolate the chemical shifts of the protons neighboring the potential N-6/N-7 binding site, i.e., of H-8 and H-11 (see Chart I), to infinite dilution (δ_0); in this way we obtained the shifts for these protons in the monomeric $Zn(\epsilon\text{-ATP})^{2-}$ complex. These values are listed in the third column of Table II together with the corresponding shift values of δ_0 for $Mg(\epsilon\text{-ATP})^{2-}$ and uncomplexed ϵ-ATP^{4-}. The latter are similar, i.e., there is no indication for a significant ring back-binding in $Mg(\epsilon\text{-ATP})^{2-}$; this agrees with the low coordination tendency of Mg^{2+} toward the N-6/N-7 site in ϵ-adenosine[11] and with the observations described in section 2.

However, for H-8 of $Zn(\epsilon\text{-ATP})^{2-}$ there is a significant downfield shift. This is convincing evidence for an *intra*molecular base–metal ion interaction in this unstacked complex, and for an intramolecular (and therefore concentration-independent) equilibrium between an "open" isomer, $M(\epsilon\text{-ATP})_{op}^{2-}$, and a "closed" species, $M(\epsilon\text{-ATP})_{cl}^{2-}$:



(7)

$$K_I = [M(\epsilon\text{-ATP})_{cl}^{2-}]/[M(\epsilon\text{-ATP})_{op}^{2-}] \quad (8)$$

(36) Khan, M. M. T.; Martell, A. E. *J. Am. Chem. Soc.* **1966**, *88*, 668–671.
(37) Mariam, Y. H.; Martin, R. B. *Inorg. Chim. Acta* **1979**, *35*, 23–28.
(38) Sigel, H.; McCormick, D. B. *Acc. Chem. Res.* **1970**, *3*, 201–208.
(39) It was reasoned that the possibly small increases in stability of $Mg(ATP)^{2-}$ and $Ca(ATP)^{2-}$, compared with the corresponding $M(PNTP)^{2-}$, might be due to a water molecule bridging N-7 of the adenine moiety and the phosphate-coordinated metal ion (see the discussion on p 257 of ref 23), but for a clarification of this point certainly more experimental data are needed.

Table II. Evidence for a Zn^{2+}/ϵ-Adenine Moiety Interaction in the Monomeric $Zn(\epsilon\text{-ATP})^{2-}$ Complex from a Comparison of the Chemical Shifts (δ_0) (ppm) of H-8 and H-11 for Monomeric 1,N^6-Ethenoadenine Derivatives (D_2O; 27 °C; I = 0.1, $NaNO_3$)

	ϵ-ATP			downfield shift $\Delta\delta'$ for Zn^{2+}/ϵ-Ado[b]	est% $Zn(\epsilon\text{-ATP})_{cl}^{2-}$ (eq 16)
H	system	δ_0 of H-8, or H-11	downfield shift,[a] $\Delta\delta_0$		
H-8	ϵ-ATP^{4-}	8.658 ± 0.007[c]			
	$Mg(\epsilon\text{-ATP})^{2-}$	8.673 ± 0.009[c]			
	$Zn(\epsilon\text{-ATP})^{2-}$	8.87 ± 0.02[d]	0.20	0.15	(133)
H-11	ϵ-ATP^{4-}	7.628 ± 0.007[c]			
	$Mg(\epsilon\text{-ATP})^{2-}$	7.661 ± 0.010[c]			
	$Zn(\epsilon\text{-ATP})^{2-}$	7.68 ± 0.01[d]	~0.02	0.11	(~18)
av[e]			0.11	0.13	85

[a] Shift difference $\Delta\delta_0$ for H-8 or H-11 between $Zn(\epsilon\text{-ATP})^{2-}$ and $Mg(\epsilon\text{-ATP})^{2-}$. [b] Downfield shifts obtained upon complexation between ϵ-adenosine and Zn^{2+}; the values are taken from Table VI of ref 11. With these $\Delta\delta'$ values and $\Delta\delta_0$, the percentage of $Zn(\epsilon\text{-ATP})_{cl}^{2-}$ is calculated [% closed isomer = $(\Delta\delta_0/\Delta\delta') \times 100$]; this then allows also the calculation of the intramolecular equilibrium constant K_I (eq 8). [c] Value from Table S1 of ref 22. [d] Graphically extrapolated (see Figure S2 in ref 22); the error is estimated. The given value agrees well with the average of the corresponding values given in Tables S1 and S2 of ref 22. [e] See discussion in the text (section 3).

Table III. Extent of Intramolecular Macrochelate Formation in Metal Ion Complexes of ϵ-ATP^{4-}: Estimations of the Intramolecular Dimensionless Equilibrium Constant K_I and of the Percentage of the Closed Isomer $M(\epsilon\text{-ATP})_{cl}^{2-}$ [Percentages of $M(\epsilon\text{-AMP})_{cl}$ and $M(ATP)_{cl}^{2-}$ Given for Comparison] (I = 0.1; 25 °C)

$M(\epsilon\text{-ATP})^{2-}$	log $K^M_{M(\epsilon\text{-ATP})}$ (eq 4)[a]	log $K^M_{M(\epsilon\text{-ATP})op}$[b]	log Δ (eq 10)	K_I (eq 8 and 9)	% $M(\epsilon\text{-ATP})_{cl}^{2-}$ (eq 7)	% $M(\epsilon\text{-AMP})_{cl}$ (eq 7)[c]	% $M(ATP)_{cl}^{2-}$ (eq 7)[d]
$Ca(\epsilon\text{-ATP})^{2-}$	3.74	3.73	~0	~0[e]	~0[e]		~0[e]
$Mg(\epsilon\text{-ATP})^{2-}$	4.24	4.10	~0.1	~0[e]	~0[e]	~0[e]	~0[e]
$Mn(\epsilon\text{-ATP})^{2-}$	5.10	4.68	0.42	1.6	62	69	38
$Co(\epsilon\text{-ATP})^{2-}$	~5.1	4.58	~0.5	~2	~70	~97	57
$Ni(\epsilon\text{-ATP})^{2-}$	~5.8	4.34	~1.5	~30	~97	~99	74
$Cu(\epsilon\text{-ATP})^{2-}$	>8	5.67	>2.3	>200	>99.5	99.9	76/68
$Zn(\epsilon\text{-ATP})^{2-}$	5.44	4.82	0.62	3.2	76	92	62

[a] Values from Table I. [b] The stability constants, $K^M_{M(PNTP)}$ (see Table I), of the pyrimidine nucleoside 5′-triphosphate (PNTP) complexes were used as a basis for the stability of $M(\epsilon\text{-ATP})^{2-}_{op}$ (because the pyrimidine residue does not participate in complex formation)[23,32,34,37] after correcting for the small differences in basicity between the ligands; i.e., log $K^M_{M(\epsilon\text{-ATP})op}$ = log $K^M_{M(PNTP)}$ + 0.05 (due to $pK^H_{H(\epsilon\text{-ATP})} - pK^H_{H(PNTP)} = 6.50 - 6.45 = 0.05$). [c] From ref 21. [d] From ref 23; the second value given for $Cu(ATP)^{2-}$ from ref 34. [e] See text in section 4 and ref 39.

One may attempt to estimate the position of intramolecular equilibrium 7 and hence to calculate a value for the dimensionless equilibrium constant K_I (eq 8) by comparing the downfield shifts, $\Delta\delta_0$, for H-8 and H-11 of $Zn(\epsilon\text{-ATP})^{2-}$ (Table II, column 4) with the corresponding shift values, $\Delta\delta'$, obtained for complete complexation of Zn^{2+} at the N-6/N-7 site of ϵ-adenosine (column 5). It is evident that the downfield shift, $\Delta\delta_0$, of H-8 is larger for $Zn(\epsilon\text{-ATP})^{2-}$ than for $Zn(\epsilon\text{-Ado})^{2+}$, which would indicate more than 100% ring back-binding in $Zn(\epsilon\text{-ATP})^{2-}$. On the other hand, $\Delta\delta_0$ for H-11 of $Zn(\epsilon\text{-ATP})^{2-}$ hardly indicates a downfield shift, while $\Delta\delta'$ for $Zn(\epsilon\text{-Ado})^{2+}$ is significant.

Clearly, the described observations can only mean that the coordination of Zn^{2+} to the N-6/N-7 site in $Zn(\epsilon\text{-ATP})^{2-}$ is somewhat different from that in $Zn(\epsilon\text{-Ado})^{2+}$. This result is understandable because Zn^{2+} in $Zn(\epsilon\text{-ATP})^{2-}$ is strongly coordinated to the triphosphate chain; due to this phosphate-binding its "mobility" is somewhat restricted and an optimal adjustment to the relatively wide N-6/N-7 site,[40] as is possible in $Zn(\epsilon\text{-Ado})^{2+}$, cannot be reached. It appears from the chemical shifts that the Zn^{2+} in $Zn(\epsilon\text{-ATP})^{2-}$ interacts with the ϵ-adenine moiety mainly at N-7. Hence, to a first approximation one may assume that weakening of the one N interaction strengthens the other; therefore, the downfield shifts given in Table II for H-8 and H-11 were averaged, and the percentage of the closed isomer was estimated on this basis. It is gratifying to note that the resulting 85% for $Zn(\epsilon\text{-ATP})_{cl}^{2-}$ is in excellent accord with the 76% calculated from the stability data obtained by potentiometric pH titrations (see section 4). However, it should be emphasized that the crucial result of this section is not so much the estimate of the percentage of $Zn(\epsilon\text{-ATP})_{cl}^{2-}$ but the clear proof through the measured downfield shifts that the macrochelated form of $Zn(\epsilon\text{-ATP})^{2-}$ does indeed exist.

(4) Comparison of the Extent of Macrochelate Formation in $M(\epsilon\text{-ATP})^{2-}$ and $M(ATP)^{2-}$ Complexes. The results described above provide a solid basis for a general estimation of the position of equilibrium 7 for $M(\epsilon\text{-ATP})^{2-}$ complexes from stability data. The intramolecular equilibrium constant K_I (eq 8) can be deduced[23,37,41,42] from the experimentally accessible overall stability constant, $K^M_{M(\epsilon\text{-ATP})}$ (eq 4), by using eq 9. $K^M_{M(\epsilon\text{-ATP})_{op}}$ is the

$$K_I = \left(K^M_{M(\epsilon\text{-ATP})}/K^M_{M(\epsilon\text{-ATP})_{op}}\right) - 1 \qquad (9)$$

stability constant of the open isomer $M(\epsilon\text{-ATP})_{op}^{2-}$ (eq 7), a constant not directly accessible by experimental determinations. However, in the present case the value is well represented by the averaged stability constants $K^M_{M(PNTP)}$ (Table I) determined for the $M(NTP)^{2-}$ complexes of pyrimidine nucleoside 5′-triphosphates, because the pyrimidine residue does not participate in complex formation;[23,32,34,37] i.e., these complexes exist only in the open form (see footnote *b* of Table III).

The reliability of the calculated value for K_I (eq 9) depends on the accuracy of the logarithms of the ratio (eq 10), and this

$$\log \Delta = \log K^M_{M(\epsilon\text{-ATP})} - \log K^M_{M(\epsilon\text{-ATP})_{op}} \qquad (10)$$

accuracy depends very much on the experimental error in the constants, which becomes more important the more similar the two constants in eq 10 are. Fortunately, in the decisive cases log Δ is greater than 0.4 log unit; i.e., reasonable estimates for the intramolecular equilibrium constant K_I (eq 7–9) and the percentage of the closed isomer $M(\epsilon\text{-ATP})_{cl}^{2-}$ may be expected. The results are given in Table III, together with the data on which they are based.

The results in Table III confirm the conclusions from section 3: there is no or only a very weak metal ion–base interaction in

(40) (a) The fact that the $M(\epsilon\text{-Ado})^{2+}$ complexes[11] are not as stable as the corresponding $M(1,10\text{-phenanthroline})^{2+}$ complexes[30] is probably due to the larger distance between N-6 and N-7 in ϵ-adenosine compared with that of N-1 and N-10 in 1,10-phenanthroline. This is evident from the angles N-6,C-6,C-5 and C-6,C-5,N-7 in ϵ-adenosine (see Chart I), which are 135.6° and 131.6°,[33] while the corresponding angles in phenanthroline are close to 120° (i.e., between 116.5° and 119.7°).[40b] (b) Nishigaki, S.; Yoshioka, H.; Nakutsu, K. *Acta Crystallogr., Sect. B* **1978**, *B34*, 875–879.

(41) Sigel, H.; Scheller, K. H.; Rheinberger, V. M.; Fischer, B. E. *J. Chem. Soc., Dalton Trans.* **1980**, 1022–1028.

(42) Sigel, H. *Angew. Chem.* **1982**, *94*, 421–432; *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 389–400.

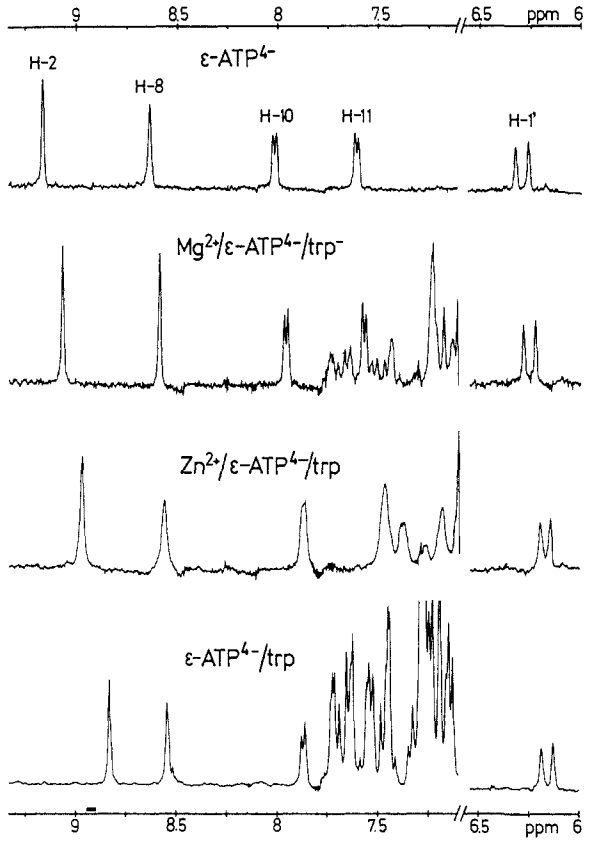


Figure 3. ^{1}H NMR spectra of L-tryptophan/ϵ-ATP systems: ϵ-ATP^{4-} (0.01 M) at pD 8.3; Mg^{2+}, ϵ-ATP^{4-}, and Trp (each 0.01 M) at pD 10.5; Zn^{2+}, ϵ-ATP^{4-}, and Trp (each 0.01 M) at pD 9.1; ϵ-ATP^{4-} (0.01 M) with a *5-fold* excess of Trp (0.05 M) at pD 8.3. The spectra were measured relative to $(CH_3)_4N^+NO_3^-$ (0.002 M) and converted to ppm relative to sodium 3-(trimethylsilyl)propanesulfonate by adding 3.188 ppm (90.025 MHz; 27 °C; I = 0.1 M, $NaNO_3$; D_2O).

$Mg(\epsilon\text{-ATP})^{2-}$ and $Ca(\epsilon\text{-ATP})^{2-}$,[39] but this interaction is very pronounced for the $M(\epsilon\text{-ATP})^{2-}$ complexes containing Zn^{2+} or one of the divalent transition elements. Most importantly, the extent of macrochelate formation is significantly larger in the ϵ-ATP^{4-} complexes than in the parent ATP^{4-} complexes, an observation in agreement with the larger metal ion affinity of ϵ-adenosine[11] compared with that of adenosine. The extent of macrochelate formation in $M(\epsilon\text{-ATP})^{2-}$ and M(ϵ-AMP) complexes is comparable to a first approximation, and this is a hint that the closed isomer is even more important in the corresponding M(ϵ-ADP)$^-$ complexes, because for the adenine nucleotides the extent of macrochelate formation also depends for all studied metal ions on the number of phosphate groups,[21] i.e., % $M(AMP)_{cl}$ < % $M(ADP)_{cl}^-$ > % $M(ATP)_{cl}^{2-}$.

(5) Intramolecular Stacking in Mixed-Ligand Complexes Containing NTP^{4-} and 2,2'-Bipyridyl. The stability of complexes like $Cu(bpy)(NTP)^{2-}$ may be quantified by determining the position of equilibrium 11. The corresponding equilibrium

$$Cu(bpy)^{2+} + Cu(NTP)^{2-} \rightleftharpoons Cu(bpy)(NTP)^{2-} + Cu^{2+} \quad (11)$$

constant $10^{\Delta \log K}$ is calculated[42-44] with eq 12. Hence, the stability

$$\Delta \log K = \log K^{Cu(bpy)}{}_{Cu(bpy)(NTP)} - \log K^{Cu}{}_{Cu(NTP)} \quad (12)$$

constant of the binary complex $K^{Cu}{}_{Cu(NTP)}$ (eq 4) has to be determined as well as the constant according to equilibrium 13.

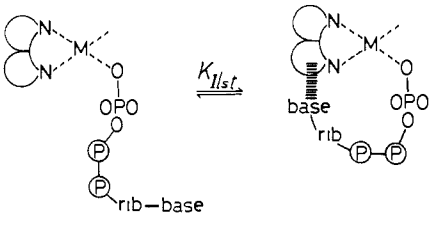
$$Cu(bpy)^{2+} + NTP^{4-} \rightleftharpoons Cu(bpy)(NTP)^{2-} \quad (13a)$$

$$K^{Cu(bpy)}{}_{Cu(bpy)(NTP)} = [Cu(bpy)(NTP)^{2-}]/([Cu(bpy)^{2+}][NTP^{4-}]) \quad (13b)$$

For the ternary pyrimidine nucleoside 5'-triphosphate (PNTP) complexes, it follows (Table I) that $\Delta \log K_{Cu/bpy/PNTP} = 6.0 - 5.62 = +0.4$; indeed, the values of $\Delta \log K$ are identical within experimental error for the complexes with CTP^{4-}, UTP^{4-}, and TTP^{4-} (see Table 5 of ref 24). This then means that equilibrium 11 is displaced to its right, which is against any statistical expectation.[43,44] For Cu^{2+} systems $\Delta \log K_{Cu/statist}$ was estimated as −0.9 log unit, assuming a square-planar coordination sphere and a bidentate coordination of the ligands.[43]

Previous experience[43,44] shows that two cooperative effects are responsible for this overall increase in stability of about 1.3 log units: (i) π-accepting heteroaromatic N bases like 2,2'-bipyridyl favor, if coordinated to a transition-metal ion, the coordination of O-donor ligands like phosphates; (ii) intramolecular aromatic-ring stacking also enhances the stability of ternary complexes. That stacking interactions occur between the pyrimidine moiety and 2,2'-bipyridyl has been shown by spectrophotometry and ^{1}H NMR shift measurements for the binary adducts (uridine)(bpy) and $(UTP)(bpy)^{4-}$ as well as for several ternary $M(bpy)(UTP)^{2-}$ and $M(bpy)(CTP)^{2-}$ complexes.[25,45]

For ternary complexes containing Cu^{2+}, 2,2'-bipyridyl, and a second ligand with O-donor atoms, generally *positive* values of about +0.1 log unit are observed for $\Delta \log K$. Hence, point (i) gives a total stability increase of about 1.0 log unit (=0.9 + 0.1), leaving for point (ii), the intramolecular stacking interaction, the remaining 0.3 log unit (=1.3 − 1.0). By using the equations published earlier,[42,46,47] one calculates that, in the intramolecular equilibrium 14, about 50% of the ternary $Cu(bpy)(NTP)^{2-}$ com-



(14)

plexes formed with the pyrimidine nucleoside 5'-triphosphates exists as the stacked isomer (i.e., $K_{1/st} \simeq 1$). This estimate comes close to the 40% estimated by ^{1}H NMR shift measurements[48] for the closed isomer of $Zn(bpy)(UTP)^{2-}$, as well as to the 55% estimated recently[34] in a different way for $Cu(bpy)(UTP)^{2-}$.

The intramolecular stack in $Cu(bpy)(ATP)^{2-}$ has been proven already in 1974.[49] As purines stack much better than pyrimidines,[32,34] a larger percentage of the stacked isomer is expected for $Cu(bpy)(ATP)^{2-}$, and for $Cu(bpy)(\epsilon\text{-ATP})^{2-}$ as well, despite the connected release of the base moieties from the coordination sphere of Cu^{2+} (section 4) by the formation of the ternary complexes. Indeed, $Cu(bpy)(ATP)^{2-}$ stacks to about 85% (eq 14; $K_{1/st} \simeq 5$)[50] and $Cu(bpy)(\epsilon\text{-ATP})^{2-}$ to about 90% (eq 14; $K_{1/st} \simeq 10$).[51,52]

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(45) Fukuda, Y.; Mitchell, P. R.; Sigel, H. *Helv. Chim. Acta* **1978**, *61*, 638–647.
(46) Fischer, B. E.; Sigel, H. *J. Am. Chem. Soc.* **1980**, *102*, 2998–3008.
(47) The above 0.3 log unit corresponds in these equations to $\Delta\Delta \log K_M = \Delta \log K_{M/exp} - \Delta \log K_{M/op}$, and the percentage of the stacked isomer is calculated via $K_{1/st} = 10^{\Delta\Delta \log K_M} - 1$.
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(50) With the reasoning outlined above for $Cu(bpy)(PNTP)^{2-}$ and taking into account the release of the adenine moiety from Cu^{2+} in $Cu(ATP)^{2-}$ (log Δ = 0.70; Table I), it holds for the stacking part = ΔΔ log K_M (ref 47) = Δ log K (eq 12; Table I) − statistical value + log Δ − π stabilization (point i) = +0.2−(−0.9) + 0.7 − 1.0 = +0.8; hence, $Cu(bpy)(ATP)_{st}^{2-}$ occurs to about 85%. This estimate is in excellent agreement with a recent calculation[34] that gave 86 ± 3%.
(51) In contrast to $Cu(bpy)(PNMP)^{2-}$ and $Cu(bpy)(ATP)^{2-}$, $Cu(bpy)(\epsilon\text{-ATP})^{2-}$ is considerably less stable than its binary parent complex $Cu(\epsilon\text{-ATP})^{2-}$; i.e., $\Delta \log K_{Cu/bpy/\epsilon\text{-ATP}} < -1.2$ (eq 12; Table I). This is due to the high affinity of the ϵ-adenine moiety toward Cu^{2+} in $Cu(\epsilon\text{-ATP})^{2-}$,[31] but this does not mean that no stacking interaction in $Cu(bpy)(\epsilon\text{-ATP})^{2-}$ occurs.[52]
(52) An analogy to ref 50 holds for the Cu^{2+}/bpy/ϵ-ATP system: stacking part = ΔΔ log K_M = (6.83–9.0; Table I and ref 31) − (−0.9) + (9.0–5.67; ref 31 and Table III) − 1.0 = +1.1. An evaluation of this system by using the stability of $Cu(ATP)^{2-}$ as a basis gives the same result.

Table IV. Evidence for an Intramolecular Purine–Indole Stack in Ternary Complexes Consisting of Mg^{2+} or Zn^{2+}, ε-ATP^{4-} or ATP^{4-}, and L-Tryptophanate:[a] Calculated Upfield Shifts for Complete Formation[b] of the Ternary Complexes, $\Delta\delta^*_{complex}$ (ppm), Approximate Percentages of the Stacked Isomers (eq 15), and Estimates for the Intramolecular Equilibrium Constants, $K_{I/st}$ (eq 16), in D_2O (I = 0.1, $NaNO_3$; 27 °C)

complex	proton	$\delta_{0/NTP}$[c]	$\Delta\delta^*_{complex}$[b]	$\Delta\delta^*_{Trp}$[d]	% $M(NTP)(Trp)_{st}^{3-}$ (eq 15)[e]	$K_{I/st}$ (eq 16)
$Mg(\epsilon\text{-ATP})(Trp)^{3-}$	H-2	9.193	0.533	1.30	41.0	
	H-8	8.658	0.286	0.38	75.3	
	H-10	8.044	0.346	0.59	58.6	
	H-1′	6.308	0.224	0.45	49.8	
	av		0.347	0.68	*51 ± 11*[f]	1.0
$Zn(\epsilon\text{-ATP})(Trp)^{3-}$	H-2	9.193	0.296	1.30	22.8	
	H-8	8.658	0.133	0.38	35.0	
	H-10	8.044	0.225	0.59	38.1	
	H-1′	6.308	0.179	0.45	39.8	
	av		0.208	0.68	*31 ± 6*[f]	0.45
$Mg(ATP)(Trp)^{3-}$	H-2	8.278	0.199	0.60	33.2	
	H-8	8.563	0.252	0.35	72.0	
	H-1′	6.163	0.131	0.37	35.4	
	av		0.194	0.44	*44 ± 19*[f]	0.8
$Zn(ATP)(Trp)^{3-}$	H-2	8.278	0.184	0.60	30.7	
	H-8	8.563	0.220	0.35	62.9	
	H-1′	6.163	0.124	0.37	33.5	
	av		0.176	0.44	*40 ± 15*[f]	0.7

[a] The experimental details for the ε-ATP systems are given in the legend to Figure 3; those of the ATP systems are very similar, and the details are given in Table 2 and Figure 2 of ref 48. The data of the ATP systems have now been reevaluated for reasons of comparison (i.e., the self-association of ATP has also been taken into account); however, the present results differ only slightly from those given earlier.[48] All the above entries are the averages of two independent experiments. [b] The formation degree of the ternary complexes $M(NTP)(Trp)^{3-}$ under the experimental conditions (Figure 3)[a] was calculated by taking into account the following species: $H_2(NTP)^{2-}$, $H(NTP)^{3-}$, NTP^{4-} (section 1), $H_2(Trp)^+$, H(Trp), Trp^-,[55] M^{2+}, $M(H{\cdot}NTP)^-$, $M(NTP)^{2-}$ (Table I), $Zn(NTP)(OH)^{3-}$ (Table I and ref 59), $M(Trp)^+$, $M(Trp)_2$,[48,55] $M(H{\cdot}NTP)(Trp)^{2-}$, and $M(NTP)(Trp)^{3-}$;[48,55] log $K^{Zn(\epsilon\text{-ATP})}{}_{Zn(\epsilon\text{-ATP})(Trp)} \simeq 4.5$, and log $K^{Mg(\epsilon\text{-ATP})}{}_{Mg(\epsilon\text{-ATP})(Trp)} \simeq 1.8$. The sources of the constants needed for the calculations are always given after the corresponding species; some of the constants were estimated as described in ref 48. As the formation degree of the ternary Zn^{2+} complexes is high (~80%), the calculations are affected in these cases very little by possible errors in the stability constants; however, the formation degree of the ternary Mg^{2+} complexes is lower (~25%), the chemical shift differences are smaller (see Figure 3), and therefore somewhat larger errors must be expected. [c] The chemical shifts of monomeric ε-ATP^{4-} and ATP^{4-} are from ref 22 and 23, respectively. [d] Upfield shifts resulting from the binary tryptophan adduct formation with ε-adenosine (from Table VII of ref 11) and ATP^{4-} (slightly altered, reevaluated data from table 2 of ref 48). [e] $(\Delta\delta^*_{complex}/\Delta\delta^*_{Trp}) \times 100$. [f] This value is considered as the best estimate (see text); the above error limit is estimated.

(6) Evidence for Intramolecular Stack Formation in Ternary Complexes Composed of ε-ATP^{4-} or ATP^{4-}, L-Tryptophanate, and Mg^{2+} or Zn^{2+}. The results of section 5 indicate participation of the ε-adenine moiety of ε-ATP in stacking interactions with other aromatic-ring systems just like the adenine moiety of ATP. As the recognition interactions[9] between nucleotides/nucleic acids and amino acids/proteins concern an essential relationship between these two classes of important ligands, it seemed desirable to study a system containing an amino acid. We have chosen tryptophanate for two reasons: (i) 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+};[53] in fact, many such purine–indole interactions are expected[54] to occur in nature. (ii) For several $M(ATP)(Trp)^{3-}$ complexes the formation of such stacks has been proven directly by spectrophotometric and ^1H NMR shift measurements and indirectly by the enhanced stability of the complexes as determined by potentiometric pH titrations,[55] and these results have been repeatedly confirmed by independent studies.[48,56]

Indeed, the ^1H NMR spectra in Figure 3 reveal immediately stacking 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. It should be noted that for the bottom spectrum in Figure 3 a 5-fold excess of tryptophan was employed, while in the two spectra shown in the middle part of the figure the ratio of all reactants is 1:1:1; hence, roughly speaking, the upfield shifts seen in the bottom spectrum should be divided by 5 if compared with the other spectra. That the formation of a metal ion bridge between aromatic-ring systems facilitates stacking considerably, especially in diluted solutions, has been shown and discussed in a different connection.[34,57]

It is important to note that the upfield shifts of the bottom spectrum in Figure 3 correspond closely to those observed for the ε-adenosine/Trp system;[11] the same is true for two further spectra (not shown) of ε-ATP/Trp systems measured at different concentrations. From these experimental data and the upfield shifts determined earlier[11] for the complete formation of the (ε-adenosine)(Trp) adduct, the stability constant for $(\epsilon\text{-ATP})(Trp)^{4-}$ was calculated: $K^{\epsilon\text{-ATP}}{}_{(\epsilon\text{-ATP})(Trp)} = 7.5 \pm 0.8\ M^{-1}$. This association constant is within experimental error identical with $K^{ATP}{}_{(ATP)(Trp)} = 6.2 \pm 1.2\ M^{-1}$ as determined[48] for $(ATP)(Trp)^{4-}$ and $K^{\epsilon\text{-Ado}}{}_{(\epsilon\text{-Ado})(Trp)} = 6.0 \pm 1.1\ M^{-1}$ for (ε-adenosine)(Trp).[11,58]

In the middle part of Figure 3 the spectra recorded for the ternary system with ε-ATP^{4-}, Trp, and Mg^{2+} or Zn^{2+} are shown. From the upfield shifts and the computed formation degree of the corresponding $Mg(\epsilon\text{-ATP})(Trp)^{3-}$ and $Zn(\epsilon\text{-ATP})(Trp)^{3-}$ 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. These shifts, together with those calculated[11] for the binary stacked adduct (ε-adenosine)(Trp), are listed in Table IV; the corresponding data for the ternary $Mg(ATP)(Trp)^{3-}$ and $Zn(ATP)(Trp)^{3-}$ complexes are also given (see footnote *a* in Table IV).

From the upfield shifts listed in Table IV it is again evident that stacking occurs within the ternary complexes, but from the considerations in section 5 it is also clear that an intramolecular

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(58) The error limits given with these constants are *twice* the standard deviation. A possible stabilizing effect of an ionic bridge between the positively charged ammonium group of $Trp^{\pm}$ and the negative phosphate residue of ε-ATP^{4-} (or ATP^{4-}) is within these error limits. Cf. also: Orenberg, J. B.; Fischer, B. E.; Sigel, H. *J. Inorg. Nucl. Chem.* **1980**, *42*, 785–792.

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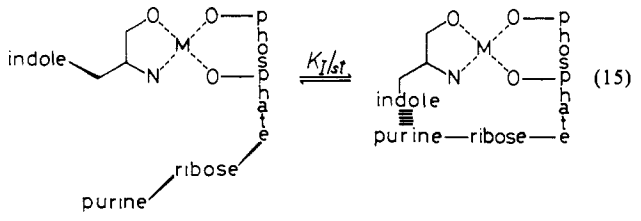
equilibrium exists between an open and a stacked form, i.e., between the two isomers indicated in equilibrium 15.



(15)

(7) Extent of Intramolecular Stacking in the M(ε-ATP)(Trp)$^{3-}$ and M(ATP)(Trp)$^{3-}$ Complexes with Mg^{2+} or Zn^{2+}. If the two isomers in equilibrium 15 are designated as $M(NTP)(Trp)_{op}^{3-}$ and $M(NTP)(Trp)_{st}^{3-}$, the constant of this intramolecular and therefore concentration-independent equilibrium is defined by eq 16.

$$K_{I/st} = [M(NTP)(Trp)_{st}^{3-}]/[M(NTP)(Trp)_{op}^{3-}] \quad (16)$$

A comparison of the relative shifts calculated for the several protons in the ternary complexes with those in the corresponding binary adducts should allow an estimation of the percentage of the stacked isomer occurring in equilibrium 15 and hence also of a value for $K_{I/st}$ (eq 16). These calculations are listed in the two right-side columns of Table IV; the percentages calculated for $M(NTP)(Trp)_{st}^{3-}$ from the individual protons differ considerably, and especially for H-2 and H-8 the differences are remarkable.

This result is actually not surprising because in the binary adduct the two aromatic moieties are free to interact in the orientation that results in the highest stability of the stack. This flexibility is considerably reduced once these two ligands are bridged by coordination to a metal ion, and thus the orientation of the two aromatic moieties forming the stack is expected to be somewhat different in the ternary complexes: this affects the chemical shifts of the protons. However, it seems plausible that a larger influence of the ring current of the indole moiety on, e.g., H-2 is accompanied by a smaller influence on H-8, and vice versa as the two rings in the stack are moved over each other or rotated; the *average* of the upfield shifts should therefore be less sensitive to different orientations of the stack in the binary adduct and in the ternary complex. Indeed, the percentages of the stacked isomer of the ternary complexes calculated with this average shift are for all four cases listed in Table IV relatively close to the results obtained with H-1′. This is very reasonable because the ribose proton H-1′ is not directly bound to the stacked purine system; hence, one expects that it is less influenced by different orientations of the aromatic rings in the stacks. We believe that the estimates given in Table IV for the percentage (printed in italics) of $M(NTP)(Trp)_{st}^{3-}$ and $K_{I/st}$ offer a good description of the true situation.

For all four ternary complexes, independent of the bridging metal ion, be it Mg^{2+} or Zn^{2+}, and also independent of the 1,N^6-etheno substituent, the percentage of the closed isomer (eq 15) 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+} and Zn^{2+} in their respective complexes, are quite alike. It should further be noted that, in the binary adducts, (ε-Ado)(Trp), (ε-ATP)(Trp)$^{4-}$, and (ATP)(Trp)$^{4-}$, H-2 is shifted most, while in all ternary $M(NTP)(Trp)_{st}^{3-}$ complexes H-8 is more strongly influenced; this indicates that the structures of the purine–indole stacks with ε-ATP^{4-} and ATP^{4-} are in each respective case very similar.

Conclusions

We can ask what we learned by comparing the properties of ε-ATP and ATP, which are the points one has to be aware of if one replaces in an enzymic study ATP by ε-ATP, and can ε-ATP be considered as a useful probe.

The answers to these questions are unfortunately not simple and cannot always be given with a clear "yes" or "no". Much will depend on the experimental conditions employed. The tendency for self-stacking is comparable for both nucleotides[60] as long as metal ions are absent and the coordination properties toward Mg^{2+}, and most probably also Ca^{2+}, are in *dilute* solutions quite alike (sections 2 and 4).

The formation of macrochelates, i.e., the simultaneous coordination of a metal ion to the phosphate chain and the base moiety, occurs for Zn^{2+} and the divalent transition-metal ions with both ε-ATP^{4-} and ATP^{4-} (section 4), as well as with ε-AMP^{2-} and AMP^{2-}.[21] The percentage of the macrochelated isomer (cl) follows for the adenine nucleotides the series[21] $M(AMP)_{cl} < M(ADP)_{cl}^{-} > M(ATP)_{cl}^{2-}$; the same order is to be expected for the ε-adenine derivatives because the percentages of the closed isomers for a given metal ion are % $M(AMP)_{cl} \simeq$ % $M(ATP)_{cl}^{2-}$ (section 4). This might appear as comforting, and for example the difference between about 38% $Mn(ATP)_{cl}^{2-}$ and 62% $Mn(\epsilon\text{-ATP})_{cl}^{2-}$ may at first sight not seem very significant; but one must be aware that this difference corresponds to 1.2 kJ/mol. If macrochelation is more pronounced, the energy differences become even more significant: 90% vs. 99% macrochelated isomers means a difference in $\Delta G°$ of 5.7 kJ/mol—and this difference might well mean inhibition of an enzymic reaction.

In mixed-ligand complexes formed by Mg^{2+} or Zn^{2+}, ε-ATP^{4-} or ATP^{4-}, and L-tryptophanate, intramolecular purine–indole stacks result (sections 6 and 7). The extent of this intramolecular stacking is comparable, indicating that in this respect the two nucleotides are interchangeable (see also section 5) as long as the larger size of the ε-adenine moiety does not give rise to steric restrictions, e.g., at the surface of a protein. At the same time one has to be aware, however, that the stacked ε-adenine moiety still has the N-6/N-7 site available for metal ion coordination; ternary complexes in which the metal ion is coordinated only to one ligand while both ligands are kept together by stacking are well-known.[11,43,45,61] To give just one example what this could mean: The affinity of the N-6/N-7 site of the ε-adenine moiety toward Zn^{2+} in the physiological pH region is larger than that of a carboxylate group [log $K^{Zn}_{Zn(\epsilon\text{-Ado})}$ = 1.5 (ref 11) > log $K^{Zn}_{Zn(RCOO)} \simeq$ 1.1 (see Table III in ref 62)]; hence, Zn^{2+} might change its site of coordination. There is no such problem with the adenine residue (log $K^{Zn}_{Zn(Ado)} \simeq -0.3$),[11,23] despite the similarity of both base residues in the affinity toward the proton (section 1).

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Registry No. ε-ATP, 37482-17-0; ATP, 56-65-5; $Mg(\epsilon\text{-ATP})^{2-}$, 70824-95-2; $Mn(\epsilon\text{-ATP})^{2-}$, 72786-22-2; $Zn(\epsilon\text{-ATP})^{2-}$, 101696-42-8; $Ca(\epsilon\text{-ATP})^{2-}$, 101696-43-9; $Co(\epsilon\text{-ATP})^{2-}$, 101696-44-0; $Ni(\epsilon\text{-ATP})^{2-}$, 101696-45-1.

(60) However, it should be noted that this is different in the presence of metal ions:[22] Mg^{2+} is promoting the self-association of ε-ATP^{4-} about twice as much as that of ATP^{4-}. On the other hand, Zn^{2+} promotes the self-association of ATP^{4-} much more strongly than Mg^{2+}, while its influence on ε-ATP^{4-} is even less than that of Mg^{2+}.

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