Acknowledgment. We thank the Fonds der Chemischen Industrie for financial support of this work.

Registry No. L'Tl(PF_6), 101165-02-0; L'Tl(ClO_4), 101165-03-1; $LTlCl_3$, 101165-04-2; L'$TlCl_3$, 101165-05-3; $LTlBr_3$, 101165-06-4; $LTlI_3$, 101165-07-5; L'TlI_3, 101165-08-6; $[L_2Tl](ClO_4)_3$, 101165-10-0; CoL_2^{2+}, 91760-59-7.

Supplementary Material Available: Tables of anisotropic thermal parameters, calculated positional parameters of H atoms, and bond angles (3 pages). Ordering information is given on any current masthead page. According to policy instituted Jan 1, 1986, the tables of calculated and observed structure factors are being retained in the editorial office for a period of 1 year following the appearance of this work in print. Inquiries for copies of these materials should be directed to the Editor.

Contribution from the Institute of Inorganic Chemistry, University of Basel, CH-4056 Basel, Switzerland

Complex Formation between Cu^{2+} and 1,N^6-Ethenoadenosine 5'-Triphosphate (ε-ATP)

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By potentiometric pH titrations and UV spectrophotometric measurements the complexes formed in the Cu^{2+}/ε-ATP 1:1 system are characterized in the pH range 3.4–8. The main species occurring are Cu(H·ε-ATP)$^-$, Cu(ε-ATP)$^{2-}$, and $Cu_2H(ε\text{-ATP})_2^{3-}$; this contrasts with the Cu^{2+}/ATP system where Cu(H·ATP)$^-$ and Cu(ATP)$^{2-}$ are dominating. Due to the intense back-binding to the N-6/N-7 site of the 1,N^6-ethenoadenine residue, the stability of Cu(ε-ATP)$^{2-}$ is much larger (log $K^{Cu}_{Cu(ε\text{-ATP})} = 9 \pm 1$) than that of Cu(ATP)$^{2-}$ (log $K^{Cu}_{Cu(ATP)} = 6.32 \pm 0.04$). Consequently, the extent of macrochelate formation (i.e., simultaneous coordination of Cu^{2+} to the phosphate chain and the base residue) reaches with Cu(ε-ATP)$^{2-}$ more than 99.5%, while for Cu(ATP)$^{2-}$ this "closed" species involving N-7 occurs only to about 70%. As a result of these differences one must conclude that ε-ATP should never be employed as a probe for ATP in the presence of Cu^{2+}.

Adenosine 5'-triphosphate (ATP^{4-}) is in nature a widely used substrate for many enzymic reactions, and there are indications that Cu(ATP)$^{2-}$ might be a natural active form of Cu^{2+}.[1] As ε-ATP is a popular probe for ATP (Chart I),[2] mainly due to its fluorescent qualities, we studied the properties of the Cu^{2+}/ε-ATP system in aqueous solution. The results show that the stabilities and structures of the Cu^{2+}/ε-ATP complexes differ so much from those of Cu^{2+}/ATP that ε-ATP should never be employed as a probe for ATP in the presence of Cu^{2+}.

Results and Discussion

The experimental data[3] from potentiometric pH titrations of Cu^{2+}/ε-ATP cannot be explained by the sole formation of Cu(H·ε-ATP)$^-$ and Cu(ε-ATP)$^{2-}$. This contrasts with the Mg^{2+}, Mn^{2+}, and Zn^{2+}/ε-ATP systems[4] where the data could well be accounted for with H^+, $H_2(ε\text{-ATP})^{2-}$, H(ε-ATP)$^{3-}$, ε-ATP^{4-}, M^{2+}, M(H·ε-ATP)$^-$, and M(ε-ATP)$^{2-}$, i.e., by considering the following two equilibria:

$$M^{2+} + H(ε\text{-ATP})^{3-} \rightleftharpoons M(H·ε\text{-ATP})^- \quad (1a)$$

$$K^{M}_{M(H·ε\text{-ATP})} = [M(H·ε\text{-ATP})^-]/([M^{2+}][H(ε\text{-ATP})^{3-}]) \quad (1b)$$

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

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

The acidity constant of the connected equilibrium 3 is calculated with eq 4.

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

$$K^{H}_{M(H·ε\text{-ATP})} = [M(ε\text{-ATP})^{2-}][H^+]/[M(H·ε\text{-ATP})^-] \quad (3b)$$

$$pK^{H}_{M(H·ε\text{-ATP})} = pK^{H}_{H(ε\text{-ATP})} + \log K^{M}_{M(H·ε\text{-ATP})} - \log K^{M}_{M(ε\text{-ATP})} \quad (4)$$

The spectrophotometric results shown in the upper part of Figure 1 also suggest that in the Cu^{2+}/ε-ATP system additional complex species are formed: considering the dependence of the absorption at 260 or 271 nm on pH it is evident that the absorption decreases to a minimum at about pH 4.5 and rises then again. Hence, at least one further complex must be formed. These observations are also quite different from those made for the Zn^{2+}/ε-ATP system.[4]

1. Estimation of Equilibrium Constants. As the titration data indicated a first inflection point after liberation of "half a proton" per ligand molecule besides the final equivalence point, we assumed that a complex $Cu_2H(ε\text{-ATP})_2^{3-}$ might be formed according to the dimerization equilibrium 5. This species would liberate "half a proton" via process 6. A first attempt to fit the experimental

$$Cu(H·ε\text{-ATP})^- + Cu(ε\text{-ATP})^{2-} \rightleftharpoons Cu_2H(ε\text{-ATP})_2^{3-} \quad (5a)$$

$$K_{D/Cu,ε\text{-ATP}} = [Cu_2H(ε\text{-ATP})_2^{3-}]/([Cu(H·ε\text{-ATP})^-][Cu(ε\text{-ATP})^{2-}]) \quad (5b)$$

$$Cu_2H(ε\text{-ATP})_2^{3-} \rightleftharpoons 2Cu(ε\text{-ATP})^{2-} + H^+ \quad (6)$$

data, on the basis of the model containing the complexes Cu(H·ε-ATP)$^-$, Cu(ε-ATP)$^{2-}$, and $Cu_2H(ε\text{-ATP})_2^{3-}$ [aside from $H_2(ε\text{-ATP})^{2-}$, H(ε-ATP)$^{3-}$, and ε-ATP^{4-}] by using the program TITFIT,[8] failed. The iterative process did not converge, because titration data were available only at pH >3.4, and here the complex Cu(H·ε-ATP)$^-$ is already fully formed. Hence, in a series of calculations the value for log $K^{Cu}_{Cu(ε\text{-ATP})}$ was kept constant and now the iteration converged readily. The corresponding results are listed in Table I.

From preliminary calculations it became immediately clear that log $K^{Cu}_{Cu(ε\text{-ATP})} > 8$ (Table I): with log $K^{Cu}_{Cu(ε\text{-ATP})} = 8.0$ the standard deviation between the experimental and calculated

(1) Tallineau, C.; Barriere, M.; Boulard, M.; Boulard-Heitzmann, P.; Pontcharraud, R.; Reiss, D.; Guillard, O. *Biochim. Biophys. Acta* **1984**, *775*, 51–56.
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(3) Equipment, materials, and experimental procedures were the same as given in ref 4. Three independent titrations with 1 mL of 0.04 M NaOH were carried out for aqueous solutions with $[Cu^{2+}] = [ε\text{-}ATP^{4-}] = 0.288$, 0.336, and 0.384 mM (volume 50 mL) in the presence of 0.72 mM HNO_3 and $NaNO_3$ ($I = 0.1$; 25 °C). In these concentrations self-stacking of ε-ATP is negligible.[5] The experiments were also done such that dephosphorylation of ε-ATP, which is metal ion promoted[6] like that of other nucleoside 5'-triphosphates,[7] was kept to a minimum.
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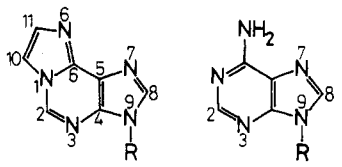
0020-1669/86/1325-1313$01.50/0

Chart I



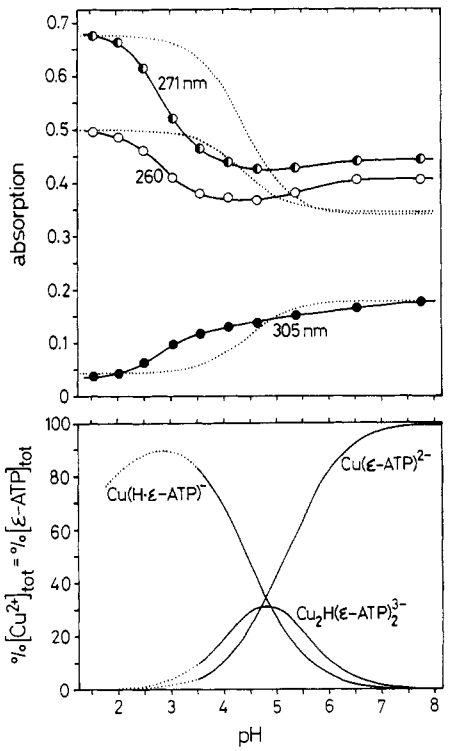


Figure 1. Comparison of the UV absorption of the Cu^{2+}/ϵ-ATP system with the formation of complex species in the same system. Upper part: Dependence of the UV absorption of Cu^{2+}/ϵ-ATP on pH in aqueous solution (measured in 2-mm cells with $[Cu(ClO_4)_2] = 3.36 \times 10^{-4}$ M $\simeq$ [ε-ATP]; 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 of ref 4). Lower part: Effect of pH on the concentration of the complexes present in an aqueous solution of $[Cu^{2+}]$ = [ε-ATP] = 3.36×10^{-4} M. Given is the percentage of the complexes based on the total Cu^{2+} (=total ε-ATP) present. The data were computed with the most probable values resulting from the potentiometric pH titrations (see Table I). In the lower pH range (dotted line) the formation of the diprotonated complex $Cu(H_2{\cdot}\epsilon\text{-ATP})$ and the dimer species $Cu_2(H{\cdot}\epsilon\text{-ATP})_2^{2-}$ (see text) seems also possible, but the stability of these complexes is not known.

consumption of NaOH, expressed in σ_{mL}/mL of NaOH used for the whole titration, of 0.015 is still rather large. However, σ_{mL} becomes considerably smaller with log $K^{Cu}_{Cu(\epsilon\text{-ATP})}$ = 9.0, while a further increase of log $K^{Cu}_{Cu(\epsilon\text{-ATP})}$ does not reduce σ_{mL} significantly anymore. In fact, log $K^{Cu}_{Cu(\epsilon\text{-ATP})}$ = 9.0 seems a reasonable estimate for the stability of $Cu(\epsilon\text{-ATP})^{2-}$ because it is 3.1 log units larger than log $K^{Cu}_{Cu(\epsilon\text{-AMP})}$ = 5.87 (±0.02)[10] and the increase in complex stability from Cu(AMP) to $Cu(ATP)^{2-}$ is also in this order [i.e., log $K^{Cu}_{Cu(ATP)}$ − log $K^{Cu}_{Cu(AMP)}$ = 6.32 (Table I) − 3.04 (ref 11) = 3.28]. In other words, replacement of a monophosphate moiety by a triphosphate residue increases the stability by about 3 log units. Hence, these reasonings allow to fix the upper limit for log $K^{Cu}_{Cu(\epsilon\text{-ATP})}$ to 10 and the lower to 8; with this the limits of the other constants in Table I are of course also fixed.

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Table I. Summary of the Attempts To Evaluate the Potentiometric pH Titration Data of the Cu^{2+}/ϵ-ATP System with a Set of the Most Probable Equilibrium Constants[a] and with Corresponding Constants for the Cu^{2+}/ATP System Given for Comparison (I = 0.1, $NaNO_3$; 25 °C)[b]

log $K^{Cu}_{Cu(\epsilon\text{-ATP})}$	log $K^{Cu}_{Cu(H\cdot\epsilon\text{-ATP})}$	$pK^{H}_{Cu(H\cdot\epsilon\text{-ATP})}$	log $K_{D/Cu_2H(\epsilon\text{-ATP})_2}$	σ_{mL}
		Values at Fixed log $K^{Cu}_{Cu(\epsilon\text{-ATP})}$		
8.00	6.22	4.72	3.92	0.0150
8.50	6.77	4.77	3.71	0.0106
9.00	7.29	4.79	3.60	0.0085
10.00	8.31	4.81	3.49	0.0073
11.00	9.31	4.81	3.46	0.0072
		Most Probable Values[a]		
9 ± 1	7.3 ± 1	4.8 ± 0.1	3.6 ± 0.3	
		Corresponding Values for ATP[9]		
6.32 ± 0.04	3.57 ± 0.08	3.74 ± 0.09		

[a] See text. [b] Acidity constants: $pK^{H}_{H_2(\epsilon\text{-ATP})}$ = 4.45 ± 0.02, $pK^{H}_{H(\epsilon\text{-ATP})}$ = 6.50 ± 0.01;[4] $pK^{H}_{H_2(ATP)}$ = 4.01 ± 0.01, $pK^{H}_{H(ATP)}$ = 6.49 ± 0.01.[9]

It should be emphasized that calculations with more complicated models, i.e. the additional considerations of $Cu_2(\epsilon\text{-ATP})_2^{4-}$, $Cu_2(H{\cdot}\epsilon\text{-ATP})_2^{2-}$, or $Cu_2(\epsilon\text{-ATP})$ together with the species $Cu(\epsilon\text{-ATP})^{2-}$, $Cu(H{\cdot}\epsilon\text{-ATP})^{-}$, and $Cu_2H(\epsilon\text{-ATP})_2^{3-}$, did not significantly improve the fit of the experimental data. Clearly, this does not mean that $Cu(\epsilon\text{-ATP})^{2-}$, $Cu(H{\cdot}\epsilon\text{-ATP})^{-}$, and $Cu_2H(\epsilon\text{-ATP})^{3-}$ are the only complexes formed in the Cu^{2+}/ϵ-ATP system, but it means that under our experimental conditions in the pH range from 3.4 to 5.8, or better up to pH 8, no other complexes could be determined. The extension of the pH range up to 8 is based on the upper part of Figure 1, from which it is evident that $Cu(\epsilon\text{-ATP})(OH)^{3-}$ is not formed to any significant extent up to pH 8.[12] That hydroxo complex formation is retarded in the Cu^{2+}/ϵ-ATP system is expected; as in $Cu(\epsilon\text{-ATP})^{2-}$, the four equatorial binding sites of Cu^{2+} are practically saturated (see section 3).

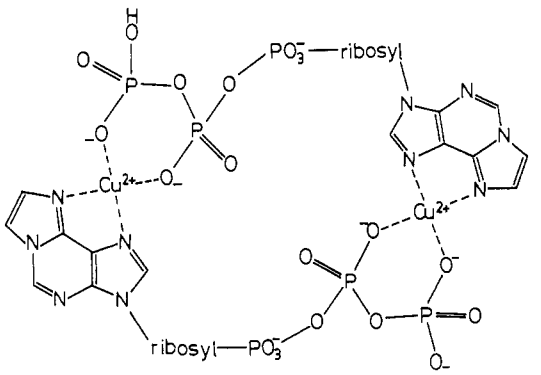
2. Further Considerations about the Cu^{2+}/ϵ-ATP System. Since $pK^{H}_{Cu(H\cdot\epsilon\text{-ATP})}$ = 4.8, the proton in $Cu(H{\cdot}\epsilon\text{-ATP})^{-}$ must be mainly located at the γ phosphate group ($pK^{H}_{H_2(\epsilon\text{-ATP})}$ = 4.45; Table I) allowing a base–metal ion interaction.

The distribution of complex species as a function of pH, calculated with the most probable values of Table I for the conditions of the spectrophotometric measurements, is shown in the lower part of Figure 1. A comparison of the two parts of Figure 1 reveals a contradiction: according to the spectrophotometric titration (upper part), complexation begins only at pH >2, while the calculations (lower part) give for pH 2 already an 80% formation of $Cu(H{\cdot}\epsilon\text{-ATP})^{-}$ [compare, e.g., the decrease in absorption at 260 or 271 nm with the plotted concentration for $Cu(H{\cdot}\epsilon\text{-ATP})^{-}$]. Hence, in this pH range an additional species must be formed on account of $Cu(H{\cdot}\epsilon\text{-ATP})^{-}$; this can only be a complex carrying a proton at N-6 (thus explaining the retarded decrease in absorption) *and* at the terminal γ phosphate group, i.e. $Cu(H_2{\cdot}\epsilon\text{-ATP})$. Deprotonation of N-6 in $Cu(H_2{\cdot}\epsilon\text{-ATP})$ at pH >2 would then account for the observed absorption changes in this pH range and the formation of $Cu(H{\cdot}\epsilon\text{-ATP})^{-}$.

However, in the pH range above 4, the two parts of Figure 1 correlate well. This is gratifying, but also expected, because from the calculations summarized in Table I it is obvious that $pK^{H}_{Cu(H\cdot\epsilon\text{-ATP})}$ and log $K_{D/Cu_2H(\epsilon\text{-ATP})_2}$ are practically independent of the value of log $K^{Cu}_{Cu(\epsilon\text{-ATP})}$. It is evident that the decreasing concentration of the dimer, $Cu_2H(\epsilon\text{-ATP})_2^{3-}$, parallels the increase in absorption at 260 and 271 nm following the minimum observed at pH about 4.5. That at pH >6.5 the absorption of $Cu(\epsilon\text{-ATP})^{2-}$ differs somewhat from the absorption of the free ligand (dotted lines) with a neutral 1,N^6-ethenoadenine moiety is to be expected

(12) In the Zn^{2+}/ϵ-ATP system the formation of $Zn(\epsilon\text{-ATP})(OH)^{-}$ is clearly indicated by UV spectrophotometric measurements (cf. Figure 2 in ref 4).

Chart II

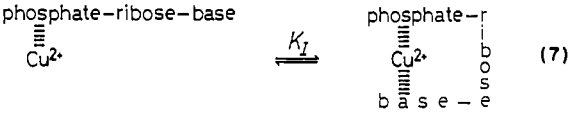


due to the intense ring back-binding of Cu^{2+} in $Cu(\epsilon\text{-ATP})^{2-}$ (see section 3); furthermore, heteroaromatic N bases of this type[13] have π-accepting properties regarding Cu^{2+}.

The present results prove, despite the shortcomings of this study, that $Cu(H\cdot\epsilon\text{-ATP})^{-}$ and $Cu(\epsilon\text{-ATP})^{2-}$ dimerize according to equilibrium 5; hence the question for the structure of this dimer arises. As $\log K_{D/Cu_2H(\epsilon\text{-ATP})_2} \sim 3.6$ (Table I), this dimer cannot be a simple stack of the ϵ-adenine residues.[14] Chart II shows a tentative and simplified structure of the $Cu_2H(\epsilon\text{-ATP})_2^{3-}$ dimer; this structure corresponds to dimeric Cu^{2+} complexes observed with peptide-like ligands,[16-18] and it takes into account the rather pronounced stability of the $Cu(\epsilon\text{-Ado})^{2+}$ complex ($\log K^{Cu}_{Cu(\epsilon\text{-Ado})} = 2.81 \pm 0.06$),[15,19] in which the metal ion is coordinated to the N-6/N-7 site. The tentative structure of Chart II suggests that diprotonated $Cu_2(H\cdot\epsilon\text{-ATP})_2^{2-}$ might also exist and that the dimers could be folded in a way allowing an intramolecular stacking interaction between the two 1,N^6-ethenoadenine moieties; such an additional interaction would contribute to the stability of the dimer(s). For the species $Cu(\epsilon\text{-ATP})^{2-}$, apparently dimerization is less favorable compared with an *intra*molecular ring back-binding, thus giving rise to marcochelate formation (see section 3).

3. Structure of $Cu(\epsilon\text{-ATP})^{2-}$ and General Conclusions. In an earlier study[20] the stability of $Cu(\epsilon\text{-ATP})^{2-}$ has been given as log $K^{Cu}_{Cu(\epsilon\text{-ATP})} = 5.69$.[21] This value is clearly too small (cf., e.g., with $\log K^{Cu}_{Cu(ATP)} = 6.32$; Table I) and not reliable; this may be due to the presence of buffers[22] in the corresponding experiments, the method used (fluorescence quenching) or both.

The much larger stability of $Cu(\epsilon\text{-ATP})^{2-}$ ($\log K^{Cu}_{Cu(\epsilon\text{-ATP})} \simeq 9$; Table I) than that of the $Cu(PNTP)^{2-}$ complexes, which contain a pyrimidine-nucleoside 5'-triphosphate ($\log K^{Cu}_{Cu(PNTP)} = 5.67$)[4,23] and in which no base–metal ion interactions occur,[23] must mean that the N-6/N-7 site of the 1,N^6-ethenoadenine moiety participates in complex formation. Indeed, this indirect conclusion is confirmed by the spectrophotometric results (section 2). Hence, the following intramolecular (and therefore concentration-independent) equilibrium between an "open" isomer, $Cu(\epsilon\text{-ATP})^{2-}_{op}$, and a "closed" species, $Cu(\epsilon\text{-ATP})^{2-}_{cl}$, must be considered:



(7)

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

The intramolecular equilibrium constant K_I may be calculated[4,23] from the experimentally determined overall stability constant $K^{Cu}_{Cu(\epsilon\text{-ATP})}$ and the constant $K^{Cu}_{Cu(PNTP)}$ representing $K^{Cu}_{Cu(\epsilon\text{-ATP})op}$, i.e. the stability of the open isomer (eq 7). The value of K_I allows then obviously the calculation of the percentage of the macrochelated isomer: more than 99.5% of the $Cu(\epsilon\text{-ATP})^{2-}$ exists in this closed form.[24] This contrasts with the 70% calculated[9,23] for the closed isomer of $Cu(ATP)^{2-}$, in which back-binding occurs to N-7 (see Chart I).

These differences are expected to be reflected in the reactivity. Indeed, the Cu^{2+}-promoted dephosphorylation of ϵ-ATP and ATP is rather different and proceeds also via different pathways: the reactive species for $\epsilon\text{-ATP}^{4-}$ is a monomeric complex[6] and for ATP^{4-} a dimeric one.[7] This example, aside from differences in stabilities and structures of the complexes, evidences further that ϵ-ATP should not be employed as a probe for ATP in enzymic reactions in the presence of Cu^{2+}.

Acknowledgment. The technical assistance of Rita Baumbusch and support by the Swiss National Science Foundation are gratefully appreciated.

Registry No. ϵ-ATP, 37482-17-0; Cu, 7440-50-8.

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(14) The self-association tendency of ϵ-adenosine is remarkable, but still much smaller: $K = 9.4 \pm 1.2\ M^{-1}$ (if only dimerization is assumed, then $K_D = 4.7\ M^{-1}$).[15]
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(19) (a) The $M(\epsilon\text{-Ado})^{2+}$ complexes[15] are not as stable as the corresponding $M(1,10\text{-phen})^{2+}$ complexes[19b] probably due to the larger distance between N-6 and N-7 (see Chart I) in ϵ-adenosine[19c] compared with N-1 and N-10 in 1,10-phenanthroline.[19d] (b) Anderegg, G. *Helv. Chim. Acta* **1963**, *46*, 2397–2410. (c) Wang, A. H.-J.; Dammann, L. G.; Barrio, J. R.; Paul, I. C. *J. Am. Chem. Soc.* **1974**, *96*, 1205–1213. (d) Nishigaki, S.; Yoshioka, H.; Nakutsu, K. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1978**, *B34*, 875–879.
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(24) This lower limit is obtained by using $\log K^{Cu}_{Cu(\epsilon\text{-ATP})} > 8$ (Table I) in the calculation, which gives $K_I > 200$ and $[Cu(\epsilon\text{-ATP})^{2-}_{cl}] > 99.5\%$. With $\log K^{Cu}_{Cu(\epsilon\text{-ATP})} = 9.0$, one obtains $K_I \sim 2000$ and $[Cu(\epsilon\text{-ATP})^{2-}_{cl}] = 99.95\%$.