MIXED-LIGAND Cu(II) COMPLEXES WITH AMP 1319

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Ternary Complexes in Solution, XIV

The Stability Increasing Effect of the Pyridyl and Imidazole Groups on the Formation of Mixed Amine-Copper(II)-Adenosine 5'-monophosphate Complexes 1, 2

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The stability constants of the ternary Cu2+ complexes containing adenosine 5'-monophosphate (AMP) and as a second ligand, 2,2'-bipyridyl, 4-(2'-pyridyl)imidazole, 2-picolylamine, 4-aminomethylimidazole, and ethylenediamine were determined by potentiometric titration in water containing 10% dioxane (f = 0.1:25 °C). For the equilibrium, Cu(amine)2+Cu(AMP) = Cu(amine) (AMP) + Cu2+, the corresponding constants are (in the order of the above amine ligands), log K =: log KCu(amine)(AMP) - log KCu(AMP) = 0.53, 0.20, 0.04, -0.35, and -0.45; hence, in the first cases the equilibrium is considerably on the side of the mixed-ligand complex. The importance of the a-system of the amine for the stability of the mixed-ligand Cu2+ complexes is obvious from the given series. The imidazole group, an important binding site for metal ions in biological systems, has clearly qualities similar to the pyridyl group (Inorg. Chem. 9, 1238 [1970]), i.e. the stability of ternary Cu2+ complexes containing these groups is increased.

Ternary complexes can be considered as models for enzyme-metal ion-substrate complexes as well as for mixed-ligand complexes occurring in biological fluids. Therefore, investigations of the stability of ternary complexes may help toward understanding the driving forces which lead to the formation of such complexes in biological systems.

From earlier studies 3,4 it is known that ligands containing oxygen as donor atoms, such as malonate or pyrocatecholate, form ternary complexes with Cu2+ complexes containing 2,2'-bipyridyl which are more stable than the corresponding binary Cu2+-O-ligand 1:1 complexes 5. The same phenomenon is observed in mixed-ligand Cu2+ systems containing 2,2'-bipyridyl and a phosphate, e.g. hydrogen phosphate, adenosine 5'-monophosphate or -triphosphate 6.

Recently, the stability of the mixed-ligand Cu2+ complexes containing pyrocatecholate and one of the following N-ligands was determined: 2,2'-bipyridyl, 4-(2'-pyridyl)imidazole, 2-picolylamine, 4-aminomethylimidazole, and ethylenediamine 7. It became apparent that the stability of the ternary N-ligand-Cu2+-pyrocatecholate complexes is strongly
dependent on the presence of an aromatic amine. This means, the stability of the ternary complexes is increased by changing an amino group against an imidazol group, and again by replacing the latter against a pyridyl group. To see, if this is generally true for N-ligand-Cu²⁺-O-ligand complexes and, especially, if it also applies for those containing phosphates, we have now studied the mixed-ligand Cu²⁺ systems containing adenosine 5'-monophosphate and one of the mentioned N-ligands. Additionally, for the amino and the imidazol groups, which are important metal ion binding sites in nature, the given series of N-ligands offers the possibility to elucidate the coordination qualities of these two binding sites in mixed-ligand complexes.

**Experimental Section**

**Materials and Apparatus**

Adenosine 5'-monophosphoric acid and the disodium salt (puriss.) were obtained from BDH Chemicals Ltd., Poole, England, and from Fluka AG, Buchs, Switzerland, respectively. All the other materials and the apparatus were the same as used earlier.³,⁷

**Measurements**

Depending on the substances available, the solubility of the reactants, and the concentrations needed, 10, 15, 25, or 50 ml of water containing 10% dioxane and the reactants (I = 0.1, T = 25°C) were titrated potentiometrically with 1 ml of 2 × 10⁻² M, 5 × 10⁻² M, 0.1 M or 0.5 M NaOH. The pH used for the calculations was the one read from the potentiograph.

**Determination of the Acidity Constants**

The acidity constants, K_H²⁺L, and K_H⁻L, were determined by titrating solutions containing HClO₄ and NaClO₄ to 0.1 M in the presence and absence of the ligands. The difference in ml NaOH used for a pair of titrations was evaluated. The constants were calculated from the degree of neutralization for at least 10 points of the titration curves. The concentrations were as follows:

- Adenosine 5'-monophosphate: K_H²⁺L, [HClO₄] = 6 × 10⁻⁴ M and [H₂AMP] = 6.7 × 10⁻⁴ M or 1.2 × 10⁻³ M. K_H⁻L, [HClO₄] = 3 × 10⁻⁴ to 3.6 × 10⁻³ M and [AMP²⁻] = 2 × 10⁻⁴ to 3.6 × 10⁻³ M.
- Ethylenediamine: K_H²⁺L, [HClO₄] = 3.5 × 10⁻⁴ to 3.7 × 10⁻³ M and [L] = 1.7 × 10⁻⁴ to 1.8 × 10⁻³ M. K_H⁻L, [HClO₄] = 1.5 × 10⁻³ M and [L] = 7.2 × 10⁻⁴ M.
- 4-Aminomethylimidazole: K_H²⁺L, [HClO₄] = 6 × 10⁻⁴ M and [H₂L] = 1.2 × 10⁻³ M or 2.4 × 10⁻⁴ M. K_H⁻L, [HClO₄] = 2 × 10⁻⁴ or 6 × 10⁻⁴ M and [H₂L] = 8 × 10⁻⁴ or 1.2 × 10⁻³ M.
- 2-Picolylamine: K_H²⁺L, [HClO₄] = 8.4 × 10⁻³ to 4.7 × 10⁻² M and [L] = 8 × 10⁻³ or 2 × 10⁻⁴ M. K_H⁻L, [HClO₄] = 4 × 10⁻⁵ or 8 × 10⁻⁵ M and [H₂L] = 4 × 10⁻³ or 8 × 10⁻³ M.

**Determination of the Stability Constants of the Binary Complexes**

The conditions of measurements for the determination of the stability constants, K_CuL, were the same as for the acidity constants, but part of NaClO₄ was replaced by Cu(ClO₄)₂. With AMP the ratio was Cu : L = 15 : 1 and 20 : 1; with ethylenediamine, 4-aminomethylimidazole, and 2-picolylamine Cu : L = 1 : 1 titrations were carried out. As usual, titrations of solutions without ligand were used as a basis for the evaluation. Additionally, these titrations showed in which pH regions hydrolysis could be neglected.

The calculation of K_CuL was done by taking into account K_CuL₂ which was obtained from titrations where L was in excess with regard to Cu²⁺ (ref. ⁵). The only exception was with AMP, despite several attempts we were not able to determine a definite value for K_CuL₂. However, the concentration of the species, Cu(AMP)₂, is certainly insignificant under the conditions employed for the determination of K_CuL, as well as in the mixed-ligand system.

**Determination of the Stability Constants of the Ternary Complexes**

The conditions of measurements for the titrations of the ternary complexes were similar as for the binary ones, but the solutions contained adenosine 5'-monophosphate, Cu²⁺, and ethylenediamine, 4-aminomethylimidazole, or 2-picolylamine in a ratio 3 : 1 : 1 or 4 : 1 : 1. A typical reaction solution consisted of [HClO₄] = 3.6 × 10⁻³ M, [Cu(ClO₄)₂] = [N – L] = 1.2 × 10⁻³ M, and [AMP²⁻] = 3.6 × 10⁻³ M. The titration of a solution of the same kind but without AMP showed the pH range where hydrolysis could be omitted. The evaluation of the titration curves was based on curves obtained from analog solutions without Cu²⁺ and L. The stability constant, β_{Cu(N-L)AMP}, of the ternary systems was computed by taking into account the species, H, H₂(N – L), H(N – L), N – L, Cu(N – L), Cu(N – L)₂, H₃(AMP), H(AMP), AMP, Cu(AMP), Cu, and Cu(N – L) AMP.³

The stability constants of the ternary complexes in the systems, AMP, Cu²⁺, and 2,2'-bipyridyl or 4-(2-pyridyl)imidazole could be calculated in a much simpler way.³,⁸ The potentiometric titrations showed that the release of protons due to the complexing between Cu²⁺ and 2,2'-bipyridyl or 4-(2-pyridyl)imidazole is completely finished before the reaction between Cu(N – L) and AMP occurs. This means the curves that resulted from the titration of a solution containing only HClO₄ and those containing additionally Cu²⁺ and N – L are superimposable. Of course, this is only true in the lower pH region which was actually used for the calculation of the stability constants. At higher pH
Table I. Negative log acidity constants of adenosine 5'-monophosphate and of several ligands with N as donor atoms, and log stability constants of their binary Cu²⁺ complexes determined in water containing 10% dioxane (I=0.1; T=25 °C).

<table>
<thead>
<tr>
<th>Ligand</th>
<th>pK_{H+}^{II}</th>
<th>pK_{H+}</th>
<th>log K_{Cu}</th>
<th>log K_{CuL}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine 5'-monophosphate</td>
<td>3.71 ± 0.01</td>
<td>6.48 ± 0.01</td>
<td>3.39 ± 0.02</td>
<td>—</td>
</tr>
<tr>
<td>2-Picolylamine</td>
<td>2.00 ± 0.02</td>
<td>8.76 ± 0.01</td>
<td>9.60 ± 0.02</td>
<td>7.84</td>
</tr>
<tr>
<td>4-Aminomethylimidazole</td>
<td>4.63 ± 0.01</td>
<td>9.41 ± 0.02</td>
<td>9.22 ± 0.01</td>
<td>8.00</td>
</tr>
<tr>
<td>Ethylenediamine</td>
<td>7.08 ± 0.01</td>
<td>9.95 ± 0.01</td>
<td>10.65 ± 0.02</td>
<td>9.22</td>
</tr>
</tbody>
</table>

Table II. Log stability constants of some ternary N-ligand-Cu²⁺-adenosine 5'-monophosphate complexes determined in water containing 10% dioxane (I=0.1; T=25 °C).

<table>
<thead>
<tr>
<th>N-Ligand</th>
<th>log β_{Cu(N-L)AMP}</th>
<th>log K_{Cu(N-L)AMP}</th>
<th>Δ log K</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,2'-Bipyridyl</td>
<td>3.92 ± 0.01</td>
<td>3.92 ± 0.01</td>
<td>0.53</td>
</tr>
<tr>
<td>4-(2',Pyridyl)imidazole</td>
<td>3.59 ± 0.03</td>
<td>3.59 ± 0.03</td>
<td>0.20</td>
</tr>
<tr>
<td>2-Picolylamine</td>
<td>13.03 ± 0.04</td>
<td>3.43 ± 0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>4-Aminomethylimidazole</td>
<td>12.26 ± 0.06</td>
<td>3.04 ± 0.06</td>
<td>0.35</td>
</tr>
<tr>
<td>Ethylenediamine</td>
<td>13.59 ± 0.06</td>
<td>2.94 ± 0.06</td>
<td>0.45</td>
</tr>
</tbody>
</table>

The range of error given is three times the standard deviation. The reproducibility of log K_{CuL} was at least ±0.1 log unit.
\[ \log K_{Cu(N-L)AMP} = \log K_{Cu(N-L)AMP} - \log K_{Cu(N-L)} \]  \hspace{1cm} \text{(7)}

The stability of ternary complexes can be characterized according to Eqn. 8, i.e. by comparing the difference in stability between the complexes defined in Eqn. 3 (with L = AMP) and 6. The values expected for \( \Delta \log K \) (Eqn. 8) are negative (general rule: \( K_{CuL} > K_{SSL} \)), because more coordination positions are available for bonding of the first ligand to a given metal ion than for the second ligand. Additionally, \( \Delta \log K \) is identical with the equilibrium constant due to Eqn. 9. These values are also listed in Table II.

\[ \Delta \log K = \log K_{Cu(N-L)AMP} - \log K_{CuAMP} \]
\[ = \log K_{Cu(AMP)(N-L)} - \log K_{Cu(N-L)} \]  \hspace{1cm} \text{(8)}

\[ Cu(N-L) + Cu(AMP) \rightleftharpoons Cu(N-L)AMP + Cu \]  \hspace{1cm} \text{(9)}

**Discussion**

In all cases reported here \( \Delta \log K \) (Table II) is considerably less negative than one might expect from the values of

\[ \log K_{Cu(N-L)AMP} - \log K_{Cu(N-L)} \]

(cf. Table I), and in two cases even significant positive values of \( \Delta \log K \) are obtained. Positive values indicate the preference of AMP to coordinate with \( Cu(N-L)^{2+} \) rather than \( Cu(aq)^{2+} \), i.e. Eqn. 9 is displaced to the right side.

The stability of the ternary N-ligand-Cu\(^{2+}\)-adenosine 5'-monophosphate complexes increases by changing the N-ligand in the series, ethylenediamine < 4-aminomethylimidazole < 2-picolylamine < 4-(2'-pyridyl)imidazole, 2-picolylamine, 4-aminomethylimidazole, and ethylenediamine (from left to right). The data are those of Tables I and II (cf. 18).

Fig. 1. Relation between \( \Delta \log K \) and \( pH_{H^+} + pH_{H^+} \) for the ternary complexes, \( (N-L)-Cu^{2+}-\text{adenosine 5'-monophosphate} \), where \( N-L = 2,2'-\text{bipyridyl, 4-(2'-pyridyl)imidazole, 2-picolylamine, 4-aminomethylimidazole, and ethylenediamine (from left to right)} \). The data are those of Tables I and II (cf. 18).

Fig. 2. Variation with pH of the concentrations (given as the percentage of the total Cu\(^{2+}\) present) of the several species present in water containing 10% dioxyne of Cu\(^{2+}\), adenosine 5'-monophosphate (AMP), and 2-picolylamine (Pic, upper part) or ethylenediamine (En, lower part); concentrations of the reactants: \( 10^{-3} \text{M} \). All data were computed with the constants given in Tables I and II. Hydrolysis was omitted in these calculations (cf. experimental section).

where the concentrations of the several species present in the ternary systems, 2-picolylamine-Cu\(^{2+}\)-AMP and ethylenediamine-Cu\(^{2+}\)-AMP, are given in their dependence on pH. Toward higher pH values the concentrations tend to approach limiting values. Due to the stability increasing effect of the pyridyl group the ternary complex in the 2-picolylamine-Cu\(^{2+}\)-AMP 1:1:1 system reaches a concentration of about 48%, while in the ethylenediamine-Cu\(^{2+}\)-AMP
1:1:1 system the corresponding complex rises only to about 26%.

The present results obtained for the N-ligand-Cu\(^{2+}\)-AMP systems confirm those of the N-ligand-Cu\(^{2+}\)-pyrocatecholate systems. This means, adenosine 5'-monophosphate behaves like a simple O-ligand. This result is in agreement with the known fact that the stability of metal ion-nucleotide complexes is determined by the coordination tendency of the phosphate groups.

In conclusion, with regard to biological systems it is important to note that the participation of an imidazole group favors the formation of mixed-ligand complexes more strongly than an amino group, provided an O-ligand is also involved. Additionally, the imidazole (like the pyridyl) group has discriminating qualities: for example, Cu(histamine)\(^{2+}\) prefers to coordinate O- rather than N-ligands. How far the described results also apply for complexes with metal ions other than Cu\(^{2+}\) cannot be decided presently. However, there is evidence that at least some metal ions of the first transition series show qualities similar to those of Cu\(^{2+}\).

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5. Abbreviations: The terms, "N-ligand" and "O-ligand", are used for ligands containing O or N as donor atoms. L, general ligand; N—L, ligand with N as donor atoms; AMP, adenosine 3'-monophosphate. Charges are omitted in all equations.
10. As mentioned, the acidity constants of the ligands in Table 1 are practically identical with those determined in water. Therefore, in Fig. 1 the constants determined in aqueous solution are used for 2,2'-bipyridyl (pK\(_{H^+}\)_L = 0.2, ref. 11; pK\(_{H^+}\)_L = 4.49, ref. 12 and 4-(2'-pyridyl)imidazole (pK\(_{H^+}\)_L = 1.33, pK\(_{H^+}\)_L = 5.49, ref. 13). There are more examples of such a dependence as given in Fig. 1; the problems connected herewith shall be discussed in more detail elsewhere.