Potentiometric studies on the stability constants of some α-amino acid-copper(II) and nickel(II) systems in ethanol-water mixture

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Received 27 May 2002; revised 13 January 2003

The stability constants for the nickel(II) and copper(II)-α-amino acid (glycine, L-alanine, L-valine, L-leucine, L-isoleucine, L-serine, L-phenylalanine and L-methionine) binary complex systems have been studied using pH-measurements in 40% ethanol-60% water mixture. The study shows that at 25°C and ionic strength at 0.10 M the following species are present in this solvent mixture: CuHL₂⁺, CuL⁺, CuL₂⁻ for copper(II) complexes and NiL⁺, NiL₂⁺, NiL₃⁻ for nickel(II) complexes. The stability of the nickel(II) complexes is lower than that of the copper(II) complexes. The study shows that the tendency of α-amino acids to form complexes with metal(II) ions is greater in 40% ethanol-60% water mixture as compared to that in only water.

The primary function of the amino acids is to serve as building blocks of living organisms, and therefore amino acids and their metal complexes have special importance in biological and chemical systems. Some metal ions present in biological fluids and tissues, e.g., sodium, potassium, calcium, copper, zinc, are necessary for the body, while some others like mercury and lead are toxic.

Vast data are available on the protonation of amino acids and the stability constants of their metal complexes in aqueous solution. Despite this, little is known about the chemistry of amino acids and metal complexes in non-aqueous media and mixed solvents, in regard to their protonation and stability constants or solvation properties. One reason for this may be that in vivo reactions were thought to take place in aqueous media. However, recently it has been suggested that solvents such as ethanol provide a better model for in vivo reactions. It is clear that the data related to the protonation constants of amino acids and the stability constants of amino acid-metal ion complexes in ethanol-water mixtures will be valuable in further understanding of the amino acid chemistry in living systems.

In order to understand better the interactions of amino acids with metal ions, the protonation constants of eight α-amino acids and the stability constants of their complexes formed with copper(II) and nickel(II) have been determined potentiometrically in 40% ethanol-60% water (v/v) mixture.

Experimental
Glycine (99%), L-alanine (99.7%), L-valine (99%), L-leucine (99%), L-isoleucine (99%), L-serine (99%), L-phenylalanine (99%) and L-methionine (99%) were purchased from Merck and used as received. Ethanol was purified as described in literature. Deionized water was used for preparation of both ethanol-water mixtures and the stock solutions of α-amino acids. All other chemicals used in this investigation were of reagent grade purity. Hydrochloric acid solution (0.10 M) was prepared in water and standardised potentiometrically against sodium carbonate. Sodium hydroxide solution (0.10 M) containing 0.10 M NaCl was prepared in 40% (v/v) aqueous ethanol solution and standardised using linear least-squares fit of Gran's plots for end-point determination obtained from titrations of hydrochloric acid with this base.

Copper(II) and nickel(II) solutions were prepared by dissolving the required quantities of copper(II) chloride and nickel(II) chloride in deionized water containing hydrochloric acid to prevent hydrolysis. These solutions were standardized by the atomic absorption spectroscopic method.

Procedure
Potentiometric titrations were performed in 40% ethanol-60% water containing 0.10 M NaCl and in jacketed glass reaction vessels as described in literature. The e.m.f of the cell was measured using an Orion 720A pH ionmeter equipped with a combined pH electrode (Ingold). The electrode was modified by exchanging its aqueous KCl solution for a solution consisting of 0.10 M NaCl saturated with AgCl. The temperature was controlled at 25.0 ± 0.1°C.
The potentiometric cell was calibrated before each experiment to obtain the pH (= -log[H\(^+\)]) values for the solvent mixture studied\(^{14,15}\). The ionic product (\(K_w = [H^+][OH^-]\)) was calculated at constant ionic strength of 0.10 \(M\) with NaCl in 40% ethanol-60% water mixture\(^{16}\). For the determination of the protonation constants, stock solutions of hydrochloric acid, amino acid and sodium chloride were introduced into a volumetric flask, followed by an appropriate amount of ethanol to obtain solutions of the desired concentration and percentage of ethanol. The contents of each flask was diluted up to the mark at the equilibrium temperature. In each solvent mixture, the concentrations of hydrochloric acid, each amino acid and sodium chloride were kept at 3.0\(\times10^{-3}\), 1.5\(\times10^{-3}\) and 0.10 \(M\), respectively. Aliquots of 50 mL were taken from the test solutions and transferred to the potentiometric cell and titrated with standard NaOH solution until a precipitate or opalescence was just observed in titration cell. 

In order to determine the stoichiometric stability constants of \(\alpha\)-amino acid-copper(II) and nickel(II) complexes, test solutions were prepared by taking suitable amounts of metal ion, hydrochloric acid and hydrochloric acid stock solutions and were titrated with sodium hydroxide solution until a precipitate or opalescence was just observed in titration cell. (pH~8). At this point, titration was discontinued, because it was observed that metal complexes of amino acids disproportionated at pH higher than 8 and solution might become unstable with regard to separation of colloid phase. The analytical concentrations of metal ion, hydrochloric acid and amino acid were 5.0\(\times10^{-4}\), 3\(\times10^{-3}\) and 1.5\(\times10^{-3}\) \(M\) respectively. During each titration the ionic strength was maintained at 0.10 \(M\) NaCl and the potential reading was taken after a suitable time for equilibration. Establishing an equilibria in these systems was relatively slow. Sodium hydroxide was added every 15 min, potential readings taken every 2 min. until stable values, within ±0.1 mV, were obtained.

The computations of the stoichiometric protonation constants of \(\alpha\)-amino acids and the stoichiometric stability constants of \(\alpha\)-amino acid-metal ion complexes from potentiometric data were done with PKAS and the BEST computer programme respectively\(^{13,17}\). BEST was used to minimize the standard deviation of fit (\(\sigma_{fi}\)) between observed and calculated pH values of the entire titration data.

Results and discussion

The stoichiometric protonation constants of glycine, L-alanine, L-valine, L-leucine, L-isoleucine L-phenylalanine, L-serine and L-methionine and the stoichiometric stability constants of their copper(II) and nickel(II) determined in 40% ethanol-60% water mixture at 25\(^{\circ}\)C are given in Table 1. The values listed in Table 1 are related to the following equilibria: For the equilibria

<table>
<thead>
<tr>
<th>(\alpha)-Amino acid</th>
<th>Protonation constant</th>
<th>Stability constants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\log K_1)</td>
<td>(\log K_2)</td>
</tr>
<tr>
<td>Glycine</td>
<td>9.39±0.01</td>
<td>2.89±0.01</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>9.55±0.01</td>
<td>2.97±0.01</td>
</tr>
<tr>
<td>L-Valine</td>
<td>9.41±0.01</td>
<td>2.95±0.01</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>9.40±0.01</td>
<td>3.01±0.01</td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td>9.47±0.01</td>
<td>2.98±0.01</td>
</tr>
<tr>
<td>L-Serine</td>
<td>9.00±0.01</td>
<td>2.87±0.01</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>8.95±0.01</td>
<td>2.95±0.01</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>8.99±0.01</td>
<td>2.85±0.01</td>
</tr>
</tbody>
</table>
L⁻ + H⁺ ⇌ HL  \[ K_1 = [HL] / [L^-][H⁺] \]
HL + H⁺ ⇌ H₂L⁺  \[ K_2 = [H₂L⁺] / [HL][H⁺] \]

the constant \( K_1 \) relates to the first of these processes and \( K_2 \) to the second one. For copper(II)-α-amino acid system processes, the constant \( \beta_{111} \) relates to the first of these processes; \( \beta_{101} \) to the second process and \( \beta_{102} \) to the third one.

\[
\begin{align*}
\text{Cu}^{2+} + \text{H}^+ + \text{L}^- & \rightleftharpoons \text{CuHL}^{2+} \quad \beta_{111} = [\text{CuHL}^{2+}] / [\text{Cu}^{2+}][\text{H}^+][\text{L}^-] \\
\text{Cu}^{2+} + \text{L}^- & \rightleftharpoons \text{CuL}^+ \quad \beta_{101} = [\text{CuL}^+] / [\text{Cu}^{2+}][\text{L}^-] \\
\text{Cu}^{2+} + 2\text{L}^- & \rightleftharpoons \text{CuL}_2^- \quad \beta_{102} = [\text{CuL}_2^-] / [\text{Cu}^{2+}][\text{L}^-]^2
\end{align*}
\]

For the complex formation processes of nickel(II), the constant \( \beta_{101} \) relates to the first of these processes; \( \beta_{102} \) to the second process and \( \beta_{103} \) to the third one.

\[
\begin{align*}
\text{Ni}^{2+} + \text{L}^- & \rightleftharpoons \text{NiL}^+ \quad \beta_{101} = [\text{NiL}^+] / [\text{Ni}^{2+}][\text{L}^-] \\
\text{Ni}^{2+} + 2\text{L}^- & \rightleftharpoons \text{NiL}_2^- \quad \beta_{102} = [\text{NiL}_2^-] / [\text{Ni}^{2+}][\text{L}^-]^2 \\
\text{Ni}^{2+} + 3\text{L}^- & \rightleftharpoons \text{NiL}_3^- \quad \beta_{103} = [\text{NiL}_3^-] / [\text{Ni}^{2+}][\text{L}^-]^3
\end{align*}
\]

(where \( \text{L}^- \) is the anionic form of α-amino acids).

The α-amino acid-copper(II) and nickel(II) complex formations were studied varying metal(II) ion concentrations to ascertain the formation of polynuclear complexes. Results show that only mononuclear species were formed in appreciable concentrations within the studied concentration range of metal ions.

All α-amino acids studied are potentially bidentate ligands towards metal ions, with a donor site on the terminal amino and carboxyl groups. Nickel(II) ion has an octahedral configuration to form its amino acid complexes. Thus at higher \( pH \), it is likely that three α-amino acid molecules, each as a bidentate ligand, coordinate to each metal ion to form a mononuclear 1:3 complex, while at acidic \( pH \) a mononuclear 1:1 species will be formed. In 40% ethanol-60% water medium nickel(II) was found to give \( \text{NiL}^+ \), \( \text{NiL}_2^- \), \( \text{NiL}_3^- \) species with all the amino acids except phenylalanine. It is thought to be due to steric effect of the phenyl ring in the structure of this amino acid. Copper(II) can form 1:2 complex (CuL₂) by binding at most two amino acid molecules since it is smaller than nickel(II) ion. At very low \( pH \) values, a protonated species, CuHL₂⁺, can form in addition to 1:1 species (CuL⁺). Using the stability constants of α-amino acid-copper(II) and nickel(II) complexes, the distribution curves of the species as a function of \( pH \) were plotted with the aid of the SPE computer program\(^\text{13}\). The distribution curves of glycine-copper(II) and glycine nickel(II) are shown in Fig. 1 and Fig. 2 respectively.

The stability constants of the studied complexes in 40% ethanol-60% water are higher than those in aqueous media\(^\text{18, 19}\). This is expected because water has a high dielectric constant of about 80D, so the electrostatic force of attraction between two ions of opposite charge is considerably reduced. Adding an organic solvent as ethanol decreases the dielectric constant of the media, resulting in a greater attraction force and hence larger stability and protonation constants.
References

12 Gran G, Analyst, 77 (1952) 661.