Binuclear Cu$^{2+}$ complex mediated discrimination between L-glutamate and L-aspartate in water†

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L-Glutamate and L-aspartate selectivity is achieved by the action of two Cu$^{2+}$ metal ions rightly disposed in a cyclophane-type macrocyclic framework; electrochemical sensing of glutamate has been achieved by adsorption of the copper complexes on graphite electrodes.

L-Glutamate–L-aspartate discrimination is a key feature of presynaptic receptors.† Therefore, over the last years a great deal of research effort has been devoted to individuate the recognition features that operate in aminergic receptors.‡ However, recognition of zwitterionic species in water is still a challenge in host–guest chemistry. In this communication we offer means by which L-glutamate and L-aspartate discrimination can be achieved by the action of metal ions rightly disposed in a macrocyclic framework.

The receptor we deal with is the cyclophane 2,6,10,13,17,21-hexaaazapentadeca-(2,6)-pyridinophane (L) (Scheme 1). L can take up six protons in the pH range 2–11, 3.8 being its average protonation degree at pH 7.4.‡ This characteristic facilitates its interaction with oppositely charged species. Additionally, the amino and ammonium groups of L has at this pH could contribute to the binding of anions as hydrogen bond donors and/or acceptors while the pyridine aromatic moiety might provide π–ammonium interactions.\(^5\)

![Scheme 1](Image 2)

**Scheme 1** Discrimination of L-glutamate over L-aspartate by addition of two equivalents of Cu(II) per mole of ligand.

A potentiometric study performed in 0.15 mol dm$^{-3}$ NaClO$_4$ shows that in the pH range 2–11, L forms adduct species [H, L]$(\text{HGlu})^x$, with $x$ varying from 4 to 8. The values of the stepwise formation constants are in the range ca. 2–3 logarithmic units.\(^5\) A plot of the percentage of complexed amino acid per mole of ligand does not show any significant difference between the two amino acids (see lower curves in Fig. 1). However, addition of two equivalents of Cu$^{2+}$ per mole of ligand has a dramatic effect on the selective discrimination of L-glutamate over L-aspartate. pH-metric studies show the formation of mixed metal complexes of $\text{Cu}^2\text{H}_2\text{L}([\text{CF}_3\text{SO}_3])_y$ with $x$ varying from 3 to 1 and $y$ from 1 to 2, which prevail in solution above pH 4 (ref. 7 and ESI†). As shown by the percentages of complexed amino acids, the ternary complexes are much more stable in the case of L-glutamate.

![Fig. 1](Image 3)

**Fig. 1** Plot of the percentages of complexed glutamate and aspartate by L as free receptor and as Cu$^{2+}$ complex.
The relevant NMR features of these signals are reported in Table 1. Signals (a) and (b) show linewidths, measured at half-height, of around ~100 Hz, while signals (c) and (d) have linewidths of 1339 Hz. The \( T_1 \) values are relatively short, varying from <1 ms, in the case of signals (c) and (d), to 4.3 ms for signal (b). These results are consistent with the presence of a spin-coupled binuclear \( \text{Cu}^{2+} \) complex.\(^{11,13}\) The signals (c) (~3.2 ppm) and (d) (~9.8 ppm), which integrate for twenty-four protons, were assigned on the basis of the very short \( T_1 \) and \( T_2 \) values to the \( \alpha\text{-CH}_2 \) protons closest to the dicopper site (see Scheme 1). As described in a previous paper,\(^{12}\) \( \alpha\text{-CH}_2 \) protons in spin-coupled \( \text{Cu}^{2+} \) complexes have similar broad linewidths and very short \( T_1 \) values. Signal (a), which integrates for eight protons and exhibits a short \( T_1 \) value (2.1 ms), can be assigned to the \( \beta\text{-CH}_2 \) protons of the macrocyclic ligand. The other signal, (b), was assigned by exclusion to the protons of the pyridine ring.

When the spectrum of the ternary system \( \text{Cu}_3\text{L–Glu} \) is recorded in \( \text{D}_2\text{O} \), a new group of signals [(b’) and (d’)] appears (Table 1 and ESI†). These new signals have relatively short \( T_1 \) values (2.2 and 4.5 ms) and can be assigned to the \( \beta \)- and \( \gamma\text{-CH}_2 \) methylene groups of the complexed amino acid (see Table 1 and Scheme 1). On the other hand, the integration of the signals in experiments performed with different \( \text{Cu}_3\text{L–Glu} \) molar ratios show the formation of complexes of 1:1 and 1:2 stoichiometries in agreement with the potentiometric studies.

The \( ^1\text{H} \) NMR spectrum of the \( \text{Cu}_2\text{L–Asp} \) system at \( \text{pH} 6.5 \) shows a similar pattern of chemical shifts (Table 1). Nevertheless, the integration of signals [(c’) and (d’)] that correspond to the coordinated amino acid is less than in the case of Glu, suggesting that the amount of ternary complexes formed is smaller. Furthermore, variable temperature \( ^1\text{H} \) NMR spectra of \( \text{Cu}_3\text{L–Glu} \) and \( \text{Cu}_2\text{L–Asp} \), registered from 283 to 323 K, show that all isotropically shifted signals are temperature dependent and follow an anti-Curie behavior typical of antiferromagnetically coupled systems\(^{11} \) except the signals of the \( \alpha\text{-CH}_2 \) protons of the macrocyclic ligand, which show a Curie behaviour (see Table 1).

In order to check whether the amino acids bind through the amino group and one of the carboxylate groups or through the two carboxylate groups (ligation mode \( \text{Cu–O–N–Cu} \) or \( \text{Cu–O–O–Cu} \)), we have recorded the spectrum of the system \( \text{Cu}_2\text{L–Glu} \) in non-deuterated water. In this case, two new signals at 2.9 and 3.1 ppm appear, indicating the presence of two exchangeable NH protons, which supports the \( \text{Cu–O–Cu} \) ligation mode. Interestingly enough, preliminary studies with the \( \alpha\text{-,\beta-diaicids} \) succinic and glutaric having, respectively, the same separation between the carboxylic functions as aspartic and glutamic amino acids, yield a similar behavior, supporting the \( \text{Cu–O–O–Cu} \) binding mode.

The possibility of selectively sensing Asp and Glu was investigated using adsorbates and monolayer deposits of the 2:1 \( \text{Cu}^{2+}\text{L} \) complexes over glassy carbon electrodes. Adsorbate-modified electrodes were prepared, following literature procedures,\(^{14} \) by immersion of the bare carbon electrode into aqueous solutions of \( \text{CuSO}_4\cdot5\text{H}_2\text{O} \) plus \( \text{L}6\text{HBr} \) in a 2:1 ratio at different pH values. Monolayer electrodes were prepared by evaporation of a drop of that solution over the surface of the carbon electrode. Preparation and electrochemical response of self-assembled adsorbates and monolayers have been extensively studied.\(^{15–17} \) Upon immersion into 0.15 mol dm\(^{-3} \) \( \text{NaClO}_4 \) solutions in the pH range 5.5–8.5, the response was similar for both adsorbate-modified and monolayer-modified electrodes, consisting of a well-defined reduction peak at \( -0.40 \text{ V vs. AgCl} \) (3 mol dm\(^{-3} \) \( \text{NaCl} \)/Ag, as shown in Fig. 2a.

The electrochemical response of the modified electrodes was almost unchanged in solutions of aspartic acid (0.2–2.0 mmol dm\(^{-3} \)); the reduction peak being slightly shifted (20–30 mV) toward more negative potentials and followed by a weak shoulder near to \(-0.60 \text{ V} \) (Fig. 2b). In contrast, their response showed significant changes in the presence of Glu. As can be seen in Fig. 2c, a prominent reduction peak at \(-0.25 \text{ V} \) appears, preceding the reduction peak at \(-0.40 \text{ V} \). This electrochemistry can be described in terms of the reduction of surface-confined binary \( \text{Cu}_2\text{L} \) complexes (peak \( ca. -0.40 \text{ V} \) and that of ternary \( \text{Cu}_3\text{L–Glu} \) or \( \text{Cu}_2\text{L–Asp} \) ones. An analogous response is observed in ternary \( \text{Cu}_2\text{L–Glu} \) aqueous solutions. In agreement with potentiometric data, glutamic acid forms much more stable ternary complexes than aspartic acid. Accordingly, the reduction process of surface-confined \( \text{Cu}_2\text{L–Glu} \) ternary complexes at \(-0.25 \text{ V} \) appears concomitantly with the reduction peak at \(-0.40 \text{ V} \) corresponding to binary \( \text{Cu}_2\text{L} \) complexes. Comparable results were obtained upon immersion of monolayer electrodes in solutions of succinic and glutaric acids.

Again, electrodes modified with 2:1 \( \text{Cu}^{2+}\text{L} \) solutions showed significant differences for succinic (Suc) and glutaric (Glr) acids at neutral pH. Here, two well-defined peaks at \(-0.06 \) and \(-0.47 \text{ V} \) were obtained upon immersion of monolayer electrodes in solutions of succinic acid (ESI†).

This voltammetric response can be described in terms of the sequential reduction of the surface-confined parent \( \text{Cu}^{2+} \) complex to \( \text{Cu}^+ \) and Cu metal. In contact with glutaric acid solutions, however, both signals become resolved into separated peaks at \(-0.08 \) and \(-0.12 \text{ V} \), and \(-0.52 \) and \(-0.72 \text{ V} \), respectively, (ESI†).

Table 1 400 MHz \( ^1\text{H} \) NMR hyperfine-shifted resonances in \( \text{D}_2\text{O} \) at 40 °C and pH = 6.5 for \( \text{Cu}_2\text{L} \) and for \( \text{Cu}_2\text{L–Glu} \) and \( \text{Cu}_2\text{L–Asp} \) complexes, determined for 1:2 molar ratios

| System | Signal | \( \delta \) (ppm) | No. protons | Assignment | Temperature dependence | \( T_1 \) (ms) | \( \Delta v_{1/2} \) (Hz) | \( T_2 \) (ms)\
<table>
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<tr>
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<tbody>
<tr>
<td>( \text{Cu}_2\text{L} )</td>
<td>a</td>
<td>9.0</td>
<td>8</td>
<td>( \beta\text{-CH}_2 )</td>
<td>Anti-Curie</td>
<td>2.1</td>
<td>200</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>3.1</td>
<td>3</td>
<td>( \text{H}_{\text{m,p}} ) (Py)</td>
<td>Anti-Curie</td>
<td>4.3</td>
<td>81</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>-3.2</td>
<td>24</td>
<td>( \alpha\text{-CH}_2 )</td>
<td>Curie</td>
<td>&lt;1</td>
<td>1339</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>d</td>
<td>-9.8</td>
<td></td>
<td></td>
<td>Curie</td>
<td>&lt;1</td>
<td>1339</td>
<td>0.24</td>
</tr>
<tr>
<td>( \text{Cu}_5\text{L–Glu} )</td>
<td>b’</td>
<td>5.3(^{a})</td>
<td>~ 3 x 2</td>
<td>( \beta’\text{-CH}_2 ) (Glu)</td>
<td>—</td>
<td>2.2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>d’</td>
<td>2.5</td>
<td>~ 2 x 2</td>
<td>( \gamma\text{-CH}_2 ) (Glu)</td>
<td>Anti-Curie</td>
<td>4.5</td>
<td>144</td>
<td>2.2</td>
</tr>
<tr>
<td>( \text{Cu}_2\text{L–Asp} )</td>
<td>c’</td>
<td>3.1</td>
<td>~ 3 x 2</td>
<td>( \beta’\text{-CH}_2 ) (Asp)</td>
<td>Anti-Curie</td>
<td>3.3</td>
<td>192</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>d’</td>
<td>2.0</td>
<td>~ 3 x 2</td>
<td>( \beta’\text{-CH}_2 ) (Asp)</td>
<td>Anti-Curie</td>
<td>2.9</td>
<td>—</td>
<td>—</td>
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\(^{a}\) Measured from the line width at half-height. \(^{b}\) Measured at 288 K. \(^{c}\) Overlap prevents measurement of this value.
In agreement with the foregoing set of considerations, peak splitting can be attributed to the reduction of binary CuL and ternary Cu2L–Glr surface-confined complexes.

In conclusion, the systems here presented provide means for discriminating between glutamic and aspartic amino acids by means of their complexation as bridging M–O–O–M ligands to a pyridinoline binuclear Cu2+ complex with unsaturated coordination sites and matching dimensions. Moreover, the alteration of the voltammetric response of the Cu2+ complex in the presence of glutamic acid permits its electrochemical sensing. Similar arguments can be applied for distinguishing between glutaric and succinic acids.

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Notes and references


6. Potentiometric measurements were carried out at 298.1 K in 0.15 mol dm−3 NaClO4. The program HYPERQUAD (P. Gans, A. Sabatini and A. Vacca, Talanta, 1996, 43, 1739) was used to compute the equilibrium constants. Protonation constants for t-glutamate (A−): A−H+ ≡ HA−, log K = 9.47; A−H2 ≡ H2A−, log K = 12; H3A− ≡ H+ + H2A−, log K = 2.32; protonation constants for l-aspartate (A−): A−H+ ≡ HA−, log K = 9.65; HA− + H+ ≡ H2A−, log K = 3.82; H2A− + H+ ≡ H3A−, log K = 2.0. System t-glutamate (A−): L: H2L6+ + HA− ≡ H2Al6−, log K = 1.89(4); H2L6+ + AH2− ≡ H2Al6−; log K = 2.27(3); H2L6+ + AH3− ≡ H2Al6−; log K = 2.26(2); H2L6+ + AH4− ≡ H2Al6−; log K = 2.92(2); System t-aspartate (A−): L: H2L6+ + AH− ≡ HAl6−, log K = 2.27(2); H2L6+ + AH2− ≡ H2Al6−; log K = 2.29(2); H2L6+ + AH3− ≡ H3Al6−; log K = 2.91(2); H2L6+ + AH4− ≡ H4Al6−; log K = 2.58(2).

7. System Cu2+–l-glutamate (A−): L: ML6+ + A− ≡ MLA6−, log K = 8.92(7); ML6+ + HA− ≡ MLA5− + H+; log K = 8.73(6); ML6+ + AH− ≡ MLA4−, log K = 7.84(4); ML6+ + H2A− ≡ MLA3− + H+; log K = 7.56(3); ML6+ + H3A− + HA− ≡ MLA2− + H2; log K = 5.94(6); ML6+ + H3A− + AH2− ≡ MLA+ + H2; log K = 6.44(6); ML6+ + AH− ≡ MLA0− + H+; log K = 9.04(5); ML6+ + ML4+ + H2A− + MLA2− + H+; log K = 8.09(2); ML6+ + ML2+ + 2A− ≡ MLA0− + H2; log K = 14.04(2); ML6+ + ML2+ + 2A− ≡ MLA0− + H+; log K = 5.77(7); MLA0− + H2 + 2L ≡ MLA0− + H+ + 2L; log K = 10.44(1); MLA0− + 2L + 2H ≡ 2MLA− + 2OH−; log K = 11.37(9). System Cu2+–l-aspartate (A−): L: ML6+ + AH− ≡ MLA5−, log K = 2.50(1); ML6+ + AH− + AH− ≡ MLA4−; log K = 3.54(1); ML6+ + AH− + AH2− ≡ MLA3−; log K = 3.43(8); ML6+ + H2A− ≡ MLA5−; log K = 3.06(2); ML6+ + AH− ≡ MLA4−; log K = 4.15(8); ML6+ + AH2− ≡ MLA3−, log K = 5.09(7); ML6+ + AH3− + AH− ≡ MLA2−; log K = 5.30(6); ML6+ + AH3− + 2AH− ≡ MLA0− + 2L; log K = 8.88(1); ML6+ + AH2− + A2− ≡ MLA− + 2L; log K = 5.04(9).


Fig. 2 SQWVs† at a GCE modified by a deposit obtained via evaporation of 50 μL of a 2.0 × 10−3 mol dm−3 CuSO4·5H2O plus 1.0 × 10−3 mol dm−3 L-6HBr solution at pH 6.5 immersed into a) 0.15 mol dm−3 NaClO4; b) idem plus 2.0 × 10−2 mol dm−3 aspartic acid; c) idem plus 2.0 × 10−3 mol dm−3 glutamic acid, all at pH 7.5. Potential step increment 4 mV; square wave amplitude 25 mV; frequency 5 Hz.