The Copper(II) Complex with the Imidazole-bound Histamine Derivative of β-Cyclodextrin as a Powerful Chiral Discriminating Agent

Vincenzo Cucinotta,[a] Franca D'Alessandro,[a] Giuseppe Impellizzeri[a] and Graziella Vecchio[b]

[a] *Dipartimento di Scienze Chemiche, Università di Catania, viale A. Doria 8, 95125 Catania, Italy*
[b] *Istituto per lo Studio delle Sostanze Naturali di Interesse Alimentare e Chimico-Farmaceutico, CNR, viale A. Doria 6, 95125 Catania, Italy*

The newly synthesized 6-derivative of β-cyclodextrin with histamine, bound through the N-1 of imidazole was used as a ligand for copper(II) ion, the ligand being characterized by NMR spectroscopy and the copper(II) complex by EPR spectroscopy; the complex was used in chiral ligand exchange chromatography and was able to separate L- and D-tryptophan.

The ability of cyclodextrins to include guest molecules of suitable size and shape,[1] together with their chirality, makes applications of this class of molecules stereoselective, and the inclusion of chiral compounds gives rise to diastereoisomeric complexes,[2–4] thus providing a way to discriminate between enantiomers. However, as far as amino acids are concerned, only their derivatives have been separated by unmodified or by alkylderivatized cyclodextrins.[5,6]

In order to improve their chiral recognition, cyclodextrins have been chemically modified. Enantioselective binding of tryptophan by α-cyclodextrin[7] or by a difunctionalized β-cyclodextrin[8] has been claimed. However, chiral discrimination has recently been achieved in ligand exchange chromatography (LEC) by adding a histamine-modified β-cyclodextrin able to coordinate copper(II) ion to the eluent.[9] Some of us have previously shown that the discrimination seems to

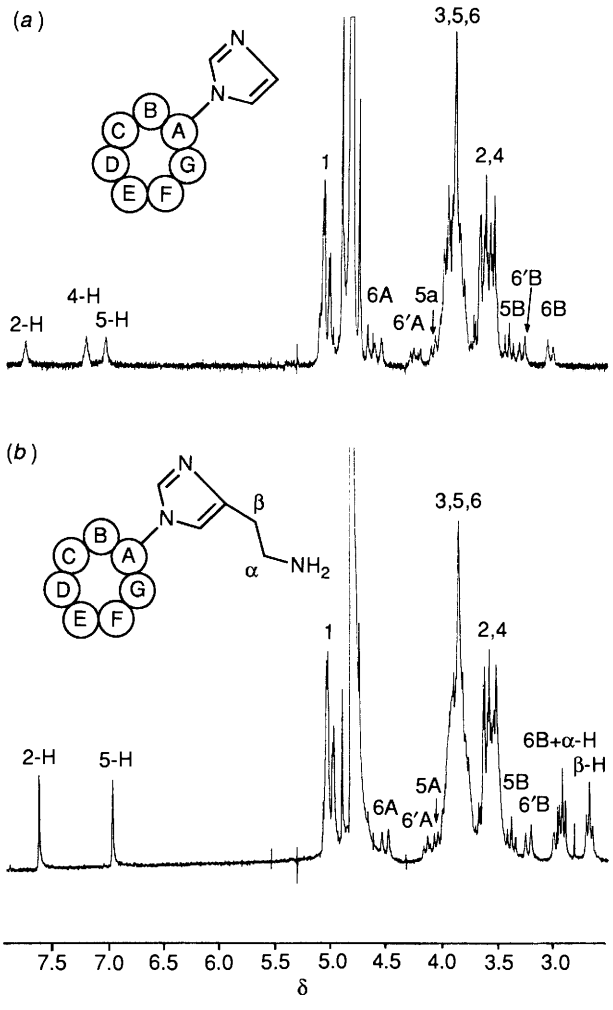

Fig. 1 ^{1}H NMR spectrum of (*a*) CDIm; (*b*) CDmh

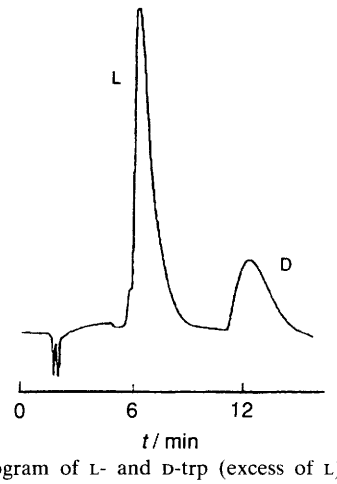

Fig. 2 Chromatogram of L- and D-trp (excess of L). See text for conditions.

occur mainly in the mobile aqueous phase, and the enantioselectivity may be directly related to differential complexation in the aqueous phase.[9]

Here, we report the synthesis and the characterization of 6-deoxy-6-[4-(2-aminoethyl)imidazolyl]cyclomaltoheptaose (CDmh). 6-Deoxy-6-(imidazolyl)cyclomaltoheptaose (CDIm) was synthesized for comparison. The copper(II) complex of CDmh was tested and compared with the corresponding copper(II) complex of 6-deoxy-6-[2-(4-imidazolyl)ethylamino)]cyclomaltoheptaose (CDhm) as a chiral discriminating agent in LEC chromatography on tryptophan enantiomers. CDmh was synthesized by the reaction of 6-deoxy-6-iodocyclomaltoheptaose with histamine in anhydrous dimethylformamide (DMF) at 80 °C, under a nitrogen stream. After 24 h, the DMF was evaporated off and the reaction mixture purified by CM-Sephadex C-25 (NH_4^+ form) column chromatography (eluent NH_4HCO_3, 0–0.2 mol dm^{-3}). CDmh was eluted after the other histamine derivative.[10] The purity of the product was checked by HPLC: yield 7%; TLC (SiO_2 plates) R_f = 0.15, eluent PrOH–H_2O–AcOEt–NH_3 (5:3:2:1); FAB MS, *m/z* 1228 (M + 1).

CDIm was synthesized by the reaction of 6-iodo-6-deoxycyclomaltoheptaose with imidazole at 70 °C for 48 h. The product was isolated by column chromatography (CM-Sephadex C-25, eluent: NH_4HCO_3, 0–0.1 mol dm^{-3}), FAB MS *m/s* 1185 (*M* + 1).

Comparison of the ^{1}H NMR spectra at 250 MHz in D_2O of CDmh and of CDIm (Fig. 1), assigned by COSY spectroscopy, shows that, apart from the presence of the four methylene protons and the absence of the 4-proton of imidazole, the two spectra are almost superimposable. As with the cyclo-(L-histidyl-L-histidyl) derivative,[11] the multiplet at δ 4.6–3.9 was assigned to the 5- and 6-protons of the *A* ring (the substituted ring) owing to a through-the-chain effect, while the two doublets at δ 3.1–3.5 were assigned to the 6-protons of the *B* ring. As a consequence, these 6-*B* upfield shifts are also ascribed to the ring-current effect of the 6-bonded imidazole placed almost perpendicularly to the cavity, as also found for CDIm.

EPR spectra, recorded on a Bruker ER 200D spectrometer, in frozen 9:1 water–methanol solutions of CDmh copper(II) complexes, were recorded for a 1:1 metal–ligand molar ratio. A single complex species was obtained at pH 6.5, with the following parameters: $g_{\|} = 2.300$, $A_{\|} = 173 \times 10^{-4}$ cm^{-1}, $g_{\perp} = 2.060$, $A_{\perp} = 165 \times 10^{-4}$ cm^{-1}. Comparing the case of $[Cu(hm)]^{2+}$ [12] (hm = histamine) and $[Cu(CDhm)]^{2+}$ [10] shows that in the latter complex a distortion in the copper(II) coordination is present in comparison to the other two complexes, as shown by its parallel hyperfine coupling constant. This distortion was ascribed to a cavity effect, as already discussed.[10] In contrast, in the case of the copper(II) complex with CDmh, the magnetic parameters are almost identical with those of $[Cu(hm)]^{2+}$, and the complex has its usual elongated octahedral geometry and can be formulated as $[Cu(L)(H_2O)_4]^{2+}$. The other species existing at pH 9.2 is probably a hydroxo complex in which a molecule of water in the complex has been deprotonated and the complex can be formulated as $[Cu(CDhm)(OH)(H_2O)_3]^+$.

The copper(II) complex of CDmh was used as a chiral additive in the mobile phase in LEC with HPLC. The mobile phase was prepared by dissolving the complex (6×10^{-5} mol dm^{-3}) and sodium acetate (3×10^{-3} mol dm^{-3}) in H_2O–MeOH (60:40), at pH 7. A conventional achiral reversed-phase Spherisorb ODS-2 (3 μm, 150 × 4.6 mm) was equilibrated and eluted (flux 0.8 cm^3 min^{-1}) with the prepared mobile phase. The D- and L-trp were separated, as shown in Fig. 2, with an enantioselectivity factor of 2.4 (elution order $K_L < K_D$). The D- and L-alanine system was eluted much faster and was not separated under our experimental conditions.

If we compare the results of this investigation with a previous study,[9] in which CDhm–copper(II) complex was used, it is immediately apparent that the elution order is opposite in two cases. Furthermore, the CDmh complex is much more efficient as a chiral discriminating agent as shown by the α values.

As regards the opposite behaviour of CDhm and CDmh complexes in the order of elution, this would be expected if in the ternary complexes the amino acid is forced to assume the *cis*-disposition (adjacent amino-groups). If the copper(II) coordination plane is almost parallel to the cavity, the interconversion in the mutual disposition of the cavity and of

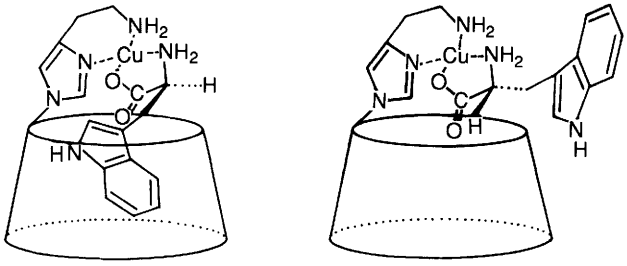

Fig. 3 Schematic representation of the Cu^{II}–CDmh ternary complexes with (*a*) L-trp and (*b*) D-trp

the histamine chain between CDhm and CDmh which is due to their different bonding gives rise to *cis* ternary complexes of amino acids where the enantiomer, whose side chain is included, is different in the two cases. Fig. 3 shows these CDhm ternary complexes.

Lastly, we emphasise the high chiral discriminating ability of this system, which may be appreciated when considering the very low concentration of the CDmh complex necessary to obtain the separation described. In comparison with the CDhm complex, the improvement is evident. In our opinion, the bond of histamine through imidazole N-1, compared with the amino bond, confers more rigidity to the molecule, which favours a more efficient inclusion of the right enantiomer in the ternary complex. Such rigidity is only apparent in ternary complexes because, as shown both by NMR studies of the ligand and by EPR studies of the binary complex, it is the very coordination of the second ligand that causes it. In the free ligand, the end of the chain is not restrained, thus not forcing the chain into a fixed position. In the binary complex, it is the small size of the coordinated water molecules which permits the copper(II) ion to assume the best steric disposition. As soon as the tryptophan molecule substitutes the in-plane coordinated water molecules, a second restraint is created, and now the histamine chain, restrained at both ends is no longer able to move freely.

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