Chiral Recognition and Separation of Amino Acids by Means of a Copper(II) Complex of Histamine Monofunctionalized β-Cyclodextrin

Roberto Corradini,[†] Arnaldo Dossena,[†] Giuseppe Impellizzeri,[‡] Giuseppe Maccarrone,[‡] Rosangela Marchelli,*[,†] Enrico Rizzarelli,*[,‡,§] Giorgio Sartor,[⊥] and Graziella Vecchio[§]

Contribution from the Dipartimento di Chimica Organica e Industriale dell'Università, I-43100 Parma, Italy, Dipartimento di Scienze Chimiche dell'Università, I-95125 Catania, Italy, Istituto per lo Studio delle Sostanze Naturali di Interesse Alimentare e Chimico-Farmaceutico del CNR, I-95125 Catania, Italy, and Istituto di Chimica Biologica dell'Università di Parma, I-43100 Parma, Italy

Received December 16, 1993. Revised Manuscript Received August 16, 1994®

Abstract: The copper(II) complex of a histamine-modified cyclodextrin (6-deoxy-6-*N*-histamine-β-cyclodextrin, CDhm) was used for the chiral recognition of amino acids. HPLC separation of the enantiomers of unmodified aromatic amino acids (Phe, Trp, and Tyr) was obtained by using the complex $[Cu(CDhm)]^{2+}$ as additive to the eluent and an achiral column C_{18}. Evidence for enantioselectivity was provided by thermodynamic and spectroscopic measurements. Potentiometric studies of the ternary complexes formed by $[Cu(CDhm)]^{2+}$ and D- or L-amino acids showed that enantioselectivity in the complexation of aromatic amino acids occurs also in aqueous solution, the stability constants of the complexes containing the D-enantiomers of Trp, Phe, and Tyr being larger than those of the corresponding L-ones. In contrast, aliphatic amino acids showed small, if any, differences in the stability of diastereomeric ternary complexes and were not separated by HPLC. Calorimetric studies were carried out in order to determine the enthalpy and entropy contribution to enantioselectivity: the overall complexation process was found to be enthalpically and entropically favored. For the complexes containing aromatic amino acids, however, the enthalpy contribution was found to be more favorable for the D-enantiomers, while entropy was less favorable. These results are consistent with a model in which the complexation of the D-enantiomers is favored by the preferential inclusion of the aromatic side chain in the cyclodextrin cavity. Accordingly, the CD spectra of the complexes containing aromatic D-amino acids showed much higher intensity ($\Delta\epsilon$) than those of the corresponding L-ones, the difference $\Delta(\Delta\epsilon)$ increasing as the size of the side chain increased. Furthermore, the fluorescence of D-Trp in the ternary complex was found to be smaller than that of L-Trp. Fluorescence lifetime measurements suggested that enantioselectivity in fluorescence could arise from the more efficient quenching of D-Trp by copper(II) ion, due to conformational constraints holding the indole moiety near the metal ion.

Introduction

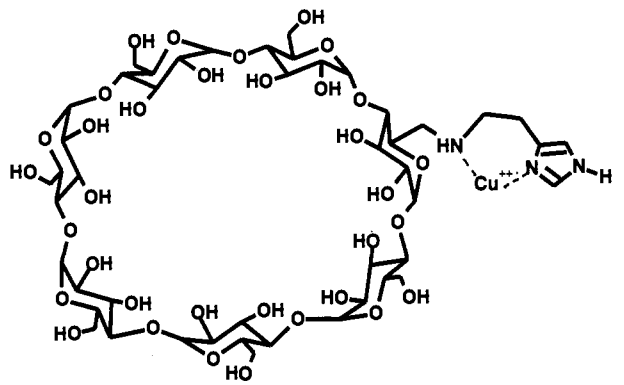
The α-, β-, and γ-cyclodextrins (CDs) are six-, seven- and eight membered α-1,4-linked cyclic oligomers of D-glucopyranose with different internal annular diameters.[1,2] Due to their ability to form inclusion complexes, they have been used as biomimetic models to study substrate binding[3] and enzymatic catalysis[4,5] and as a particular reaction medium.[6,7] Recently, cyclodextrins have also been employed for enantiomeric separations and analytical purposes by using various chromatographic procedures, in particular high performance liquid chromatography (HPLC)[8] and gas chromatography (GC).[9] Two different approaches have been used for the separation of enantiomers by HPLC: in the former α-, β-, or γ-cyclodextrins have been covalently linked to silica to form chiral stationary phases (CSPs),[10] in the latter cyclodextrins have been directly added to the mobile phase,[11] a technique first introduced in thin layer chromatography.[12] For enantiomeric separation to occur in solution, it is generally assumed that the molecular dimensions and shape of the chiral selector have to fit those of the selectand, in such a way that at least three discriminating interactions (either attractive or repulsive) occur.[13] By using cyclodextrins or their alkyl derivatives, chiral recognition has been performed for a number of drugs,[8,14] derivatives of metallocenes, carboxylic acids and other organic molecules[15] as well as derivatized amino acids (HPLC,[16-18] TLC,[19] HPCE,[20] and GC[21]). Cyclodextrins, bearing a positive and a negative charge on the C(6) carbons of adjacent A and B

† Dipartimento di Chimica Organica e Industriale dell'Università di Parma.
‡ Dipartimento di Scienze Chimiche dell'Università di Catania.
§ Istituto per lo Studio delle Sostanze Naturali di Interesse Alimentare e Chimico-Farmaceutico del CNR di Catania.
⊥ Istituto di Chimica Biologica dell'Università di Parma.
® Abstract published in *Advance ACS Abstracts,* October 1, 1994.

(1) Bender, M. L.; Komiyama, M. *Cyclodextrin Chemistry*; Springer Verlag: Berlin, 1978.
(2) Saenger, W. In *Inclusion Compounds*; Atwood, J. L., Davies, J. E. D., Mac Nicol, D. D., Eds.; Academic Press: London, 1984; Vol. 2, p 235.
(3) Breslow, R. *Acc. Chem. Res.* **1991**, *24*, 317.
(4) Tabushi, I. *Acc. Chem. Res.* **1982**, *15*, 66.
(5) Breslow, R.; Zhang, B. *J. Am. Chem. Soc.* **1992**, *114*, 5882.
(6) Menger, F. M.; Dulany, M. A. *Tetrahedron Lett.* **1985**, *26*, 267.
(7) Le Noble, W. J.; Srivastava, S.; Breslow, R.; Trainor, G. *J. Am. Chem. Soc.* **1983**, *105*, 2745.
(8) Menges, R. A.; Armstrong, D. W. In *Chiral Separation by Liquid Chromatography;* ACS Symposium Series 471, Ahuja, S., Ed.; Washington, D.C., 1991, p 67. Armstrong, D. W.; Ward, T. J.; Armstrong, R. D.; Beesley, T. E. *Science* **1986**, *232*, 1132.
(9) Schurig, V.; Nowotny, H. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 939.
(10) Armstrong, D. W.; Yang, X.; Han, S. M.; Menges, R. A. *Anal. Chem.* **1987**, *59*, 2594.
(11) Sybilska, D.; Zukowski, J.; Bojarski, J. *J. Liq. Chromatogr.* **1986**, 9, 591.
(12) Hinze, W. L.; Armstrong, D. W. *Anal. Lett.* **1980**, *13*, 1093.
(13) Ogston, A. G. *Nature* **1948**, *162*, 963. Dalgliesh, C. E. *J. Chem. Soc.* **1952**, 3940.
(14) Armstrong, D. W.; Li, W.; Stalcup, A. L.; Secor, H. V.; Izac, R. R.; Seeman, J. I. *Anal. Chim. Acta* **1990**, *234*, 365.
(15) Ward, T. J.; Armstrong, D. W. *J. Liq. Chromatogr.* **1986**, *9*, 407.
(16) Debowski, J.; Jurczak, J.; Sybilska, D.; Zukowski, J. *J. Chromatogr.* **1985**, *329*, 206.
(17) Hinze, W. L.; Riehl, T. E.; Armstrong, D. W.; De Mond, W.; Alak, A.; Ward, T. J. *Anal. Chem.* **1985**, *57*, 237.
(18) Han, S. M.; Armstrong, D. W. *J. Chromatogr.* **1987**, *389*, 256.
(19) Armstrong, D. W.; He, F. Y.; Han, S. M. *J. Chromatogr.* **1988**, *448*, 345.
(20) Guttman, A.; Paulus, A.; Cohen, A. S.; Grinberg, N.; Karger, B. L. *J. Chromatogr.* **1988**, *448*, 41.

0002-7863/94/1516-10267$04.50/0

Chart 1



[Cu(CDhm)]$^{2+}$

(or B and A) glucose rings, have been used as artificial receptors for the enantioselective recognition of D,L-tryptophan,[22] based on triple recognition (two polar sites and the hydrophobic cavity). However, no evidence for chiral discrimination for unmodified amino acids has been reported in HPLC so far with β-cyclodextrins, although aromatic amino acids were separated by an α-cyclodextrin stationary phase.[10]

Enantiomeric separation of unmodified amino acids was achieved in HPLC by means of chiral copper(II) complexes dissolved in the eluent with achiral reversed phase columns,[23,24] according to the mechanism of ligand exchange chromatography (LEC).[25] The usefulness of this approach as well as evidences on the mechanism of ligand exchange have recently been reported by some of us, by using copper(II) complexes of L-amino acid amides.[26] However, as far as we know, there are no reports on the use of functionalized β-cyclodextrins complexed to metal ions as mobile phase additives, except two preliminary communications from our laboratories.[27,28]

In the present study, we present a detailed investigation on the chiral recognition and separation ability in HPLC of the copper(II) complex of 6-deoxy-6-*N*-histamine-β-cyclodextrin [Cu(CDhm)]$^{2+}$ (Chart 1) toward unmodified aromatic amino acids, in particular, phenylalanine, tyrosine, and tryptophan (AaO$^-$ = PheO$^-$, TyrO$^-$, and TrpO$^-$).

Quantitative information and evidence of the mechanism of chiral recognition were obtained by means of a combined thermodynamic (potentiometric and calorimetric measurements) and spectroscopic (UV–vis, CD, and fluorescence) approach. The stability constants of the diastereomeric ternary complexes [Cu(CDhm)(D-AaO)]$^+$ and [Cu(CDhm)(L-AaO)]$^+$ were compared to the elution order found in the HPLC separation ($k'_D < k'_L$). The thermodynamic stereoselectivity is discussed in enthalpy and entropy terms.[29] Comparison with the analogous ternary complexes of [Cu(CDhm)]$^{2+}$ with L- and D-alanine (L-/D-AlaO$^-$), which do not show different stabilities, indicates the possibility of a significant interaction of the aromatic amino acid side chains with the cavity of the cyclodextrin molecule. CD and fluorescence data are consistent with the thermodynamic parameters and provide evidence for the different complexation mode of the enantiomers in aqueous solution.

Experimental Section

Chemicals. Copper(II) nitrate was prepared from copper(II) basic carbonate by adding a slight excess of HNO_3. The concentrations of the stock solutions were determined by EDTA titrations with murexide as indicator. The excess HNO_3 was determined by the Gran's method[30] and by the ACBA computer program.[31] Copper(II) sulfate pentahydrate was from Aldrich. The concentrations of HNO_3 and KOH stock solutions were determined by titration with the primary standard tris(hydroxymethyl)aminomethane (THAM) and potassium hydrogen phthalate, respectively. Potassium nitrate (Suprapur Merck) was used without further purification. All solutions were prepared with doubly distilled water. All amino acids (Aldrich or Fluka) were high purity products used without further purification. Their purity was checked by means of potentiometric titrations with a standard KOH solution and was always higher than 99.8%. Polarimetric tests gave substantially identical results.

Monohydrate copper(II) acetate (RPE-ACS grade) and HPLC grade solvents used for eluent preparation (HPLC or RS-grade) were obtained from C. Erba (Italy). Sodium acetate was obtained from Rudi-Pont. KI was from Merck, and acrylamide was from United States Biochemical Co. Glycogen for time-resolved fluorescence experiments was from Aldrich. CDhm was synthesized as previously reported.[32]

Methods. Chromatographic separations were carried out on a Waters HPLC chromatograph, equipped with a Model 6000 A pump and a Model 440 UV detector, set at 280 or 254 nm, using a Radialpak C_{18} column (10 × 0.8 cm). The mobile phase was prepared as previously described,[26] by dissolving copper acetate and CDhm in a water:methanol = 70:30 mixture. The pH was adjusted to 7.5 by addition of 1 M NaOH, and the solution was filtered on a Nucleopore PE membrane (0.4 μm pore size). The dead volume (t_0) was calculated by injection of acetic acid.

The overall stability constants reported in Table 2 were obtained by computer-controlled potentiometric titrations, performed with a Metrohm digital pH meter (Model 654) with a titration cell (2.5 cm^3) thermostated at 25.0 ± 0.2 °C. All solutions were kept under an atmosphere of nitrogen, bubbled through a solution of the same ionic strength at the same temperature as the solution under study. The KOH solution was added from a Hamilton burette equipped with 0.25 or 0.50 cm^3 syringes. Changes in pH were measured using a combined microelectrode (Metrohm 125) calibrated in hydrogen-ion concentration by titrating HNO_3 solutions. All solutions had an ionic strength of 0.10 mol dm^{-3} (KNO_3). The β-cyclodextrin derivative (L), the copper(II) ion (M), and the amino acid (L') concentrations ranged from 0.003 to 0.006 mol dm^{-3}. Duplicate or triplicate titrations were carried out at a 1:1:1 = L:M:L' ratio. Other details were as previously described.[33]

ΔH^0 data reported in Table 3 were obtained by calorimetric measurements, performed at 25.000 ± 0.001 °C using a Tronac 450 isoperibolic calorimeter equipped with a titration Dewar (25 cm^3). The calorimetric system was calibrated by titrating THAM with HCl according to Grenthe.[34] Titrations were carried out by adding HNO_3 (0.2–0.4 mol dm^{-3}) to the solutions and the ligands. Reaction heats, corrected for the heat of dilution, determined by separate experiments, were calculated by considering the calorie unit as equivalent to 4.184 J. ΔG^0 values were calculated from the stability constants obtained by potentiometry and ΔS^0 values from ΔH^0 and ΔG^0. Other experimental details were as previously reported.[35]

Electronic and CD spectra were recorded on a Perkin-Elmer Lambda 5 spectrophotometer and on Jasco J-600 and J-500 A dichrographs, respectively. Calibration of the CD instrument was performed with a 0.06% solution of ammonium camphorsulfonate in water ($\Delta\epsilon$ = 2.40 at 290.5 nm). Optical absorption and circular dichroism spectra were recorded at 25 °C on freshly prepared aqueous solutions of the binary and ternary systems. The spectral range between 200 and 700 nm was covered by using quartz cells of various path lengths so that dilution of

(21) Koenig, W. A.; Lutz, S.; Wenz, G. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 979.
(22) Tabushi, I.; Kuroda, Y.; Mizutani, T. *J. Am. Chem. Soc.* **1986**, *108*, 4514.
(23) Gil-Av, E.; Tishbee, A.; Hare, P. E. *J. Am. Chem. Soc.* **1980**, *102*, 5115.
(24) Weinstein, S. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 218.
(25) Davankov, V. A.; Navratil, J. D.; Walton, H. F. *Ligand Exchange Chromatography*; CRC Press: Boca Raton, FL, 1988. Davankov, V. A. *J. Chromatogr.* **1994**, *666*, 55.
(26) Galaverna, G.; Corradini, R.; De Munari, E.; Dossena, A.; Marchelli, R. *J. Chromatogr.* **1993**, *657*, 43.
(27) Impellizzeri, G.; Maccarrone, G.; Rizzarelli, E.; Vecchio, G.; Corradini, R.; Marchelli, R. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1348.
(28) Cucinotta, V.; D'Alessandro, F.; Impellizzeri, G.; Vecchio, G. *J. Chem. Soc., Chem. Commun.* **1992**, 1743.
(29) Cucinotta, V.; Purrello, R.; Rizzarelli, E. *Comments Inorg. Chem.* **1990**, *11*, 85.
(30) Gran, G. *Analyst* **1952**, *77*, 661.
(31) Arena, G.; Rizzarelli, E.; Sammartano, S.; Rigano, C. *Talanta* **1979**, *26*, 1.
(32) Bonomo, R. P.; Cucinotta, V.; D'Alessandro, F.; Impellizzeri, G.; Maccarrone, G.; Vecchio, G.; Rizzarelli, E. *Inorg. Chem.* **1991**, *30*, 2708.
(33) Bonomo, R. P.; Cali, R.; Cucinotta, V.; Impellizzeri, G.; Rizzarelli, E. *Inorg. Chem.* **1986**, *25*, 1641.
(34) Grenthe, I.; Ots, H.; Ginstrup, O. *Acta Chem. Scand.* **1970**, *24*, 1067.
(35) Borghesani, G.; Pulidori, F.; Remelli, M.; Purrello, R.; Rizzarelli, E. *J. Chem. Soc., Dalton Trans.* **1990**, 2095.

Table 1. HPLC Capacity Factors ($k' = (t - t_0)/t_0$) and Enantioselectivity Factors ($\alpha = k'_L/k'_D$) for the Chiral Separation of Amino Acids with $[Cu(CDhm)]^{2+}$ Added to the Eluent[b]

AA	k'_D	k'_L	$\alpha = k'_L/k'_D$
Ala	1.8	1.8	1
Pro	1.9	1.9	1
His	2.5	2.5	1
Val	2.9	2.9	1
Leu	5.6[a]	5.8[a]	0.96[a]
Tyr	3.6	4.0	1.10
Phe	6.9	7.8	1.12
Trp	8.4	10.4	1.23

[a] Elution order not well defined (shoulders). [b] Eluent: $[Cu(CDhm)]^{2+}$ (7.5×10^{-5} M), H_2O:MeOH = 70:30, pH = 7.5, flow rate = 1 cm³/min, detector, UV (254 nm); column: Radialpak Cartridge RP18 (10 × 0.8 cm).

the solution was not required. Results are reported in terms of ϵ (molar absorption coefficient) and of $\Delta\epsilon$ (molar CD coefficient) in dm³ mol⁻¹ cm⁻¹.

Fluorescence spectra were recorded on a Jasco FP 770 or on a Perkin Elmer MPF 44A instrument on a 0.2 × 1 cm quartz cell thermostated at 25 °C. Temperature was controlled by Braun Thermomix 1441-Frigomix 1495 units.

Concentrated stock solutions of $[Cu(CDhm)]^{2+}$ (1×10^{-2} mol dm⁻³) and D- and L-Trp (1×10^{-2} mol dm⁻³) were prepared at pH = 7.5 in a 0.3 M aqueous sodium acetate. To D- and L-Trp (1×10^{-4} mol dm⁻³) solutions, prepared by dilution of the former with the same medium, were added aliquots of concentrated $[Cu(CDhm)]^{2+}$. Fluorescence intensity was corrected first for the dilution effect and then for absorption filter effect, by means of the expression: $F_{corr} = F_{obs}10^{[(A_{em}+A_{ex})/2]}$, where F_{obs} is the observed fluorescence intensity and A_{ex} and A_{em} are the absorbance of the sample at the excitation and emission wavelengths, respectively. Results were reported in Stern–Volmer graphs by plotting the ratio F_0/F of the fluorescence intensity, at the maximum emission, in the absence and in the presence of the quencher (corrected for absorbance), versus the concentration of the $[Cu(CDhm)]^{2+}$ complex.

For the quenching experiments of the ternary complexes [Cu(CDhm)-(L-TrpO)]⁺ and [Cu(CDhm)(D-TrpO)]⁺ (0.1 mol dm⁻³) by KI and acrylamide, the samples were prepared by dilution of concentrated stock solutions at pH = 7.5 (in 0.3 mol dm⁻³ sodium acetate). Two cubic centimeters of the diluted solutions were put in a 1 × 1 cm quartz cell and KI (2.0 mol dm⁻³), or acrylamide (2.0 mol dm⁻³) concentrated solutions in doubly distilled water were added by means of a Gilson automatic pipette. Fluorescence intensity was corrected for filter effects as reported above. For KI quenching, data were treated in accordance with Lehrer,[36] by plotting the ratio $F_0/\Delta F$ ($\Delta F = F_0 - F$) versus 1/[KI] and calculating with linear regression the intercept (inverse of the accessibility) corresponding to infinite concentration. In the case of acrylamide quenching, data were found to be consistent with a model including interaction volumes.[37]

Fluorescence lifetime measurements were carried out on a single-photon-counting instrument equipped with a nanosecond pulse flash lamp (Edinburgh Instrument, mod F199), modified in order to allow N_2 to flux at a rate of 1 cm³/min; Jasco and Farrand monochromators and Philips XP2020Q photomultiplier were used for fast detection. Fast NIM electronics were from EG&G, Tennelec, and Silena. Decays were recorded on a Silena BS27n multichannel analyzer (512 channels, 86 ps/channel). Alternate measurements of the sample and of a scattering solution (glycogen) were made in order to compensate for lamp fluctuations and drift.

Samples were prepared by mixing freshly made standard solutions of D- or L-tryptophan and of the $[Cu(CDhm)]^{2+}$ complex at pH = 7.5 (in 0.3 mol dm⁻³ sodium acetate). The excitation wavelength was set at 295 nm, and measurements for each sample were made at different wavelengths in order to cover the emission spectrum of tryptophan. Data analysis of the fluorescence decays was carried out as reported in the literature.[38] Fluorescence lifetimes were calculated from decay data, utilizing a global approach[39] by means of a three-lifetime model and utilizing the following equation:

$$F(t) = \sum_i \alpha_i e^{-t/\tau_i}$$

Decay associated spectra (DAS) were obtained by means of the equation

$$F_i = \frac{\alpha_i \tau_i}{\sum_i \alpha_i \tau_i} F_{tot}$$

and were normalized to the value of the maximum total fluorescence of L-Trp.

Calculations of electrode system E^0 values, ligand purity, and HNO_3 excess in metal stock solutions were performed by the least-squares ACBA[31] computer program. Calculation of the formation constants of the copper-(II) complexes was performed by means of the least-squares SUPER-QUAD[40] computer program. The enthalpies of formation were computed by means of the least-squares DOEC[41] program. The species distribution as a function of pH reported in Figure 1 was obtained by using the DISDI[42] computer program and was calculated from the stability constants reported in Table 2. The electronic, CD, and fluorescence spectra were measured at pH 7.5, where the complex to be characterized was nearly 100%, as calculated by DISDI. Errors are expressed as three times the standard deviation. The thermodynamic data concerning the proton and the copper-(II) binary complex formation have been previously reported.[32]

The molecular graphics were drawn by means of the SYBYL program (available at the Centro di Calcolo Elettronico of the University of Parma). The models (reported in Figure 2) built with correct bond distances and angles were proposed to account for the experimental data and were not energy minimized.

Results and Discussion

Enantioselectivity of the $[Cu(CDhm)]^{2+}$ Complex toward Amino Acids in HPLC. Enantioselectivity of the $[Cu(CDhm)]^{2+}$ complex toward amino acids was first tested in HPLC (reversed phase) by adding the complex to the eluent. In fact, also weak discriminant interactions are amplified in the chromatographic system.[25] As previously found for a tryptophan enantiomeric mixture,[27] good enantiomeric resolution of the aromatic amino acids phenylalanine and tyrosine was obtained, with elution order $k'_D < k'_L$, whereas the aliphatic amino acids were not resolved (Table 1).

In agreement with a ligand exchange mechanism, the present results may be interpreted in terms of the formation of diastereomeric ternary complexes $[Cu(CDhm)(AaO)]^+$ of different stabilities. According to a model previously proposed for ligands such as cyclodextrins,[43] in which the chiral selector resides entirely in the mobile phase, the enantioselectivity factors (α) depend on the relative stabilities of the ternary complexes in solution and the enantiomer forming the more stable complex should elute first.

This can be rationalized as described in Scheme 1: the amino acid AaO^- undergoes a partition equilibrium between the mobile phase $(AaO^-)_m$ and the stationary phase $(AaO^-)_s$, whereas the binary complex $[Cu(CDhm)]^{2+}$ and the ternary complexes $[Cu(CDhm)(AaO)]^+$, being insoluble in an apolar environment, are assumed to reside entirely in the aqueous mobile phase.

From Scheme 1, it is evident how the capacity factors for the different amino acids ($k'_{Tyr} < k'_{Phe} < k'_{Trp}$) mostly depend upon their partition coefficients, whereas the capacity factors of the two enantiomers of the same amino acid (k'_D and k'_L) mainly depend upon the difference between the stability constants of the two ternary complexes $[Cu(CDhm)(L\text{-}AaO)]^+$ and $[Cu(CDhm)(D\text{-}AaO)]^+$; thus the enantiomer which is involved in the more stable ternary complex in the mobile phase gives less interactions with the column and elutes first.

(36) Lehrer, S. S. *Biochemistry* **1971**, *10*, 3254.
(37) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Plenum Press: New York, 1983.
(38) O'Connor, D. V.; Phillips, D. *Time-correlated Single Photon Counting*; Academic Press: London, 1984.
(39) Knutson, J. R.; Beechem, J. M.; Brand, L. *Chem. Phys. Lett.* **1983**, *102*, 501.
(40) Gans, P.; Sabatini, A.; Vacca, A. *J. Chem. Soc., Dalton Trans.* **1985**, 1195.
(41) Rigano, C.; Rizzarelli, E.; Sammartano, S. *Thermochim. Acta* **1979**, *33*, 211.
(42) Maggiore, R.; Musumeci, S.; Sammartano, S. *Talanta* **1976**, *23*, 43.
(43) Davankov, V. A.; Kurganov, A. A.; Ponomareva, T. M. *J. Chromatogr.* **1988**, *452*, 309.

Scheme 1

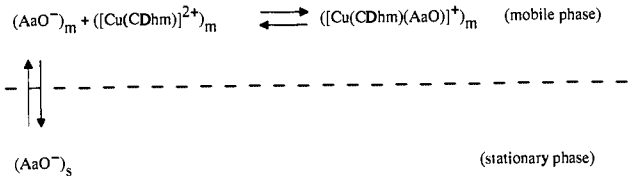


Table 2. Stability Constants of Copper(II) Ternary Complexes of CDhm with Aliphatic or Aromatic Amino Acids at 25 °C and I = 0.1 mol dm^{-3} (KNO_3)[a] Calculated from Potentiometric Titrations[b]

AaO$^-$	log β_{1110}	log β_{1111}	log β_{1112}	log β_{1113}	ref
L-AlaO$^-$	15.53	20.68	25.12		27
D-AlaO$^-$	15.51	20.67	25.12		27
L-LeuO$^-$	14.89(2)	19.3(2)	23.7(1)		this work
D-LeuO$^-$	14.96(2)	19.54(3)	23.73(7)		this work
L-NValO$^-$	14.80(2)	19.54(5)	23.70(8)		this work
D-NValO$^-$	14.87(5)	19.7(1)	24.0(1)		this work
L-PheO$^-$	15.68(2)	20.56(1)	24.89(1)		this work
D-PheO$^-$	15.85(1)	20.50(1)	24.89(1)		this work
L-TyrO$^-$	14.82(1)	19.22(2)			this work
D-TyrO$^-$	15.22(1)	19.24(2)			this work
L-TrpO$^-$	16.12	20.78	25.03		27
D-TrpO$^-$	16.47	20.82	25.02		27
L-HisO$^-$	16.78(3)	22.65(2)	27.00(3)	30.9(1)	this work
D-HisO$^-$	16.70(4)	22.54(4)	26.96(4)	30.7(1)	this work

[a] σ in parentheses. [b] See Discussion in the text for the explanation of indices.

Table 3. Thermodynamic Parameters[a] for the Formation of the [Cu(CDhm)(AaO)]$^+$ Species at 25 °C and I = 0.1 mol dm^{-3} (KNO_3)[b]

AaO$^-$	$-\Delta G^0$ (kcal/mol)	$-\Delta H^0$ (kcal/mol)	ΔS^0 (cal/mol deg)	ref
L-AlaO$^-$	21.20	14.2	23	44
D-AlaO$^-$	21.17	13.8	25	44
L-PheO$^-$	21.40(5)	14.2(3)	24(1)	this work
D-PheO$^-$	21.63(8)	15.2(3)	22(1)	this work
L-TrpO$^-$	22.00	15.5	22	44
D-TrpO$^-$	22.48	17.5	17	44

[a] Calculated by the DOEC program (see Experimental Section). [b] σ in parentheses.

On this basis and from the elution order ($k'_D < k'_L$) reported in Table 1, one can predict that the enantioselectivity order shown by the [Cu(CDhm)]$^{2+}$ complex in solution should be $\beta_D > \beta_L$ for aromatic amino acids and $\beta_D = \beta_L$ for the aliphatic ones. Within the aromatic series, the enantioselectivity factors (α) increased in the order Tyr < Phe < Trp, suggesting a dependence on the lipophilicity and/or the size of the amino acid aromatic side chain.

Thermodynamic Stereoselectivity in Ternary Complexes of [Cu(CDhm)]$^{2+}$ with Amino Acids. The reaction of CDhm (L) with copper(II) and amino acids is represented in eq 1

$$m\mathrm{Cu} + l\mathrm{L} + l'\mathrm{L}' + h\mathrm{H} \rightleftarrows \mathrm{Cu}_m\mathrm{L}_l\mathrm{L}'_{l'}\mathrm{H}_h \qquad (1)$$

where L′ is the anionic form of an amino acid (with charges omitted for simplicity), the stability constant $\beta_{ml'h}$ being defined by eq 2

$$\beta_{ml'h} = [\mathrm{Cu}_m(\mathrm{L}_l\mathrm{L}'_{l'}\mathrm{H}_h)]/[\mathrm{Cu}]^m[\mathrm{L}]^l[\mathrm{L}']^{l'}[\mathrm{H}]^h \qquad (2)$$

In the pH range explored in the present work, [Cu(CDhm)(AaO)]$^+$ is the main species, whereas two or three protonated species were identified in the acidic region (Figure 1 and Table 2).

The stability constants of the mixed-ligand complexes were measured by potentiometry and are reported in Table 2. These values reveal two distinct trends: when the amino acid contains an aromatic moiety, the ternary complexes (unprotonated species) of the D-enantiomer are significantly more stable than those of the L-enantiomer. In contrast, in alanine, leucine, norvaline, and histidine ternary complexes, stereoselectivity is absent or unsignificant. The protonated species apparently do not show any stereoselectivity.

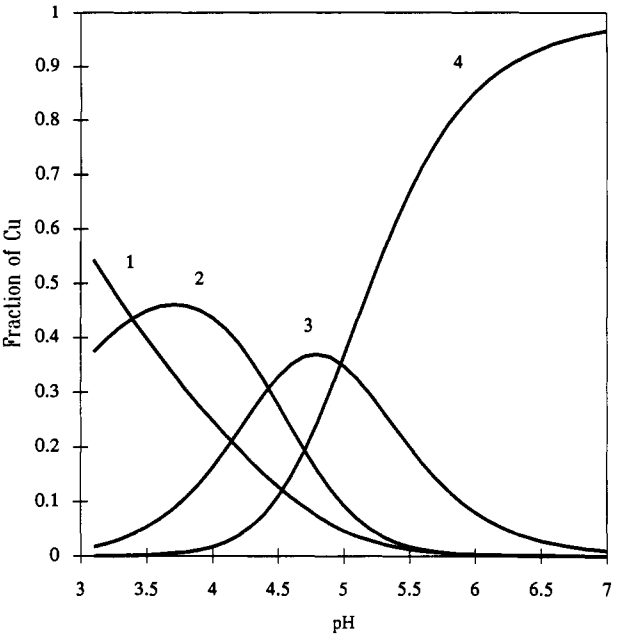


Figure 1. Distribution diagram for the Cu^{2+}-CDhm-L-Phe system (1:1:1 ratio, c = 8 × 10^{-3} mol dm^{-3}): (1) free copper(II); (2) [Cu(CDhm)(L-PheO)(H)$_2$]$^{3+}$; (3) [Cu(CDhm)(L-PheO)(H)]$^{2+}$; and (4) [Cu(CDhm)(L-PheO)]$^+$ calculated from the stability constants, obtained by potentiometry and reported in Table 2, by means of the DISDI program.

Thermodynamic parameters, the ΔG^0 values obtained from the potentiometric data (log β), the ΔH^0 values obtained by calorimetry, and the calculated ΔS^0 values are reported in Table 3.

From these data, the formation of ternary complexes [Cu(CDhm)(AaO)]$^+$ appears to be enthalpically and entropically favored.

The involvement of all potential donor atoms (three nitrogens and one oxygen) in the coordination sphere of the copper(II) ion was inferred for the alaninate and tryptophanate systems[44] by comparing the data concerning the equilibrium 3 with those for simple CDhm metal complex formation:[32]

$$\mathrm{Cu}^{2+} + \mathrm{CDhm} + \mathrm{AaO}^- \rightleftarrows [\mathrm{Cu}(\mathrm{CDhm})(\mathrm{AaO})]^+ \qquad (3)$$

Also for the phenylalaninate system, the formation of ternary species is enthalpically and entropically favored, more than that of the previously investigated binary [Cu(CDhm)]$^{2+}$ complex, where only two nitrogen atoms are involved in the metal coordination. The difference in the enthalpy contribution between the mixed complexes and the binary complex implies that in the former one additional nitrogen is bonded to the metal ion, since ΔH^0 for RCO_2^--bonding is near zero or endothermic. The large positive difference in ΔS^0 cannot be due to further nitrogen binding but to the RCO_2^--coordination with consequent charge neutralization. Therefore, these differences suggest that the phenylalaninate anions are coordinated in a bidentate fashion. If the above considerations allow the coordination set of donor atoms to be defined, the differences in the ΔH^0 and ΔS^0 values between the L- and D-aromatic amino acids deserve further discussion. In Figure 2 the molecular models of the ternary complexes [Cu(CDhm)(L-TrpO)]$^+$ and [Cu(CDhm)(D-TrpO)]$^+$ proposed to account for the observed thermodynamic effects are reported. In the crystal structures of cyclodextrins the O(5)–C(5)–C(6)–O(6) torsion angle has been found to adopt either a *+gauche* or a

(44) Corradini, R.; Impellizzeri, G.; Maccarrone, G.; Marchelli, R.; Rizzarelli, E.; Vecchio, G. In *Chemistry and Properties of Biomolecular Systems*; Rizzarelli, E.; Theophanides, T., Eds.; Kluwer: Dordrecht, 1991; p 209.

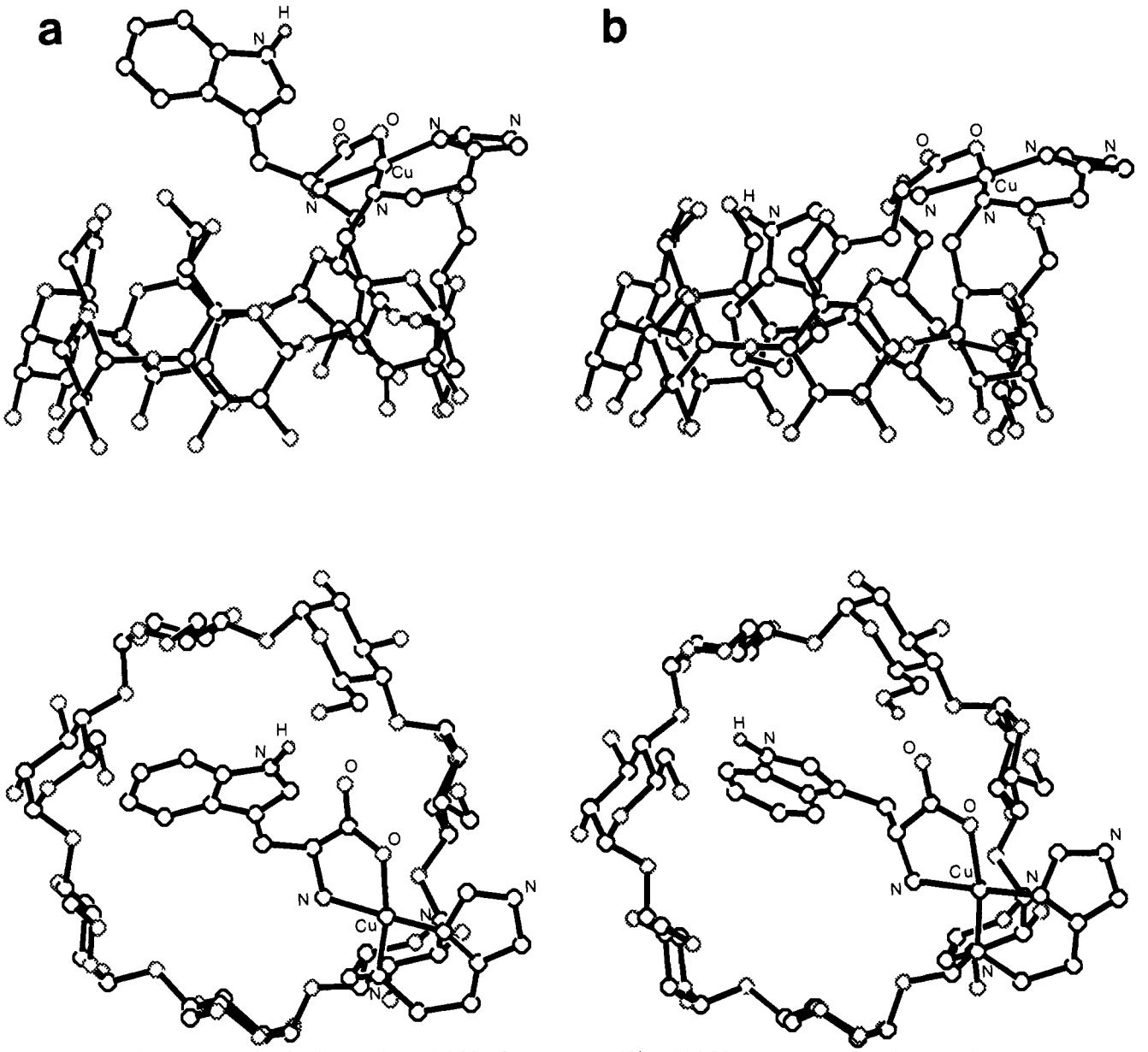


Figure 2. Proposed molecular models for the complexes: (a) [Cu(CDhm)(L-TrpO)]$^+$ and (b) [Cu(CDhm)(D-TrpO)]$^+$; above: front view; below: top view.

-gauche conformation, thus giving rise to a counterclockwise orientation of the O(6)H groups,[2] whereas the *anti* conformation has never been observed, probably on account of the repulsive interactions between the O(6) group and the atoms of the adjacent glucose ring. Thus, it is reasonable to propose the same orientation for the more bulky histamine group.

We also assume that, in the copper(II) complex in solution, the amino groups of the two ligands be arranged in a *cis*-position, as found in the crystal structures of ternary complexes with histamine and aromatic amino acids.[45] On these bases, only the aromatic moiety of the D-isomer can interact with the CD-cavity, thus accounting for the greater ΔH^0 values observed for the D-isomers of all aromatic amino acids.

It is well-known that noncovalent interactions are the forces behind host–guest or inclusion complex formation.[46,47] Elucidation of these interactions is necessary to understand the mechanisms of molecular recognition. The literature data unfortunately justify the following sentence: "There can be no better example of the continuing state of uncertainty with respect to the physical principles that govern molecular recognition than the conflicting explanations offered for the behavior of lipophilic particles in water: in addition to (or even in place of) the entropy factor..., claims are made for a purely enthalpic basis for the solvophobic effect".[48] The literature on cyclodextrin complexes shows that at least two different mechanisms govern the formation of inclusion compounds in aqueous solution. Calorimetric measurements suggest negligibly small entropy contributions for the complexes of α- and β-cyclodextrin with adamantane-1-carboxylate.[49,50] In contrast, association with γ-cyclodextrin is favorable only because of entropy factors, since here the large size of the cavity relative to the α- and β-CD compounds makes it unsuitable for effective dispersive forces. Similar conclusions can be drawn from equilibrium studies with *p*-nitrophenol and its anion.[51] Anion formation, with the resulting accumulation of a highly polarizable charge, is considerably more advantageous with the narrower

(45) Yamauchi, O.; Odani, A.; Kohzuma, T.; Masuda, H.; Toriumi, K.; Saito, K. *Inorg. Chem.* **1989**, *28*, 4066.
(46) Rigby, M.; Smith, E. B.; Wakeham, W. A.; Maitland, G. C. *The Forces Between Molecules*; Oxford Science Publications, Clarendon Press: Oxford, 1986.
(47) Tanford, C. *The Hydrophobic Effect*, 2nd ed.; Wiley: New York, 1980.
(48) Schneider, H.-J. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1417.
(49) Barone, G.; Castronuovo, G.; Del Vecchio, P.; Elia, V.; Muscetta, M. *J. Chem. Soc., Faraday Trans. 1* **1986**, 2089.
(50) Cromwell, W. C.; Bystrom, K.; Eftink, M. R. *J. Phys. Chem.* **1985**, *89*, 326.
(51) Buvari, A.; Barcza, L. *J. Chem. Soc., Perkin Trans. 2* **1988**, 543.

α-cyclodextrin cavity, since here dispersive forces, which require close contact, predominate.

Previously, we have found that solvophobic interactions between side chain moieties of two different ligands coordinated to the same metal ion favor mixed complex formation by means of a favorable enthalpy contribution.[35,44,52,53]

Analogously, also in the case of CDhm, solvophobic interactions between the cavity and the aromatic side chains of the amino acids seem to be crucial for enantioselectivity, which is enthalpy driven. The entropy changes seem to be less favorable for the D-enantiomers, which are supposed to be included in the cavity ($\Delta S^0_D - \Delta S^0_L$ = +2, −2, −5 cal mol^{-1} deg^{-1} for alanine, phenylalanine, and tryptophan, respectively): this could be interpreted also as the result of the loss of internal rotational freedom of the side chain, which predominates over the desolvation effect.

The HPLC and potentiometric data are substantially consistent insofar, as only the aromatic amino acids were well resolved and the elution order was found to be $k'_D < k'_L$. However, quantitative correlations cannot be drawn, since smaller $\Delta\Delta G^0_{D,L}$ were obtained by the HPLC measurements ($-\Delta\Delta G^0_{D,L} = RT \ln \alpha$). This is most probably due to the nature of the mobile phase: in fact, a methanol:water = 30:70 mixture had to be used in order to obtain acceptable peak shapes and measurable retention times. Under less polar conditions, the formation of inclusion complexes should be disfavored, leading to smaller enantioselectivity. In addition, the solvent effect may be responsible also for the different enantioselectivity order within the aromatic series obtained by thermodynamic measurements or by HPLC.

CD Evidences for Stereoselectivity. To obtain further information on the preferential inclusion of the D-enantiomers in the cyclodextrin cavity, in particular for the tyrosine system (the low solubility of the amino acid prevents direct calorimetric measurements), and to gain evidence about the noncovalent interactions responsible for stereoselectivity, electronic and CD spectra were recorded. In order to analyze the CD spectra of copper(II) mixed-ligand complexes of the type [Cu(CDhm)(AaO)]$^+$, it is useful to consider the contribution of the binary complexes [Cu(AaO)]$^+$ and [Cu(CDhm)]$^{2+}$, according to the distribution data obtained by means of the DISDI program.[42] In the Cu–CDhm system (Table 4), the CD spectrum contains a broad maximum in the visible region at 592 nm and a CD minimum in the near UV at 272 nm.

Following Schugar's[53] analysis of the electronic spectra of various copper(II) imidazole chromophores, the 272 nm CD band, which has been observed in the spectra of various binary and ternary systems containing copper(II) and L-histidine,[54,55] can be attributed to charge-transfer transitions from the two highest occupied π-orbitals of the imidazole moiety to the copper(II) d vacancy. The visible and UV spectra derived from aliphatic and aromatic amino acids have been previously reported as well as their CD spectral data.[56,57]

The absorption and CD spectral data of the ternary complexes [Cu(CDhm)(AaO)]$^+$ are summarized in Table 4. For the mixed complexes containing aliphatic amino acids the shapes of the CD curves are clearly independent from the absolute configuration of the amino acids. The spectra of the diastereomeric complexes with the same aliphatic amino acid [Cu(CDhm)(L-AaO)]$^+$ and [Cu(CDhm)(D-AaO)]$^+$ are practically superimposable in the visible region, while they are slightly different in the near-UV region. For the mixed complexes containing aromatic amino acids, the CD spectra are remarkably different both in the visible and UV regions, being the CD absorption of the [Cu(CDhm)(D-AaO)]$^+$ species much higher than that of [Cu(CDhm)(L-AaO)]$^+$.

Several effects must be considered to account for the CD absorptions. The inclusion of several chromophores in the cyclodextrin cavity has been shown to give rise to induced circular dichroism (ICD), due to the presence of many chiral centres. In several cases, it has been possible to correlate on theoretical bases both the sign and the intensity of the observed ICD and the orientation of the transition moment related to the cyclodextrin axis.[58] In the visible region, the d–d transitions of the mixed [Cu(CDhm)(AlaO)]$^+$ complexes are not much different from the binary [Cu(CDhm)]$^{2+}$complex, suggesting that the perturbation induced by an apolar aliphatic amino acid is negligible. In the case of tryptophan the higher CD intensity observed for the ternary complex [Cu(CDhm)(D-TrpO)]$^+$ in the visible region might be attributed either to a deeper inclusion of the copper(II) chromophore in the cyclodextrin cavity or to a more rigid conformation with the amino acid side chain perturbing the copper(II) symmetry. In the UV region, a similar effect is observed for a band at about 260 nm ($\Delta\Delta\epsilon = -13.1$). Moreover, by comparing the bands near 260 and 650 nm exhibited by the mixed complexes with those of the corresponding [Cu(L-AaO)]$^+$ and [Cu(D-AaO)]$^+$ binary species,[57] it can be excluded that the CD absorptions could be simply determined by the [Cu(AaO)]$^+$ residues.

If we take the difference between the molar CD coefficients at the maximum wavelengths in the visible region ($\Delta(\Delta\epsilon)$), as a parameter to differentiate the diastereomeric species, we observe

Table 4. Electronic and CD Spectral Parameters for CDhm, [Cu(CDhm)]$^{2+}$, and for the Ternary Complexes [Cu(CDhm)(AaO)]$^+$ with Aliphatic and Aromatic Amino Acids at pH = 7.5

	UV–vis λ_{max} (nm) (ϵ)	CD λ_{max} (nm) ($\Delta\epsilon$)
CDhm	207 sh (6400)	206 (−0.67)
[Cu(CDhm)]$^{2+}$	249 (2680)	272 (−1.8); 592 (0.33)
[Cu(CDhm)(AaO)]$^+$		
AaO$^-$		
L-AlaO$^-$	613 (85)	220 (−0.6); 256 (−2.50)
	243 (3630)	585 (0.38)
D-AlaO$^-$	614 (84)	265 (−1.40)
	245 (3770)	585 (0.40)
L-LeuO$^-$	257 sh (8000)	252 (−5.45); 297 (−5.45)
	610 (78)	592 (0.85)
D-LeuO$^-$	257 sh (8000)	250 (−5.09); 297 (−5.09)
	620 (68)	590 (0.84)
L-NValO$^-$	260 (3000)	252 (−1.36)
	625 (79)	585 (0.23)
D-NValO$^-$	253 (3200)	219 (−0.97); 251 (−1.42)
	624 (65)	588 (0.24)
L-PheO$^-$	248 (3127)	287 (−0.92)
	646 (47)	507 (−0.05); 580 (0.04)
		657 (−0.08)
D-PheO$^-$	248 (3158)	260 (−4.97)
	646 (57)	599 (0.97)
L-TyrO$^-$	270 (4150)	240 (2.33); 307 (−2.51)
	646 (110)	518 (−0.04); 647 (−0.20)
D-TyrO$^-$	270 (5100)	259 (−5.21)
	638 (77)	593 (1.10)
L-TrpO$^-$	270 (7640)	215 (−10.49); 259 (1.8)
	614 (78)	647 (−0.25)
D-TrpO$^-$	270 (7800)	218 (16.62); 260 (−11.3)
	622 (96)	612 (1.7)

(52) Arena, G.; Rizzarelli, E. In *Metal Complexes in Solution*; Jenne, E. A., Rizzarelli, E., Romano, V., Sammartano, S., Eds.; Piccin: Padua, 1986; p 105 and references therein.

(53) Fawcett, T. G.; Bernarducci, E. E.; Krogh-Jespersen, K.; Schugar, H. J. *J. Am. Chem. Soc.* **1980**, *102*, 2598. Schugar, H. J. In *Copper Coordination Chemistry: Biochemical and Inorganic Perspectives*; Karlin, K. D., Zubieta, J., Eds.; Adenine Press: New York, 1982; p 43.

(54) Arena, G.; Bonomo, R. P.; Casella, L.; Gullotti, M.; Impellizzeri, G.; Maccarrone, G.; Rizzarelli, E. *J. Chem. Soc., Dalton Trans.* **1991**, 3203.

(55) Casella, L.; Gullotti, M. *J. Inorg. Biochem.* **1983**, *18*, 19; *Inorg. Chem.* **1983**, *22*, 242.

(56) Hawkins, C. J.; Wong, C. L. *Aust. J. Chem.* **1970**, *23*, 2237. Hawkins, C. J. *Absolute Configuration of Metal Complexes*; Wiley-Interscience: New York, 1971; Chapter 5.

(57) Tsangaris, J. M.; Chang, J. W.; Martin, R. B. *J. Am. Chem. Soc.* **1969**, *91*, 726. Phan, C. V.; Tosi, L.; Garnier, A. *J. Inorg. Nucl. Chem.* **1981**, *43*, 971. Garnier-Suillerot, A.; Albertini, J. P.; Collet, A.; Faury, L.; Pastor, J. M.; Tosi, L. *J. Chem. Soc., Dalton Trans.* **1981**, 2544.

(58) Kodaka, M. *J. Phys. Chem.* **1991**, *95*, 2110.

Table 5. Difference between the Molar CD Coefficients $\Delta(\Delta\epsilon)$ (Absolute Values) of the [Cu(CDhm)(L-AaO)]$^+$ and [Cu(CDhm)(D-AaO)]$^+$ Complexes

AaO$^-$	λ_{nm} (L)	λ_{nm} (D)	$\Delta(\Delta\epsilon)$
AlaO$^-$	585	585	0.02
LeuO$^-$	590	592	0.01
NValO$^-$	580	585	0.01
PheO$^-$	599	657	1.05
TyrO$^-$	593	647	1.30
TrpO$^-$	612	647	1.95

an increase with the bulkiness of the aromatic amino acid side chain (Table 5).

The progressive differentiation in the CD spectra of the diastereomeric couples may be related to some stereoselective interactions between the coordinated ligands. Since such interactions involve the amino acid side chains, they can only be of noncovalent nature within the cyclodextrin cavity. It is expected that the inclusion of the aromatic side chain in the cavity restricts the conformational mobility of the participating groups. The opposite sign of the circular dichroism observed for the diastereomeric complexes depends on the different relative orientation of the transition vectors in the two systems.

Enantioselective Fluorescence Quenching of Tryptophan by [Cu(CDhm)]$^{2+}$. In order to obtain independent evidence for the involvement of the cyclodextrin cavity in the recognition process, fluorescence measurements were carried out on copper(II) ternary complexes of CDhm with L- or D-tryptophan. In fact, the fluorescence spectrum of tryptophan has been shown to be sensitive to the polarity of the microenvironment in which it is located and has been used in many studies as a probe for the conformation of proteins and peptides.[59] As for many fluorophores, the indole fluorescence of Trp is quenched by copper(II) ion: this effect has been used as a measure of the stability constants of copper(II) complexes.[60,61] In a recent work, some of us have shown that the fluorescence of dansyl derivatives of amino acids undergoes enantioselective quenching by chiral copper(II) complexes and that fluorescence measurements can be used for the study of enantioselectivity in the formation of ternary complexes in solution.[62] We thus performed the same type of experiments by adding increasing amounts of the [Cu(CDhm)]$^{2+}$ complex to a solution of D- or L-tryptophan. The results are reported in Figure 3 as a Stern–Volmer plot (F_0/F versus the concentration of the complex). Enantioselectivity in fluorescence quenching is also observed in this case, with the D-enantiomer being more extensively quenched than the L-one. However, the data could not be fitted by simple linear regression, but rather by a second-order curve. Such a model is consistent with the occurrence of both dynamic (i.e., collisional) and static quenching.

Quenching by KI and Acrylamide. The fluorescence of D- and L-tryptophan in the 1:1:1 ternary complexes was not completely quenched by [Cu(CDhm)]$^{2+}$. Since the distribution diagram shows that at pH = 7.5 this complex is the major species present, the residual fluorescence observed must be due to the ternary complex [Cu(CDhm)(TrpO)]$^+$. This assumption is supported by the results obtained by quenching the solutions containing the ternary complexes with KI and acrylamide (see Figure 4a,b).

Both quenchers are achiral and have been used for the measurement of accessibility of tryptophan residues in proteins,[37] since only those exposed to the bulk solvent are quenched. In both cases, preferential quenching of the fluorescence of the L-enantiomer was observed. The quenching by KI is consistent with a model in which both collisional as well as static quenching occur, due to electrostatic interactions between the iodide ion and the positively charged complex. Data treatment according to Lehrer[36] ($F_0/\Delta F$ versus $1/[KI]$) allowed us to calculate accessibility as the inverse of the intercept at $1/[KI] = 0$ in Figure 5.

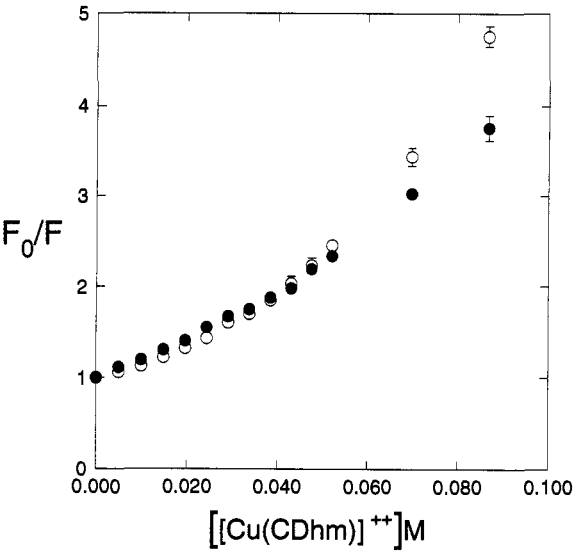


Figure 3. Fluorescence quenching of D- (○) and L-Trp (●) by [Cu(CDhm)]$^{2+}$.

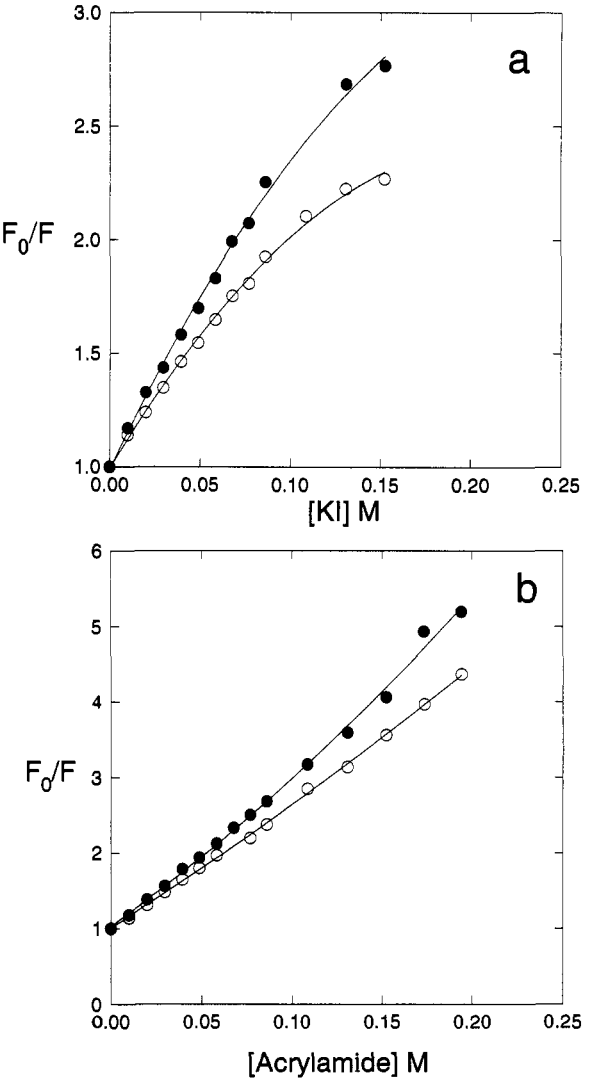


Figure 4. (a) KI quenching of [Cu(CDhm)(D-Trp)]$^+$ (○) and [Cu(CDhm)(L-TrpO)]$^+$ (●); (b) acrylamide quenching of [Cu(CDhm)(D-TrpO)]$^+$ (○) and [Cu-(CDhm)(L-TrpO)]$^+$ (●).

(59) Cantor, C. R.; Schimmel, P. R. *Biophysical Chemistry*; Freeman & Company: New York, 1980.
(60) Chen, R. F. *Anal. Lett.* **1986**, *19*, 963.
(61) Tabak, M.; Sartor, G.; Cavatorta, P. *J. Luminescence* **1990**, *46*, 291. Szabo, A. G.; Rayner, D. M. *J. Am. Chem. Soc.* **1980**, *102*, 554.
(62) Corradini, R.; Sartor, G.; Marchelli, R.; Dossena, A.; Spisni, A. *J. Chem. Soc., Perkin Trans. 2* **1992**, 1979.

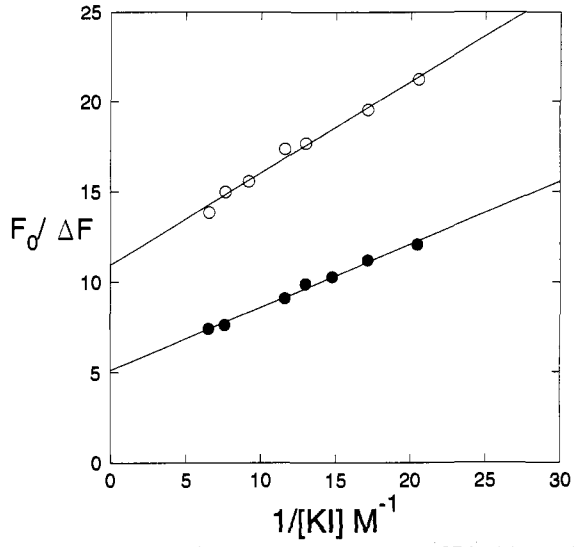


Figure 5. Lehrer plot of KI quenching: (○) [Cu(CDhm)(D-TrpO)]$^+$ and (●) [Cu(CDhm)(L-TrpO)]$^+$.

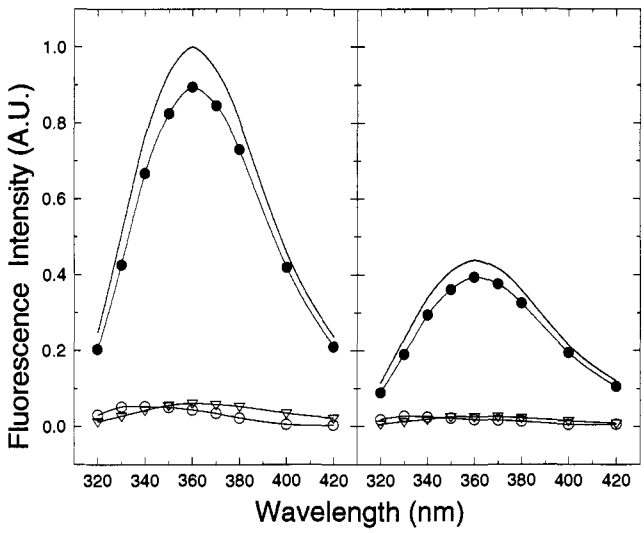


Figure 6. Decay associated spectra: (left panel) [Cu(CDhm)(L-TrpO)]$^+$ (●) 3.06 ns, (○) 0.36 ns, (▽) 12.43 ns components; (right panel) [Cu(CDhm)(D-TrpO)]$^+$ (●) 2.32 ns, (○) 0.16 ns, (▽) 12.43 ns components.

The accessibility of the indole moiety in the ternary complex containing L-tryptophan turned out to be almost double than that of the complex containing D-tryptophan, thus supporting the hypothesis that in the former the amino acid side chain is more exposed to the quencher, whilst in the latter it is shielded by the cyclodextrin cavity.

Similar conclusions can be drawn for quenching by a neutral species such as acrylamide (Figure 4b), although in this case the data are consistent with a model which takes into account "interaction volumes".[37]

Time-Resolved Fluorescence. Fluorescence lifetimes of the ternary complexes were measured at different wavelengths. Figure 6 reports the decay associated spectra (DAS) thus obtained. A three-component model was used, but fluorescence turned out to be mainly due to one component having a lifetime of 3.06 ns for L-Trp and of 2.32 ns for D-Trp.

The lower fluorescence intensity of the D-enantiomer observed in the DAS can be ascribed either to a closer interaction between the copper(II) ion and the indole moiety or to a more favorable quencher-fluorophore orientation, as predicted by the Foerster's theory.[59] This could be consistent with the proposed model, since by inclusion in the cavity the indole ring could be kept in a fixed position closer to the metal ion. In the case of the L-enantiomer, the relatively freer rotational motion could allow more conformations in which the indole moiety is located far from the metal ion.

Therefore, the results of both static and dynamic fluorescence experiments are consistent with the interpretation that residual fluorescence is due to the ternary complexes and that quantum yields are affected by the preferential inclusion of the side chain of the D-enantiomer within the cyclodextrin cavity.

Conclusions

In this paper we have shown the enantioselective binding of aromatic amino acids to the copper(II) complex of histamine functionalized β-cyclodextrin. The thermodynamic stereoselectivity was found to be due to the more favorable enthalpy contribution in the formation of ternary complexes of the D-isomers, suggesting noncovalent interactions between the amino acid side chain and the cyclodextrin cavity. HPLC was used as a rapid method for screening the occurrence of enantioselectivity, although $\Delta\Delta G^0$ smaller than those found by potentiometry were obtained, on account of the different medium composition. Different $\Delta\epsilon$ values for ternary complexes containing D- and L-aromatic amino acids were found in the CD spectra, being in particular higher for the D-enantiomers. This behavior is fully consistent with the preferential inclusion of the D-enantiomer, in agreement with both the stability constants and the chromatographic elution order observed. The enantioselective fluorescence effects found by titration of D- and L-tryptophan with [Cu(CDhm)]$^{2+}$ and the different degree of accessibility of the indole moiety of the two enantiomers to achiral quenchers (KI and acrylamide) confirm the involvement of the cavity in the recognition process.

According to the present results, evidence is achieved about the inclusion of the aromatic side chains of D-amino acids, although the differences between the thermodynamic parameters for the D- and L-enantiomers are not as large as could be expected for a multisite receptor. This is probably due to the presence of a *cis–trans* equilibrium regarding the disposition of the two amino nitrogens in the copper coordination sphere, usually invoked in copper(II) amino acid complexes with an estimated difference between the *cis*- and *trans*-isomers ranging from 0.43 to 2.17 kcal/mol.[63,64] However, the fact that we observe thermodynamic stereoselectivity in solution, chromatographic resolution, and enantioselective fluorescence effects suggests that the *cis–trans* equilibrium is shifted toward the *cis* configuration. Our experimental approach provides no evidence for differential interactions between the hydroxyl groups of the cyclodextrin and the coordinating groups of the amino acid (NH_2, RCO_2^-), the indole N–H in tryptophan, and the OH group of tyrosine. Indeed, chiral discrimination can be brought about by weak forces, such as those involved in the inclusion phenomenon, and by noncovalent interactions, as invoked recently for α-cyclodextrin–tryptophan systems.[65]

Acknowledgment. We thank CNR, Progetto Finalizzato Chimica Fine II, and MURST for partial support and the Centro Interdipartimentale di Misure (CIM)-Università di Parma, where some of the spectroscopic measurements were performed. We wish to thank Lorenza Carima for her valuable contribution and Tiziana Campagna for technical assistance.

(63) Ablov, A. W.; Diyakon, I. A.; Ivanova, V. Ya.; Proskina, N. N.; Chapurina, L. F. *J. Inorg. Chem. (Engl. Transl.)* **1965**, *10*, 339.
(64) Delf, B. W.; Gillard, R. D.; O'Brien, P. *J. Chem. Soc., Dalton Trans.* **1979**, 1301.
(65) Lipkowitz, K. B.; Raghothama, S.; Yang, J. *J. Am. Chem. Soc.* **1992**, *114*, 1554.