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Coordination Properties of 6-Deoxy-6-[1-(2-amino) ethylamino]-β-cyclodextrin and the Ability of Its Copper(II) Complex to Recognize and Separate Amino Acid Enantiomeric Pairs

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Abstract. The functionalized cyclodextrin 6-deoxy-6-[1-(2-amino)ethylamino]-β-cyclodextrin was synthesized, and an NMR, EPR, pH-metric, and calorimetric investigation was carried out in aqueous solution in order to ascertain its behaviour towards protonation and copper(II) complex formation. The thermodynamic parameters of the ternary complex formation with alanine, phenylalanine and tryptophan enantiomeric pairs were also determined (25° C and $I = 0.1$ mol dm^{-3}). No thermodynamic enantioselectivity was observed in these systems, while a chiral, though poor, discrimination was observed in LEC: c.d. spectra also show enantiomeric stereoselectivity. The results of the present investigation, compared with previously reported results, suggest the occurence of a *cis*-complex ⇌ *trans*-complex equilibrium in such systems.

Key words: Copper(II) complex, functionalized cyclodextrin, LEC.

1. Introduction

Cyclodextrins are a family of natural hosts, owing to their interior cavity, which provides a relatively hydrophobic environment in which organic and inorganic molecules can be trapped [1]. The formation of such adducts has been studied extensively in recent years for their importance in catalysis, in chromatography, in pharmaceutical applications, and as models for protein-ligand complexes and artificial enzymes [2].

The synthesis of functionalized cyclodextrins has provided more efficient models of natural enzymes and receptors [3]. Furthermore, the presence of attached potentially coordinating groups, which promote the formation of stable

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metal complexes, has encouraged the use of functionalized cyclodextrins as multiple recognition artificial receptors [4–6].

Metal complexes of cyclodextrins functionalized with polyamines have been shown to act as metal enzyme models endowed with specific metal-substrate binding interaction [7–9].

Recently, we have reported the ability of a copper(II) complex with a cyclodextrin derivative 6-deoxy-6-[2-(4-imidazolyl)ethylamino]-β-cyclodextrin (CDhm) [10] to discriminate between *L*- and *D*-tryptophan, as reflected by the different stability of the ternary metal complexes in aqueous solution [11–13]. A strictly correlated compound, 6-deoxy-6-[4-(2-aminoethyl)imidazolyl]-β-cyclodextrin (CDmh), shows an even higher chiral discriminating ability [14], though in LEC (ligand exchange chromatography) the order of elution of the two enantiomers is opposite in the two systems. These results have been explained by invoking a '*cis*-effect', where in the copper(II) coordinating plane the amino group of the amino acid is adjacent to the amino group of the other ligand. The fact that the discriminating ability of CDmh is higher than that of CDhm has been ascribed to a more rigid structure of the CDmh ternary complex [14]. With the '*cis*-effect' in mind, it is interesting to study the behavior of the analogous ternary complexes when the substituted cyclodextrin has two very similar coordinating groups, like the primary and secondary amino groups of 6-deoxy-6-[1-(2-amino)ethylamino]-β-cyclodextrin (CDen).

Here we report the synthesis of this compound, a detailed potentiometric and calorimetric investigation in aqueous solution (25 °C and $I = 0.1$ mol dm^{-3}, with addition of KNO_3) on CDen; and the characterization of the different species in proton–CDen and copper(II)–CDen systems by NMR and EPR investigation, respectively. In addition, the ternary copper(II) systems with CDen and *L*- and *D*-amino acids (alanine, phenylalanine or tryptophan) were investigated by potentiometry, calorimetry and c.d. spectroscopy. The chiral discriminating ability of the copper(II)–CDen complex was tested on the same enantiomeric pairs of amino acids in LEC.

2. Experimental

2.1. Materials

Commercially available reagents were used directly unless otherwise noted. β-Cyclodextrin (Sigma) was dried *in vacuo* (10 mm Hg) for 24 h at 80 °C using a P_2O_5 trap. Pyridine was distilled after boiling under reflux over KOH for 10 h and over BaO for 12 h. *N*, *N*-Dimethylformamide was distilled under reduced pressure after boiling under reflux over CaH_2 for 10 h.

Each solvent was stored over molecular sieves. TLC was carried out on silica gel plates 60F-254 (Merck). CD derivatives were detected with UV light and the anisaldehyde reagent. Merck Lichroprep RP-8 (40–63 μm) was used for reverse-

phase column chromatography.

L- and *D*-amino acids (Sigma) were all high purity products. Their purity was checked by means of potentiometric titration with standard KOH solution and in all cases it proved higher than 99.8%. Polarimetric tests gave almost identical results. Copper(II) nitrate was a 'reinst' (Merck) product. The concentration of stock solutions of this salt was determined by ethylenediaminetetraacetate titration. Stock solutions of HNO_3 and KOH were made up from concentrated HNO_3 (Suprapur, Merck) and from Normex Carlo Erba vials, respectively. Their concentrations were determined potentiometrically by titrating with tris(hydroxymethyl)-aminomethane and potassium hydrogenphthalate, respectively. All solutions were prepared with CO_2-free freshly bidistilled water. The ionic strength was adjusted to 0.1 mol dm^{-3} by adding KNO_3 (Suprapur, Merck). Grade A glassware was employed throughout.

2.2. SYNTHESIS

6-Deoxy-6-[1-(2-amino)ethylamino]-β-cyclodextrin was synthesized using a slightly different procedure from that reported in the literature [8, 9]. A solution of CD (1 g) in ethylenediamine (3 m*l*) was stirred for 7 h at 70 °C under nitrogen, then concentrated *in vacuo* at 40 °C. The syrup residue was stirred with acetone, the resulting solid was collected by filtration, precipitated from aqueous solution with acetone, and purified by elution from a column (20 × 600 mm) of CM-Sephadex C-25 column (NH_4^+ form) with water (400 mL), then with a 0–0.2 M NH_4HCO_3 linear gradient (total volume 1.1 L). The appropriate fractions were combined and concentrated to give CDen (0.4 g), $R_f = 0.23$ (PrOH / H_2O / NH_3 5 : 3 : 1). ^{13}CNMR (D_2O) δ (ppm) 102 (C-1); 84 (C-4A); 81.8 (C-4); 73.8 (C-2); 72.7 (C-5); 72.5 (C-3); 71.2 (C-5A); 61 (C-6); 49.7 (C-6A); 48.6 (α CH_2); 39.7 (β CH_2).

2.3. POTENTIOMETRIC MEASUREMENTS

The potentiometric measurements were carried out by means of two fully automated, computer-controlled meters (Metrohm E 654) using a combined glass microelectrode (Metrohm 125). All experiments were carried out at 25 °C using 2.5 mL thermostated cells. All solutions were magnetically stirred and maintained under an atmosphere of inert nitrogen, previously bubbled through 0.1 mol dm^{-3} KNO_3 solutions. The electrodes were standardized on the pH = -log[H^+] scale by titrating HNO_3 with CO_2-free KOH.

Solutions containing, in turn, CDen (protonation), CDen and the copper(II) ion (simple complex formation), CDen, the metal ion and an amino acid (mixed complex formation) were titrated with standard KOH using Hamilton burettes equipped with 0.25 or 0.50 mL syringes, calibrated with mercury. Each experiment was simultaneously run in both potentiometric apparatuses to avoid systematic

errors and to check for reproducibility. β-Cyclodextrin derivative (L) and amino acid (L') concentrations ranged from 0.002 to 0.007 mol dm^{-3}. Duplicate or triplicate titrations were carried out at 1 : 1 : 1 Cu/L/L' ratios. Other details were as previously described [15, 16].

2.4. Calorimetric Measurements

The calorimetric titrations were performed at 25.000 ± 0.001 °C using a Tronac 450 isoperibolic calorimeter equipped with a 25 mL titration dewar. Solutions containing, in turn, the cyclodextrin derivative, CDen and the metal ion, CDen, the copper(II) ion and the amino acid were titrated with standard HNO_3. The titration data, corrected for all non-chemical energy terms determined in separate experiments, were refined to obtain the final $\Delta H°$ of each system, using the computer program *DOEC* (see Section 2.8: Calculations). Other experimental details were as previously reported [15, 16].

2.5. NMR Measurements

^{1}HNMR spectra (600 MHz) were recorded with a Bruker AMX-600 spectrometer and ^{13}CNMR spectra (62.9 MHz) with a Bruker AC-250 spectrometer on D_2O solutions without a reference compound; the ^{1}HNMR spectra were referenced to water since most of the usual reference compounds interact with the β-CD cavity. The solutions of mono- and diprotonated CDen were prepared by adding the stoichiometric amount of DCl in D_2O solution.

2.6. EPR Measurements

Frozen solution EPR spectra were recorded on a Bruker ER 200 D X-band spectrometer, with a standard low temperature apparatus. DPPH was used as a calibrant and the magnetic field was measured by means of the Gauss meter (ER 035 M), which leaves markers at preselected values. Spin Hamiltonian parameters were obtained directly from the experimental spectra and are reported in Table I. In the case of the mono-complex, the solutions had 1 : 1 metal to ligand ratios, the pH value being about 6. To promote the formation of the bis-species, a solution containing isotopically pure 63 $Cu(NO_3)_2$ (5×10^{-3} mol dm^{-3}) and the ligand in a 1 : 4 ratio was analyzed. In this latter case the pH of the solution was about 7. After having adjusted the pH to the desired value, methanol up to 5% was added to these aqueous solutions to increase spectral resolution. All the measurements were carried out at 150 K.

2.7. LEC experiments

Chromatographic separation was performed on a Hewlett Packard series 1050 HPLC, using a Spherisorb ODS-2 (3 μm, 150 × 4.6 mm), setting the UV detector

TABLE I

Spin Hamiltonian parameters for copper(II) complexes with CDen and en in water–methanol (95 : 5) solution at 150 K.

Complex	$g_{\parallel}$	$A_{\parallel}$ (cm^{-1})	$g_{\perp}$	$A_{\perp}$ (cm^{-1})	Ref.
$[Cu(CDen)]^{2+}$	2.290(2)	0.0176(2)	2.063(5)	0.0014(5)	this work
$[Cu(CDen)_2]^{2+}$	2.215(2)	0.0189(2)	2.054(5)	0.0018(5)	this work
$[Cu(en)(H_2O)_4]^{2+}$	2.281	0.0181	2.058	0.0024	[17]
$[Cu(en)_2(H_2O)_2]^{2+}$	2.209	0.0203	2.047	0.0028	[17]

at 254 nm.

The mobile phase was prepared by dissolving the complex (7×10^{-5} mol dm^{-3}) and sodium acetate (3×10^{-3} mol dm^{-3}) in H_2O/CH_3OH (60 : 40) mixture, at pH 7 [18].

2.8. CALCULATIONS

The calculations concerning the electrode system were performed by the computer program *ACBA* [19] which refines the parameters of an acid-base titration by using a nonlinear least squares method minimizing the function $U = \sum(v_{i,\text{expt}} - v_{i,\text{calc}})$, v being the volume of titrant added.

All other potentiometric data were handled by the program *SUPERQUAD* [20] which minimizes the error-square sum of the differences between measured and calculated electrode potentials. The ΔH° values of complex formation were calculated by the program *DOEC* [21]. Throughout the paper, errors in thermodynamic parameters are expressed as three times the standard deviations. The thermodynamic parameters pertinent to the proton and copper(II) complexes of amino acids have been reported elsewhere [16].

3. Results and Discussion

NMR data confirmed the identity of our compound, showing that the ethylenediamine is N-bonded to atom 6C of β-CD. The ^{1}HNMR spectrum in Figure 1 has been assigned on the basis of the COSY spectrum. Hydroxyl substitution in β-CD causes upfield shifts in protons of the substituted moiety. The triplet at 3.5 ppm is assigned to the H-4A, the doublet at 3.09 to H-6A, the multiplet at 2.9 to the other H-6A and the multiplets at 2.97 and 2.83 ppm are assigned to methylene protons of the ethylenediamine chain. The diastereotopicity of the H-6A protons and the high multiplicity of CH_2 amine proton resonances suggest a rigidity of the functionalized moiety, as has already been proposed for other amine CD derivatives [10, 24]. This may reflect the occurrence of an intra-chain hydrogen bond, involving the two nitrogens of the thylenediamine chain. The ^{1}HNMR spectrum

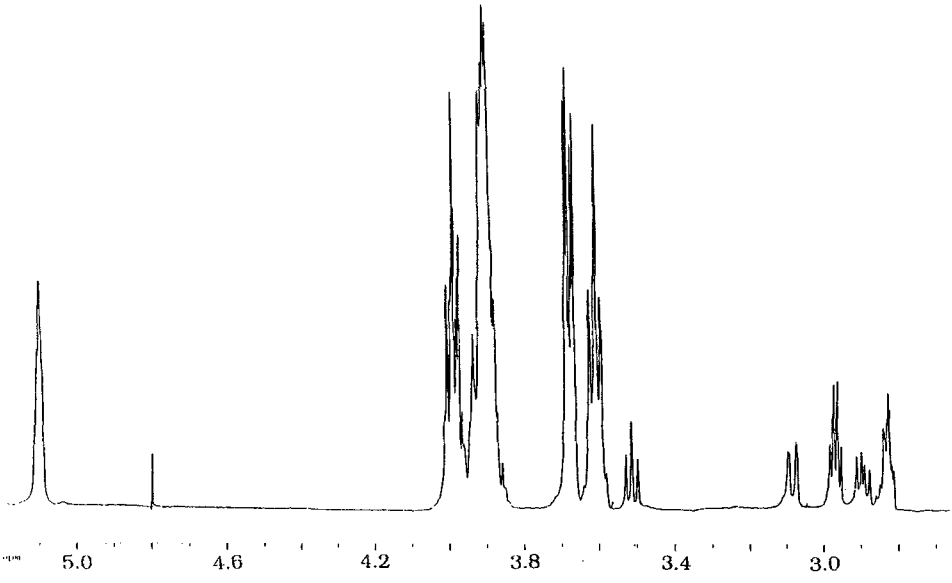
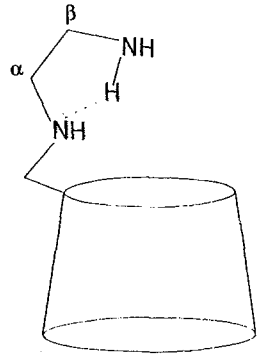


Fig. 1. ^{1}HNMR spectrum (600 MHz, D_2O) of CDen.

of $[CDenH]^+$ suggests that the first protonation involves the NH_2 group and this spectrum is not very different from that of CDen. The resonances of the CH_2 of the ethylenediamine moiety are shifted downfield at 3.1 and 2.9 ppm, respectively, as a consequence of the protonation. Furthermore they show the same multiplicity as is found in CDen, suggesting that the hydrogen bond does not break as a consequence of the first protonation. On the basis of this, an intra-chain hydrogen bond sketched in Formula 1 can be proposed.



Formula 1.

The second step of protonation, involving the secondary amine nitrogen, causes differences of the ^{1}H spectra (Figure 2). The protonation of the -NH group breaks the hydrogen bond, and induces an asymmetry on the CDen molecule. It is interesting to observe that, while in the case of CDhm [10], the first protonation

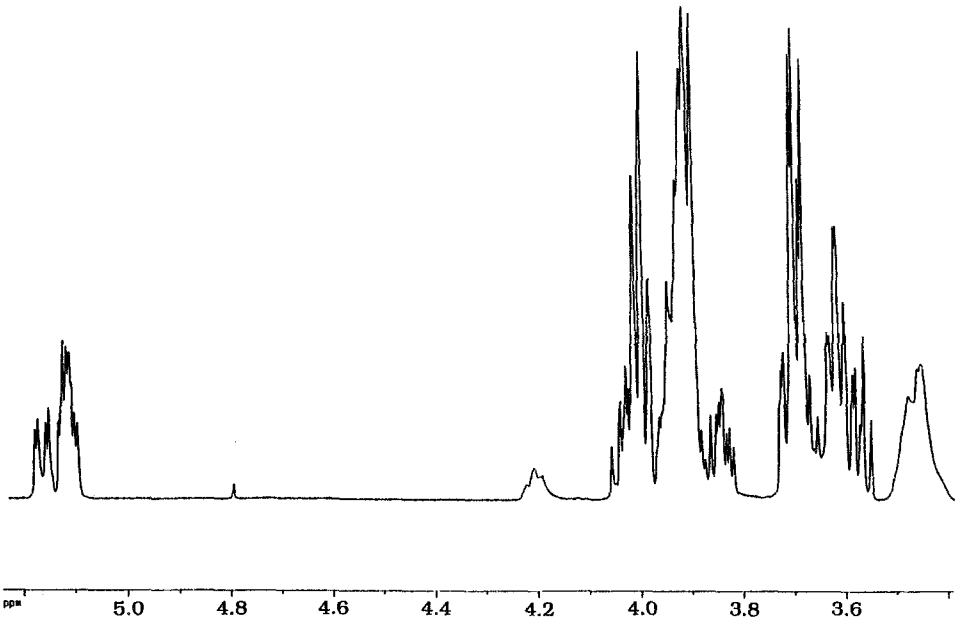


Fig. 2. ^{1}HNMR spectrum (600 MHz, D_2O) of $[CDenH_2]^{2+}$.

was sufficient to break the hydrogen bond, because the imidazole N-1 atom does not act as a hydrogen acceptor, in the case of CDen the hydrogen bond is formed between two amino groups, both of them being able to accept hydrogen bonds. As a consequence, the hydrogen bond persists to and beyond the first protonation and breaks only when all the electronic lone pairs are involved in the protonation process. The $[CDenH_2]^{2+}$ spectrum is much more complex than the spectra of the other two species and this asymmetry appears even in the H-1 and H-2 regions, relatively far from the protonation centres, as inferred from the COSY spectrum. On the basis of CPK-models we can hypothesize a hydrogen bond of the NH_2^+ group with a primary hydroxyl group of a B or G glucosidic ring. The additional methylene peak observed in the diprotonated species ^{13}C spectrum, compared to the corresponding spectra of the other species, may be ascribed to C-6B or C-6G atom confirming this hypothesis. At 4.2 ppm the H-5A appears and this downfield shift accompanies the protonation of the NH-group, in the β position with respect to the C-5A atom, as observed for CDhm [10], and should be ascribed to an electronic through-the-chain effect.

The proton and copper(II) complex formation constants for CDen are reported respectively in Tables II and III, together with the values for some other amines (en = ethylenediamine, CH_3en = *N*-methylethylenediamine). $\Delta G°$, $\Delta H°$ and $\Delta S°$ values for proton and copper(II) complex formation are given in the same tables. The protonation of amine nitrogens is mainly favoured on enthalpy grounds,

TABLE II

Thermodynamic parameters of protonation of CDen, en, CH_3en at 25 °C and I = 0.1 mol dm^{-3} (KNO_3).

Equilibrium	log K	$-\Delta G^{\circ *}$	$-\Delta H^{\circ *}$	$\Delta S^{\circ **}$	Ref.
CDen + H^+ = $[(CDen)H]^+$	8.92 (1)	12.18 (1)	9.8(2)	8(1)	this work
$[(CDen)H]^+ + H^+ = [(CDen)H_2]^{2+}$	5.56 (5)	7.58 (7)	9.1(2)	-5(1)	this work
en + H^+ = $[(en)H]^+$	9.89	13.49	11.88	5.6	[22]
$[(en)H]^+ + H^+ = [(en)H_2]^{2+}$	7.10	9.69	10.90	-4.0	[22]
$CH_3en + H^+ = [(CH_3en)H]^+$	10.21	13.94	11.25	9.0	[23]***
$[(CH_3en)H]^+ + H^+ = [(CH_3en)H_2]^{2+}$	7.27	9.9	10.30	-1.3	[23]***

* kcal mol^{-1},

** cal mol^{-1} deg^{-1},

*** I = 0.5 mol dm^{-3}

as expected. Comparing the thermodynamic parameters accompanying the first protonation of the CDen amino group with the analogous parameters of en and CH_3en, it is evident that the basicity decrease is almost completely due to a less favourable enthalpy contribution. From the NMR study we know that a hydrogen bond is present in the unprotonated species. Owing to this bond, the nitrogen lone pair of the primary amine is less disposed to interact with the incoming proton. This fact could explain the decreased basicity in comparison to the en and CH_3en molecules. The decrease in basicity of the secondary amine nitrogen is also due to a less favourable enthalpy value, but, in addition, an unfavourable entropy contribution explains the more relevant decrease of the log K value of the second protonation step compared to en and CH_3en. Also in this case these enthalpy and entropy changes can be explained considering the NMR results. According to these, we observe two effects that influence the protonation process in opposite ways: the breaking of intrachain hydrogen bonding (enthalpy unfavoured) and the interaction of the diprotonated species with the oxygen atoms of the cyclodextrin residue (enthalpy favoured). Furthermore, this latter process involves a decrease of conformational freedom that implies an unfavourable entropy contribution. In addition, it may be considered that the proximity of nitrogen basic sites to the relatively hydrophobic cyclodextrin cavity could determine a decrease in basicity [25].

The $[Cu(CDen)]^{2+}$ complex formation is significantly less favoured than that of the $[Cu(en)]^{2+}$ species due to a lesser exothermic enthalpy contribution. The difference in ΔH° values of copper(II) complexes parallels that found in the proton complex formation of amine and reflects the basicity decrease. Even if the bis-complex $[Cu(CDen)_2]^{2+}$ is less stable in comparison with the analogous en and CH_3en complex species, the differences in enthalpy values are less marked,

TABLE III

Thermodynamic parameters for copper(II) complexes of CDen, en, CH_3en at 25 °C and I = 0.1 mol dm^{-3} (KNO_3).

Equilibrium	log K	$-\Delta G°$*	$-\Delta H°$*	$\Delta S°$**	Ref.
$CDen + Cu^{2+} = [Cu(CDen)]^{2+}$	7.81 (1)	10.65 (1)	7.9(2)	9.2(4)	this work
$[Cu(CDen)]^{2+} + CDen = [Cu(CDen)_2]^{2+}$	6.17 (1)	8.41 (1)	10.1(2)	-5.6(4)	this work
$en + Cu^{2+} = [Cu(en)]^{2+}$	10.46	14.28	12.5	6	[22]
$[Cu(en)]^{2+} + en = [Cu(en)_2]^{2+}$	9.01	12.30	12.30	0	[22]
$CH_3en + Cu^{2+} = [Cu(CH_3en)]^{2+}$	10.49	14.32	11.5	9.5	[23]***
$[Cu(CH_3en)]^{2+} + CH_3en = [Cu(CH_3en)_2]^{2+}$	8.71	11.89	12.2	-1	[23]***

* kcal mol^{-1},

** cal mol^{-1} deg^{-1},

*** I = 0.5 mol dm^{-3}.

while the entropy contribution is significantly unfavourable. Noncovalent interactions are favoured on enthalpy grounds and are unfavoured on entropy grounds as previously seen for proton and metal complex formation [26]. If we compare the thermodynamic parameters of the $[Cu(CDen)_2]^{2+}$ complex formation with those pertinent to the $[Cu(CDen)]^{2+}$ species, we can observe a more favourable enthalpy contribution and an unfavourable entropy contribution. Thus, the unfavourable enthalpy contribution being constant due to the intrachain hydrogen bonding breaking in the first and second step of complex formation, the more favourable enthalpy change can be ascribed to a favourable interaction between the cyclodextrin moieties, which seems to overcome crowding problems due to the steric hindrance of the cavities.

In addition, the comparison of the EPR magnetic parameters of the $[Cu(CDen)]^{2+}$ species with the analogous data relative to the $[Cu(en)]^{2+}$ complex deserves some comments (Table I). As usual, systems having aliphatic nitrogens as donor atoms do not give rise to a relevant superhyperfine pattern in copper(II) complexes, thus it is impossible to have direct evidence of the number of nitrogens bound to the metal centre. Nevertheless, the values of $g_{||}$ and $A_{||}$ are extremely sensitive to the copper(II) environment as well as to the peculiar geometry of the coordination polyhedron. In effect, the magnetic parameters of the $[Cu(CDen)]^{2+}$ complex are not very different from those obtained in the case of the $[Cu(en)(H_2O)_4]^{2+}$ species. The differences are more marked when the magnetic parameters of the bis-complexes are compared. The bulkiness of cyclodextrin molecules can be responsible for a slight distortion of the square planar arrangement.

As regards the mixed complexes, in the pH range explored in the present work, $[Cu(CDen)L]^{2+}$ is the main species. Other species have been identified, such as

TABLE IV

Thermodynamic parameters for the formation of copper(II) ternary complexes of CDen with *L/D*-alanine, phenylalanine or tryptophan at 25 °C and $I = 0.1$ mol dm^{-3} (KNO_3).

AaO^-	log β[a]	$-\Delta G^{\circ\star}$	$-\Delta H^{\circ\star}$	$\Delta S^{\circ\star\star}$
L-AlaO^-	15.48 (2)	21.11(3)	13.5(3)	25.4(4)
D-AlaO^-	15.45 (2)	21.10(3)	13.5(3)	25.4(4)
L-PheO^-	15.50 (2)	21.13(3)	12.0(3)	30.6(6)
D-PheO^-	15.55 (2)	21.20(3)	12.6(3)	29.0(4)
L-TrpO^-	16.28 (2)	22.19(3)	14.5(3)	25.8(4)
D-TrpO^-	16.26 (2)	22.17(3)	14.7(3)	25.0(4)

[a] $\beta = \dfrac{[Cu(CDen)(AaO^-)]^+}{[Cu^{2+}][CDen][AaO^-]}$

$\star$ kcal mol^{-1},

$\star\star$ cal mol^{-1} deg^{-1}

$[Cu(CDen)(L)(H)]^{2+}$ below pH 4.5. However, owing to the small percentage of this species, we were not able to determine its stability constant. The stability constants of the mixed ligand complexes do not show a distinct trend (Table IV) and stereoselectivity seems to be insignificant or absent, unlike the behaviour found in the case of the mixed complex of copper(II)–CDhm and *L/D* amino acids [12].

The ΔH° and ΔS° values indicate that the complex formation is enthalpy and entropy driven (Table IV), as expected for this kind of complex where the potential donor atoms of the amine derivative and the amino acid are involved in the coordination of the copper(II) ion. Among the ternary complexes of *L*- or *D*-amino acids the enthalpy and entropy changes do not show significant differences, thus all the thermodynamic parameters indicate the absence of stereoselectivity. However, as can be seen in Figure 3, HPLC measurements show that in the case of tryptophan, unlike that of alanine and phenylalanine, a partial separation is obtained, with the *L*-enantiomer eluting first.

Certainly, other factors must be considered in order to describe HPLC behaviour, besides complex stability in solution, such as: (i) complex affinity for the stationary phase; and (ii) complex stability at the interphase. These factors could explain the apparent discrepancy between thermodynamic parameters and LEC HPLC experiments. In particular, we must consider that a low discriminating interaction is amplified in the chromatographic experiments and Davankov [27] reported that it is possible to have separation even in the presence of very small differences (10–100 cal/mol) in the interaction between different enantiomers and the stationary phase.

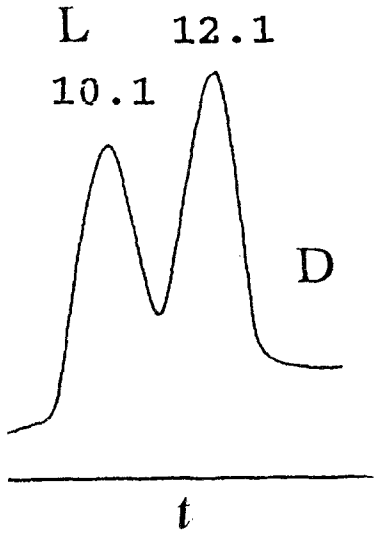


Fig. 3. Chromatogram of *D*- and *L*-Trp. See text for conditions.

The c.d. spectrum of the *L*-phenylalanine as well as that of the *L*-tryptophan ternary complex shows a stronger Cotton effect, compared to the analogous *D*-enantiomer complex, though the $\Delta(\Delta\epsilon)$ value is small (Table V). The enhancement of the Cotton effect, as usual, may be ascribed to the inclusion of the indole moiety in the cyclodextrin cavity.

If what can be deduced from LEC (showing weak enantioselectivity), and thermodynamic parameters (which do not show any enantioselectivity), can be rationalized on the basis of our own previous observations and those described in the scientific literature [27], the different diagnostic range of c.d. parameters compared to thermodynamic ones deserves further consideration. Both of these investigations were being carried out in homogeneous solution.

The weak enantioselective effect cannot be reflected in the thermodynamic parameters, the method used being insufficiently accurate in this respect.

Thus this investigation shows that c.d. spectroscopy, and LEC HPLC experiments, at least for this kind of system, are more sensitive techniques for observing such effects.

Considering the results of the present investigation, together with the results for CDhm [10–13] and CDmh [14] analogous systems, we observe both a graduation of chiral discriminating ability and inversions in the elution order between the enantiomeric tryptophans (Table VI). The proposed '*cis*'-effect satisfactorily explains the elution orders in the different systems, since molecular models show that the complex in which the indole moiety is included in the cyclodextrin cavity, is the more stable, is the first to elute, shows the stronger Cotton effect, and is always the one in which the amino acid amino group is adjacent to the more similar donor group of the functionalized cyclodextrin.

TABLE V

Electronic and c.d. spectral data [λ_{max}/nm (ϵ or $\Delta\epsilon$/dm^{-3} mol^{-1} cm^{-1})] for the ternary system Cu^{2+}–CDen–AaO^- (6×10^{-3} mol dm^{-3}).

AaO^-	pH	UV-vis		c.d.		
		λ_{max} (nm)	ϵ	λ_{max} (nm)	$\Delta\epsilon$	$\Delta(\Delta\epsilon)^*$
L-AlaO^-	7.2	246	5300	225.8	0.30	
		588	67	260.4	-0.42	
				– –	– –	
						n.d.
D-AlaO^-	7.2	244	5400	202.4	-0.38	
		588	61	241.4	0.99	
				– –	– –	
L-PheO^-	7.2	248	5415	235.8	1.40	
		588	69	286.2	-0.26	
				566.4	-0.14	
						0.39
D-PheO^-	7.2	248	5442	235.8	0.17	
		588	72	286.2	0.47	
				575	0.24	
L-TrpO^-	7.2	264	5758	232.2	1.37	
		588	42	579.2	-0.26	
						0.45
D-TrpO^-	7.2	264	5748	241	-0.71	
		588	40	560.2	0.19	

* Absolute value of $\Delta(\Delta\epsilon)$ between the c.d. spectra of the complex $[Cu(CDen)(D-AaO)]^+$ and $[Cu(CDen)(L-AaO)]^+$ at $\lambda = 577$, 570 nm PheO^- and TrpO^- amino acid, respectively (n.d. = not detectable).

TABLE VI

Enantioselectivity factors, α, for tryptophan enantiomers in LEC using Cu(II)-*L* as chiral eluent.

L	$\alpha(K'_L/K'_D)$	Ref.
CDhm	1.41	[11]
CDmh	2.4	[14]
CDen	0.8	this work

However, the difference in the efficiency of chiral discrimination, as reflected by the different α values (Table VI), observed for the different functionalized cyclodextrin complexes, remains to be explained more fully. At the beginning of the present investigation an appropriate question might have been: are the differences between a primary and a secondary amino group sufficient to observe a '*cis*'-effect? The results show that though a '*cis*'-effect is acting in these complexes, we are in a borderline case, and this fact, in turn, suggests that probably the question should be corrected thus: how stable is the '*cis*'-complex formation compared to the '*trans*'-complex formation? In other words, considering that the factors listed in order to explain the different α values between CDmh systems and CDhm systems are not sufficient to explain the CDen system behaviour, we suppose that a *cis*-complex $\rightleftharpoons$ *trans*-complex equilibrium occurs in these systems and that the more such an equilibrium is shifted towards the left, the greater is the difference between the two donor groups in the functionalized cyclodextrin. Unfortunately, owing to the co-occurrence of the other cited factors influencing α values, we have no straightforward way of quantifying the constant concerning this equilibrium, but, in any case, all the experimental data so far obtained agree with such a hypothesis, and more work is in progress in our laboratory to confirm and develop it.

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