Molecular Recognition of Amino Acids by Copper(II) Complexes of $6^A,6^X$-Diamino-$6^A,6^X$-dideoxy-β-cyclodextrin (X = B, C, D)

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The three regioisomers of β-cyclodextrin 6-difunctionalized with NH_2 groups ($6^A,6^X$-diamino-$6^A,6^X$-dideoxy-β-cyclodextrin, A,X-$CDNH_2$, X = B, C, or D) were synthesized. Their binary and ternary copper(II) complexes with amino acids were characterized by ESR and electronic spectroscopy. Furthermore, the binary copper(II) complexes were used as eluent in ligand exchange chromatography (LEC), to resolve racemates of unmodified amino acids. HPLC separation of enantiomers of aromatic amino acids was obtained only when the complex $[Cu(A,B\text{-}CDNH_2)]^{2+}$ was used as eluent. The two complexes with the other two regioisomers did not show chiral recognition ability. Circular dichroism (c.d.) spectroscopy studies of the ternary complexes with D- and L-amino acids carried out in the presence and in the absence of 1-adamantanol, suggested a recognition mechanism that involves the cyclodextrin cavity, only in the case of ternary A,B-$CDNH_2$ complexes.

Introduction

The complexing properties of the molecular cavity of cyclodextrins (CDs), have been largely investigated and have been employed in many different applications.[1−7]

Replacement of hydroxyl groups of CDs with other functional groups has been shown to remarkably improve the CDs' ability to form inclusion complexes or their catalytic properties.[1,2,6−9] The CDs and their derivatives are chiral host molecules, and it is known that they can exhibit enantioselectivity in reaction with a racemic mixture.[4,10−12] The appropriate functionalization can largely improve the selectivity in the host−guest interaction.

The investigation on the recognition properties of functionalized cyclodextrins is growing, and in this context, CDs bearing appropriate substituents as recognition sites have been synthesized.[13−16] Cyclodextrins bearing a positive and a negative charge on the C(6) carbon of adjacent A and B (or B and A) glucose rings, have been used as artificial receptors of tryptophan, based on a multisite recognition mechanism.[17] Furthermore, CDs functionalized with an amino group have been used as chiral receptors for carboxylic acids such as D/L-ibuprofen[13] and, more recently, for the selective complexation of nucleotides and nucleosides.[14]

The functionalization is a useful way to improve the CD's ability to form stable metal complexes and in this context, different appropriate functionalizing moieties have been attached to the CD cavity.[1,6,18−22] In the cyclodextrin metal complexes, the metal ion can cooperate with the binding properties of the CD cavity, as a second recognition site. In particular, copper(II) or nickel(II) complexes of cyclodextrins monofunctionalized with diamines have been used as molecular receptors, and their enantioselective binding of amino acids has been observed.[21−25] When some copper(II) complexes were investigated,[21−24] it has been suggested that the discrimination between the two amino acid enantiomers is due to a different interaction of amino acid side chain with the cavity. A preferred cis disposition of the two amino groups (one of the amino acid and the other one of the CD functionalized ligand), on the coordination plane of the copper(II) ion, determines an orientation of the amino acid side chain, which is different for the two enantiomers. The chemical differences of the two nitrogen donor atoms of CD moiety in the previously studied diamine derivatives seems to be a very

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important factor for the enantioselective recognition mechanism.[21,23] Particularly, the enantiomer which is more strongly bound can be partially included in the CD cavity, while the side chain of the other enantiomer points out of the cavity.[24]

In this paper, we report on the chiral recognition properties of the copper(II) complexes of the three regioisomers of $6^A,6^X$-diamino-$6^A,6^X$-dideoxy-β-cyclodextrin (X = B, C, or D). These complexes were used as chiral additives to the mobile phase in LEC (ligand exchange chromatography),[26] to resolve amino acid racemic mixtures. The copper(II) binary and ternary complexes of these CDs, difunctionalized with NH_2 groups, and some amino acids (L/D-Ala, D/L-Phe, D/L-Trp) were characterized by ESR, c.d., and electronic spectroscopy.

These difunctionalized ligands have no chain on the CD rim; thus a strong interaction of the guest with the cavity may be expected. Furthermore, their two nitrogen donor atoms are equivalent, unlike in the other ligands previously studied.[21,23] Consequently, the recognition mechanism invoked to rationalize the results obtained with differently substituted CDs cannot be extended to these systems. The comparison of the discriminatory properties of the copper(II) complexes with the different isomers allows the understanding of the chiral recognition process mechanism.

Experimental Section

General Details. β-Cyclodextrin and anhydrous dimethylformamide was purchased from Fluka and Aldrich, respectively. Amino acids were purchased from Sigma.

TLC was carried out on silica gel plates (Merck 60-F254). CD derivatives were detected with UV light and with anisaldehyde reagent and CD amino derivatives by the ninhydrin test.

NMR spectra were recorded with a Bruker AC-200 spectrometer at 200.13 MHz, without a reference compound.

Chromatographic separations were performed on a Hewlett Packard series 1050 HPLC, using a Spherisorb ODS-2 column (3 μm, 150 × 4.6 mm), setting the UV detector at 254 nm. The mobile phase was prepared by dissolving A,X-$CDNH_2$ (8×10^{-5} mol dm^{-3}), the $Cu(NO_3)_2$ (8×10^{-5} mol dm^{-3}), and sodium acetate (3×10^{-3} mol dm^{-3}) in a H_2O/CH_3OH (80:20) mixture, adjusting the solution to pH = 7. The dead volume was calculated by injecting water.

Electronic and c.d. spectra were recorded on a Beckman DU 650 spectrophotometer and on a JASCO J-600 dichrograph, respectively. Calibration of the c.d. instrument was performed with a 0.06% solution of ammonium camphorsulfonate in water ($\Delta\epsilon$ = 2.40 at 290.5 nm). The spectra were recorded at 25 °C, on freshly prepared aqueous solutions of the binary (Cu^{2+} −A,X-$CDNH_2$, 1:1 ratio) and ternary (Cu^{2+} −A,X-$CDNH_2$−AaO^-, 1:1:1 ratio) systems at pH = 7. The spectral range (200−700 nm) was covered using quartz cells of various path lengths, so that dilution of the solution was not required. The c.d. spectra with 1-adamantanol were carried out in a water−methanol solution (90:10) at 25 °C and at pH 7. This concentration of methanol does not modify the c.d. spectra of the ternary complexes.

ESR frozen solution spectra were performed on a Bruker instrument, Type ER 200D, driven by the Model 3220 data system. All spectra were recorded at the temperature of 150 K, achieved by the aid of a standard low temperature apparatus. The 3×10^{-3} mol dm^{-3} binary and ternary complexes solutions were prepared by mixing together aqueous solution of $^{63}Cu(NO_3)_2 \cdot 6H_2O$ and the pertinent ligands in 1:1 and 1:1:1 ratios, respectively, adjusting the pH at 7 by adding NaOH. Up to 5% methanol was added to these solutions, to increase the resolution which is known to be poor for aqueous solutions. Parallel spin Hamiltonian parameters were calculated directly from the computer output of the experimental spectra. DPPH radical (g = 2.0036) was used to standardize the klystron frequency, the magnetic field being also monitored by a Bruker gauss meter, Type ER 0.35M.

Synthesis of $6^A,6^X$-Diamino-$6^A,6^X$-dideoxy-β-cyclodextrin (X = B, C, D) (A,X-$CDNH_2$). The regioselective synthesis of $6^A,6^X$-diamino-$6^A,6^X$-dideoxy-β-cyclodextrin (X = B, C, D) was carried out as described elsewhere,[27,28] starting from the appropriate CD disulfonates.[29]

Chart 1

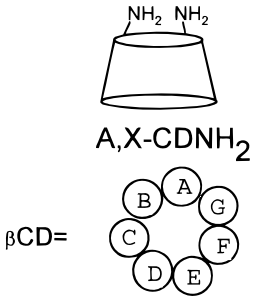

Table 1. Spin Hamiltonian Parameters for Binary Copper(II) Complexes of $[Cu(A,X\text{-}CDNH_2)]^{2+}$ in Water−Methanol (95:5) Solution at 150 K

complexes	$g_{\|\|}$	$A_{\|\|} \times 10^4$ (cm^{-1})
$[Cu(A,B\text{-}CDNH_2)]^{2+}$	2.262(2)	176(2)
$[Cu(A,C\text{-}CDNH_2)]^{2+}$	2.256(2)	175(2)
$[Cu(A,D\text{-}CDNH_2)]^{2+}$	2.262(2)	175(2)

Analytical Data. A,B-$CDNH_2$:[28] FAB MS *m*/*e* 1136 (M + 1); ^{1}H NMR (200 MHz, D_2O) δ 5.04 (m, 7H, 1-H), 4.0−3.7 (m, 24H, 3-,6-,5-H), 3.67−3.37 (m, 14H, 2-,4-H), 3.08 (d, 2H, $H6_{aA}$ and 6_{aB}; $J_{6aA,6bA} = J_{6aB,6bB}$ = 13.8 Hz), 2.85 (dd, 6bA and 6bB, 2H, $J_{6A,5A} = J_{6B,5B}$ = 7 Hz); ^{13}C NMR (50.3) δ 104.5 (C-1), 85.4 (C-4A,4B), 83.8 (C-4), 75.7−74.4 (C-5,2,3), 62.9 (C-6), 43.8 (C-6A,6B).

A,C-$CDNH_2$: R_f = 0.25 (eluent: PrOH−H_2O−AcOEt−NH_3 5:3:1:2); FAB MS *m*/*e* 1136 (M + 1) ^{1}H NMR (200 MHz, D_2O) δ 5.03 (m, 7H, 1-H), 3.95−3.7 (m, 24H, 3-,6-,5-H), 3.7−3.39 (m, 14H, 2-,4-H), 3.11 (d, 2H, $H6_{aA}$ and 6_{aB}; $J_{6aA,6bA} = J_{6aB,6bB}$ = 13.8 Hz), 2.79 (dd, 6bA and 6bB, 2H, $J_{6A,5A} = J_{6B,5B}$ = 7 Hz); ^{13}C NMR (50.3) δ 104.5 (C-1), 85.5 (C-4A,4B), 83.7 (C-4), 75.7−74.1 (C-5,2,3), 62.9 (C-6), 43.8 (C-6A,6B). Anal. Calcd for $C_{42}H_{72}N_2O_{33} \cdot 8H_2O$: C, 39.5; H, 6.9; N, 2.1. Found: C, 38.8, H, 7.0; N, 2.2.

A,D-$CDNH_2$: R_f = 0.25 (eluent: PrOH−H_2O−AcOEt−NH_3 5:3:1:2); FAB MS *m*/*e* 1136 (M + 1); ^{1}H NMR (200 MHz, D_2O) δ 5.05 (m, 7H, 1-H), 4.01−3.72 (m, 24H, 3-,6-,5-H), 3.72−3.38 (m, 14H, 2-,4-H), 3.13 (d, 2H, $H6_{aA}$ and 6_{aB}; $J_{6aA,6bA} = J_{6aB,6bB}$ = 13.8 Hz), 2.87 (dd, 6bA and 6bB, 2H, $J_{6A,5A} = J_{6B,5B}$ = 7 Hz). ^{13}C NMR (50.3) δ 104.5 (C-1), 85.5 (C-4A,4B), 83.7 (C-4), 75.7−74.5 (C-5,2,3), 62.9 (C-6), 43.9 (C-6A,6B). Anal. Calcd for $C_{42}H_{72}N_2O_{33} \cdot 8H_2O$: C, 39.5; H, 6.9; N, 2.1. Found: C, 41.0, H, 6.8, N, 2.

Results and Discussion

In order to have information on the complexing features of this class of ligands, A,X-$CDNH_2$ (X = B, C, and D), their binary and ternary copper(II) complexes have been characterized by ESR and UV−vis absorption spectroscopies.

The ESR magnetic parameters pertinent to the binary and ternary copper(II) complexes, reported in Tables 1−4, show a general trend: smaller $g_{\|\|}$ values and greater $A_{\|\|}$ constants for the ternary complexes than those of the binary ones. This suggests that the coordination sphere of the copper(II) complex is completed by the entrance of the amino acidate ligand. In analogy with similar systems, the $g_{\|\|}$ values and $A_{\|\|}$ constants are ascribable to $CuN_2(Ow)_4$ and $CuN_3O(Ow)_2$ chromophores, respectively. ESR frozen solution spectra of binary copper(II) complexes with A,X-$CDNH_2$ do not show marked differences in their parallel magnetic parameters (see Table 1); in other words, their magnetic parameters are not dependent on the

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Table 2. Spin Hamiltonian Parameters for Ternary Copper(II) Complexes of $[Cu(A,B\text{-}CDNH_2)(AaO)]^+$ in Water−Methanol (95:5) Solution at 150 K

complexes	$g_{\|}$	$A_{\|} \times 10^4$ (cm^{-1})
$[Cu(A,B\text{-}CDNH_2)(L\text{-}AlaO)]^+$	2.249(2)	187(2)
$[Cu(A,B\text{-}CDNH_2)(D\text{-}AlaO)]^+$	2.249(2)	187(2)
$[Cu(A,B\text{-}CDNH_2)(L\text{-}PheO)]^+$	2.242(2)	188(2)
$[Cu(A,B\text{-}CDNH_2)(D\text{-}PheO)]^+$	2.234(2)	191(2)
$[Cu(A,B\text{-}CDNH_2)(L\text{-}TyrO)]^+$	2.240(2)	192(2)
$[Cu(A,B\text{-}CDNH_2)(D\text{-}TyrO)]^+$	2.235(2)	194(2)
$[Cu(A,B\text{-}CDNH_2)(L\text{-}TrpO)]^+$	2.248(2)	187(2)
$[Cu(A,B\text{-}CDNH_2)(D\text{-}TrpO)]^+$	2.244(2)	186(2)

Table 3. Spin Hamiltonian Parameters for Ternary Copper(II) Complexes of $[Cu(A,C\text{-}CDNH_2)(AaO)]^+$ in Water−Methanol (95:5) Solution at 150 K

complexes	$g_{\|}$	$A_{\|} \times 10^4$ (cm^{-1})
$[Cu(A,C\text{-}CDNH_2)(L\text{-}AlaO)]^+$	2.254(2)	183(2)
$[Cu(A,C\text{-}CDNH_2)(D\text{-}AlaO)]^+$	2.255(2)	185(2)
$[Cu(A,C\text{-}CDNH_2)(L\text{-}PheO)]^+$	2.251(2)	186(2)
$[Cu(A,C\text{-}CDNH_2)(D\text{-}PheO)]^+$	2.247(2)	183(2)
$[Cu(A,C\text{-}CDNH_2)(L\text{-}TyrO)]^+$	2.254(2)	184(2)
$[Cu(A,C\text{-}CDNH_2)(D\text{-}TyrO)]^+$	2.250(2)	185(2)
$[Cu(A,C\text{-}CDNH_2)(L\text{-}TrpO)]^+$	2.247(2)	185(2)
$[Cu(A,C\text{-}CDNH_2)(D\text{-}TrpO)]^+$	2.250(2)	186(2)

Table 4. Spin Hamiltonian Parameters for Ternary Copper(II) Complexes of $[Cu(A,D\text{-}CDNH_2)(AaO)]^+$ in Water−Methanol (95:5) Solution at 150 K

complexes	$g_{\|}$	$A_{\|} \times 10^4$ (cm^{-1})
$[Cu(A,D\text{-}CDNH_2)(L\text{-}AlaO)]^+$	2.250(2)	188(2)
$[Cu(A,D\text{-}CDNH_2)(D\text{-}AlaO)]^+$	2.251(2)	186(2)
$[Cu(A,D\text{-}CDNH_2)(L\text{-}PheO)]^+$	2.246(2)	185(2)
$[Cu(A,D\text{-}CDNH_2)(D\text{-}PheO)]^+$	2.249(2)	183(2)
$[Cu(A,D\text{-}CDNH_2)(L\text{-}TyrO)]^+$	2.251(2)	185(2)
$[Cu(A,D\text{-}CDNH_2)(D\text{-}TyrO)]^+$	2.249(2)	185(2)
$[Cu(A,D\text{-}CDNH_2)(L\text{-}TrpO)]^+$	2.248(2)	185(2)
$[Cu(A,D\text{-}CDNH_2)(D\text{-}TrpO)]^+$	2.249(2)	184(2)

relative position of the two amino groups. This behavior indirectly suggests that the CD cavity can partially lose its rigidity, due to the formation of strong nitrogen−copper bonds. The CPK models also make clear that the lengthening of some hydrogen bonds in A,C-$CDNH_2$ and A,D-$CDNH_2$ ligands may occur. Furthermore, the X-ray structure of $6^A,6^B$-dideoxy-$6^A,6^B$-diamino-β-cyclodextrin has been recently obtained, and the stretching of one of the 2OH−3OH hydrogen bonds has been found in consequence to the functionalization. The copper coordination seems to force the donor atoms to assume, more or less, the same coordination distances at the narrower rim by a deformation at the wider rim.

Tables 2−4 show that the magnetic parameters of ternary complexes with A,X-$CDNH_2$ and L- or D-$AlaO^-$ are very similar. Thus, we can assume that the ternary complexes with L- or D-$AlaO^-$ constitute the blank reference point, in order to give evidence to differences which could emerge within the ternary complexes with L- or D-amino acidate enantiomers. In other words, since the methyl group of the alaninate side chain is not bulky, no weak interactions are expected to efficiently occur in these systems between the side chain substituents and the cyclodextrin cavity. Moreover, subtle differences, exceeding experimental error, are only evident for the copper(II) ternary complexes of the A,B-$CDNH_2$ isomer with amino acidate containing aromatic side chains. It is quite noteworthy, that these subtle differences within these last ternary complexes, especially involve the $g_{\|}$ values (in a lesser extent the $A_{\|}$ constants), which are always lower in the case of the D-isomers. It is well-known that a lower $g_{\|}$ value and a higher $A_{\|}$ hyperfine constant indicate a greater covalency in the coordination plane of a copper(II) complex. Thus, we can assert that in the series of the ternary complexes with A,B-$CDNH_2$ ligand, the D-amino acidate gives a more covalent complex, as can also be seen by the blue shifts of the visible absorption band maxima (see Table 5).

Chiral recognition ability of the copper(II) complexes of A,X-$CDNH_2$ (X = B, C, D), was tested in LEC-HPLC, adding the complex to the eluent. The chromatographic results when $[Cu(A,B\text{-}CDNH_2)]^{2+}$ was used as a chiral eluent are summarized in Table 6.

Only the $[Cu(A,B\text{-}CDNH_2)]^{2+}$ is able to resolve an enantiomeric mixture of some amino acids. In particular, no alifatic amino acid mixture was resolved and among the aromatic amino acids, phe and tyr show the higher α values. This trend is different from that found when some other similar systems have been used as chiral selectors.[21−24]

The *m*- and *o*-tyr racemic mixtures were not resolved, suggesting the peculiar role of the OH position in the recognition process.

On the contrary, $[Cu(A,C\text{-}CDNH_2)]^{2+}$ and $[Cu(A,D\text{-}CDNH_2)]^{2+}$ were not able to resolve any amino acids.

In keeping with the theory of LEC, the resolution of a racemic mixture of amino acids, is due to the formation of diastereoisomeric ternary complexes which have different stability constants.[26] Cyclodextrins have been described as models to study interactions in the mobile phase,[26] and thus we can consider that the chiral recognition occurs in the mobile phase. The ternary complexes eluted first, in this case $[Cu(A,B\text{-}CDNH_2)(D\text{-}AaO)]^+$, may be provided with higher stability in aqueous solution.

The c.d. spectra were carried out in order to investigate the chiral recognition process of these systems. The c.d. data of binary and ternary complexes are reported in Tables 7 and 8.

The chiral recognition ability of the alone ligands was also tested by c.d. spectroscopy, but no enantioselectivity was found.

The appearance of a c.d. band in the electronic absorption region of achiral molecules, as result of their complexation with cyclodextrins, has been widely investigated and used to describe the geometry of inclusion complexes.[30−36] In these copper(II) complexes, a chiral potential guest (amino acid) is present, and thus the variation (positive or negative) of the Cotton effect is a consequence of the interaction with the cavity, as has been found for similar systems.[23,37,38] In the case of $[Cu(A,B\text{-}CDNH_2)(AaO)]^+$, when AaO^- is $AlaO^-$, the shape of the c.d. spectra in the UV and vis regions is independent of the configuration of the $AlaO^-$. Both the ternary complexes with the L- and D-$AlaO^-$, show $\Delta\epsilon$ values of the same sign as those observed for the analogous complexes with $AlaO^-$.[23] When AaO^- is an aromatic amino acid (Tyr, Phe, and Trp), the shape and the intensity of the c.d. spectra depend on the absolute configuration of the amino acid. If we assume the binary complex $[Cu(AaO)]^+$ as a comparison, ternary systems with both amino acids (L and D) show a positive variation of $\Delta\epsilon$ in the UV region. These positive $\Delta\epsilon$ changes, as already described for achiral aromatic guest,[30−32] suggest the inclusion of the aromatic ring. In the UV region, the c.d. bands are due to the transition of aromatic rings and to charge transfer transitions. Thus, different from the described inclusion complexes,[30−38] a

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Table 5. Electronic and CD Spectral Parameters for $[Cu(A,B\text{-}CDNH_2)]^{2+}$ and for the Ternary Complexes $[Cu(A,B\text{-}CDNH_2)(AaO)]^{+}$ with Aliphatic and Aromatic Amino Acids at pH = 7

	UV−vis λ_{max} (nm) (ϵ^a)	c.d. λ_{max} (nm) ($\Delta\epsilon^a$)
$[Cu(A,B\text{-}CDNH_2)]^{2+}$	228 (1554); 680 (40)	275 (−0.18); 600 (0.45)
$[Cu(A,B\text{-}CDNH_2)(AaO)]^{+}$		
L-AlaO$^-$	234 (5150); 628 (49)	246 (−2.10); 604 (0.20)
D-AlaO$^-$	234 (5044); 620 (42)	246 (−1.13); 604 (0.27)
L-PheO$^-$	242 (5800); 613 (52)	280 (−0.70); 544 (−0.14)
D-PheO$^-$	244 (4844); 599 (57)	232 (1.11); 282 (0.92); 612 (0.30)
L-TyrO$^-$	226 (10204); 608 (65)	231 (−1.37); 268 (1.10); 307 (−0.52); 552 (0.18)
D-TyrO$^-$	226 (12546); 599 (67)	230 (6.48); 276 (−1.57); 307 (0.57); 614 (0.28)
L-TrpO$^-$	222 (21740); 270 (4946); 609 (55)	226 (2.11); 267 (−1.24); 610 (0.09)
D-TrpO$^-$	222 (16091); 272 (3845); 604 (60)	216 (5.06); 254 (−0.94); 356 (0.11); 600 (0.45)

[a] ϵ, $\Delta\epsilon$: $dm^{-3}\ mol^{-1}\ cm^{-1}$.

Table 6. HPLC Enantioselective Factor ($\alpha = K'_L/K'_D$) for the Chiral Separation of Amino Acids with $[Cu(A,B\text{-}CDNH_2)]^{2+}$ Added to the Eluent[a]

AA	$\alpha_{L/D}$	AA	$\alpha_{L/D}$
Ala	1.0	*m*-Tyr	1.0
Leu	1.0	*p*-Tyr	1.50
Phe	1.18	Trp	1.06
o-Tyr	1.0		

[a] Eluent: $[Cu(A,B\text{-}CDNH_2)]^{2+}$ (8×10^{-5} M), H_2O/MeOH (80:20), pH 7. Flow rate: 1 cm^3/min. Detector: UV (254 nm). Column: Spherisorb ODS-2 (3 μm, 150 × 4.6 mm).

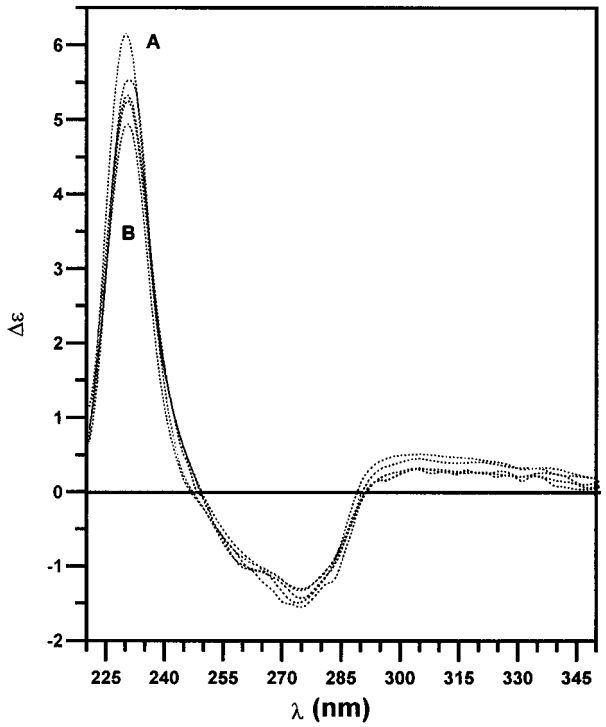


Figure 1. The c.d. spectra of $[Cu(A,B\text{-}CDNH_2)(D\text{-}TyrO)]^{+}$ (4×10^{-4} mol dm^{-3}) alone (A) upon addition (B) of 1-adamantanol (8×10^{-5} to 3×10^{-3} mol dm^{-3}) in water methanol (9:1) at pH 7 (read from A to B).

simple correlation of the sign of the band with the orientation of the guest is not easy. However, on the basis of $\Delta\epsilon$ variation in the UV region, the interaction of the amino acid side chain with the cavity can be suggested. Furthermore, the CD cavity could act as a second coordination sphere ligand of the metal ion, influencing the charge transfer (UV region) and d−d (vis region) bands. This interaction is strongly enantioselective. On the contrary, in the case of AC and AD regioisomers the spectra of ternary complexes $[Cu(A,X\text{-}CDNH_2)(AaO)]^{+}$ are "enantiomeric", in keeping with the chromatographic results.

In order to further investigate the recognition mechanism, we have used a competitive guest, 1-adamantanol, and carried

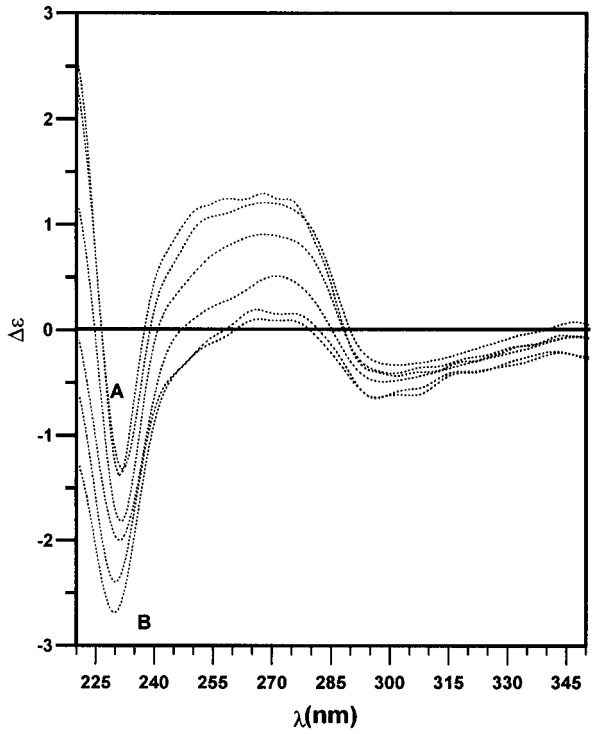


Figure 2. The c.d. spectra of $[Cu(A,B\text{-}CDNH_2)(L\text{-}TyrO)]^{+}$ (4×10^{-4} mol dm^{-3}) alone (A) upon addition (B) of 1-adamantanol (8×10^{-5} to 3×10^{-3} mol dm^{-3}) in water methanol (9:1) at pH 7 (read from A to B).

out c.d. spectra. It is known that 1-adamantanol is a good guest for the cyclodextrin cavity, and it has been used as a competitive guest.[9,39] It has the advantage of not giving coordination with copper(II) ion, thus only interacting with the cavity without modification of the complexing features of ligands. In order to verify this, ESR spectra were recorded on the final solution used for the c.d. spectra, and the preservation of the same coordination features was verified. By addition of increasing amounts of 1-adamantanol, the c.d. spectra of copper(II) in the $A,B\text{-}CDNH_2$ ternary complexes change. On the contrary, the c.d. spectra of copper(II) complexes of AC and AD isomers, remain practically unchanged. Namely, the $|\Delta\epsilon|$ of the c.d. spectra of $[Cu(A,B\text{-}CDNH_2)(AaO)]^{+}$ decrease in the case of the ternary complex with the D-enantiomer and increase with the L one (see Figures 1 and 2). The variation of $\Delta\epsilon$ found in the case of AB isomer spectra suggests a modification of the aromatic side chain interaction with the cavity, due to the inclusion of 1-adamantanol. The c.d. data suggest that the equilibrium shown in Scheme 1 occurs.

In the case of AC and AD regioisomers, the amino acidate complexation probably occurs outside of the cavity, and in

(39) Du, Y.-Q.; Nakamura, A.; Toda, F. *J. Incl. Phenom.* **1991**, *10*, 443.

Table 7. Electronic and c.d. Special Paramaters for $[Cu(A,C\text{-}CDNH_2)]^{2+}$ and for the Ternary Complexes $[Cu(A,C\text{-}CDNH_2)(AaO)]^{+}$ with Aliphatic and Aromatic Amino Acids at pH = 7

	UV−vis λ_{max} (nm) (ϵ^a)	c.d. λ_{max} (nm) ($\Delta\epsilon^a$)
$[Cu(A,C\text{-}CDNH_2)]^{2+}$	240 (793); 717 (29)	
$[Cu(A,C\text{-}CDNH_2)(AaO)]^{+}$		
L-AlaO$^-$	234 (3053); 636 (54)	240 (−0.38); 624 (−0.030)
D-AlaO$^-$	234 (2932); 637 (56)	240 (0.39); 624 (0.04)
L-PheO$^-$	240 (2145); 630 (42)	215 (−0.37); 241 (0.30); 284 (−0.07); 606 (−0.08)
D-PheO$^-$	240 (1092); 624 (32)	216 (0.54); 241 (−0.17); 280 (0.15); 606 (0.06)
L-TyrO$^-$	224 (8970); 270 (2080); 616 (62)	226 (−2.58); 262 (0.69); 302 (−0.13); 610 (−0.25)
D-TyrO$^-$	224 (8111); 270 (1889); 610 (62)	227 (3.37); 271 (−0.51); 296 (0.21); 610 (0.18)
L-TrpO$^-$	220 (4140); 270 (1140)	227 (0.08); 250 (0.06); 334 (−0.04); 628 (−0.19)
D-TrpO$^-$	218 (4172); 270 (1479)	228 (0.03); 334 (0.03); 608 (0.15)

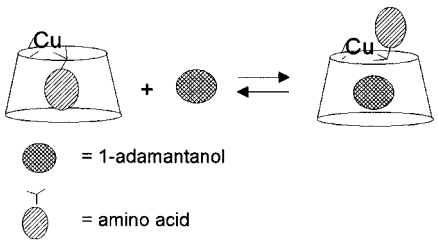
[a] ϵ, $\Delta\epsilon$: $dm^{-3}\ mol^{-1}\ cm^{-1}$.

Table 8. Electronic and c.d. Spectral Parameters for $[Cu(A,D\text{-}CDNH_2)]^{2+}$ and for the Ternary Complexes $[Cu(A,D\text{-}CDNH_2)(AaO)]^{+}$ with Aliphatic and Aromatic Amino Acids at pH = 7

	UV−vis λ_{max} (nm) (ϵ^a)	c.d. λ_{max} (nm) ($\Delta\epsilon^a$)
$[Cu(A,D\text{-}CDNH_2)]^{2+}$	230 (303); 693 (30)	
$[Cu(A,D\text{-}CDNH_2)(AaO)]^{+}$		
L-AlaO$^-$	236 (4103); 629 (38)	242 (−0.59); 626 (−0.05)
D-AlaO$^-$	236 (4079); 629 (33)	248 (0.67); 626 (0.06)
L-PheO$^-$	238 (1404); 629 (nd)	216 (0.57); 238 (−0.24); 283 (−0.02); 604 (−0.06)
D-PheO$^-$	246 (2070); 608 (39)	216 (0.57); 238 (−0.24); 285 (0.09); 604 (−0.04)
L-TyrO$^-$	224 (7773); 270 (1787); 600 (41)	226 (−2.83); 264 (0.60); 304 (−0.13); 602 (−0.24)
D-TyrO$^-$	220 (7149); 270 (1319); 598 (44)	226 (1.95); 270 (−0.29); 304 (0.14); 602 (0.13)
L-TrpO$^-$	224 (24570); 266 (8070)	430 (0.022); 614 (0.078)
D-TrpO$^-$	220 (23180); 266 (6840)	326 (0.01); 440 (−0.03)

[a] ϵ, $\Delta\epsilon$: $dm^{-3}\ mol^{-1}\ cm^{-1}$.

Scheme 1



keeping with this, the c.d. spectra were not influenced by adding 1-adamantanol.

Nevertheless, the final spectra of the $[Cu(A,B\text{-}CDNH_2)(L\text{-}AaO)]^{+}$ and $[Cu(A,B\text{-}CDNH_2)(D\text{-}AaO)]^{+}$ after the addition of a large amount of 1-adamantanol are not completely enantiomeric. Most likely, the equilibrium above is not shifted enough toward the right to completely eliminate the contribution of the intramolecular ternary complex. At the end of the titration with 1-adamantanol, a larger $\Delta\epsilon$ is observed on the spectra of the complex with D-amino acid in comparison with L, in keeping with the chromatographic and ESR data, which suggest a higher stability of this complex.

The different behavior of the binary copper(II) complexes of the three regioisomers can be rationalized on the basis of the CPK models. We can suggest that the copper(II) complexes of AC and AD isomers do not act as chiral selector because the complexation with copper(II) in the binary complexes partially "closes" the narrow CD rim, and thus no interaction of the aromatic side chain and the cavity can occur in the ternary complex formation according to the c.d. spectra.

On the contrary, in the case of A,B-$CDNH_2$ ternary complexes, the amino acid side chain can interact with the cavity. In this case, the effect of the partial closure of the upper rim can explain the best resolution obtained for amino acids with smaller aromatic side chains.

Concluding Remarks

In this paper, we have investigated the chiral recognition mechanism of the copper(II) complex of difunctionalized β-CDs. On the basis of these results, the role of the metal ion can be underlined: no enantioselective interaction was observed in the case of the ligands alone.

LEC-HPLC was used as method for screening the occurrence of enantioselectivity in the metal complexes. On the basis of the chromatographic behavior of the tyrosine isomers, when AB isomer complex was used, it can be hypothesized that the OH group in the *m*- or o-position interacts with the cavity and thus the enantioselective interaction observed in the case of *p*-Tyr or Phe is modified and destroyed.

Different $\Delta\epsilon$ values for ternary complexes containing the two amino acid enantiomers were found in the c.d. spectra only when the A,B-$CDNH_2$ was used as ligand, in keeping with the chromatographic results. This behavior suggests that inclusion of aromatic side chain can occur only in the case of the AB regioisomer. In the AC and AD regioisomers, the copper(II) complexation partially closes the entrance of the cavity, and thus the complexation with the amino acid and its inclusion cannot occur together. The involvement of the cavity in the recognition process is further confirmed by means of c.d. spectra in the presence of the competitive guest 1-adamantanol. Only the c.d. spectra of ternary complexes of AB isomer are modified in the presence of this guest.

These copper(II) complexes recognize amino acids with a different mechanism of the monofunctionalized CD.[21−24] In these diamino derivative complexes the cis effect, as has been previously described by us,[21−23] cannot act because there are no differences between the two donor atoms of the ligands (both NH_2 groups).

The involvement of the cavity in the recognition process is confirmed further by means of c.d. spectra in the presence of the competitive guest 1-adamantanol. Only the c.d. spectra of ternary complexes of the AB isomer are modified in the presence of this guest.

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