Enantioselective Folding at the Cyclodextrin Surface

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Spectroscopic and kinetic studies of β-cyclodextrin-linked L- and D-phenylalanine cyanoethyl esters in aqueous solution reveal an unusual intramolecular complexation mode where the hydrophobic portion of the amino acid resides outside the host cavity; L- and D-derivatives show different binding geometries and energies.

The ability of cycloamyloses (cyclodextrins, CyD) to engage in chiral recognition has engendered a diversity of applications ranging from chromatographic separations to asymmetric catalysis.[1] The mechanism of recognition by CyD generally rests upon internal inclusion, since their hydrophobic cavites often provide energetically favourable binding sites for organic guest molecules. It was not until kinetic studies of Tee[2] that the possibility of extra-cavity binding became an alternative explanation for CyD recognition properties.

We have synthesized derivatives of β-CyD covalently linked to L- and D-phenylalanine esters through a succinoyl spacer **1**.† Preliminary molecular modelling indicated that flexibility of the latter could allow formation of an unstrained intramolecular complex with the phenyl ring being included in the CyD cavity from the primary side.‡ In order to define the inclusion modes, we performed an NMR study of **1** in D_2O. The ^1H spectra showed no concentration dependence (0.5–10 mmol dm^{-3}) of the chemical shifts, indicating an absence of intermolecular complexation. ^1H NMR titration of **1** with cyclohexanol (known to form inclusion complexes with β-CyD) yielded plots of δ *vs.* [**1**] from which the limiting values of complexation-induced chemical shifts (CIS), along with binding constants K, were estimated by nonlinear regression. As indicated in Fig. 1, the external guest entering the cavity of **1** exerts both shielding and deshielding effects on the aminoacid protons, the shifts ranging from δ −0.2 to +0.5. Absolute CIS values differ between L- and D-**1** especially for the proton at the chiral centre adjacent to NH and CO groups [δ 0.47 (L-) and 0.12 (D-)]. Complexation of cyclohexanol with both L- and D-**1** was found to be 10–20-fold weaker than with native β-CyD (K = 500)[3]. This fact and the appreciable CIS values of the substituent protons show that intramolecular association between the amino acid ester and CyD moieties hinder the interaction of cyclohexanol with the CyD cavity. The different CIS of L- and D-**1**, and their difference in binding constants (K_L/K_D = 1.5, Fig. 1), indicate enantioselectivity of this intramolecular complexation.

Aromatic protons of the phenylalanine were less affected by the complexation than those near asymmetric carbon. This led to the suggestion that complexation modes other than internal inclusion could be occuring in the complexes. NOESY spectra

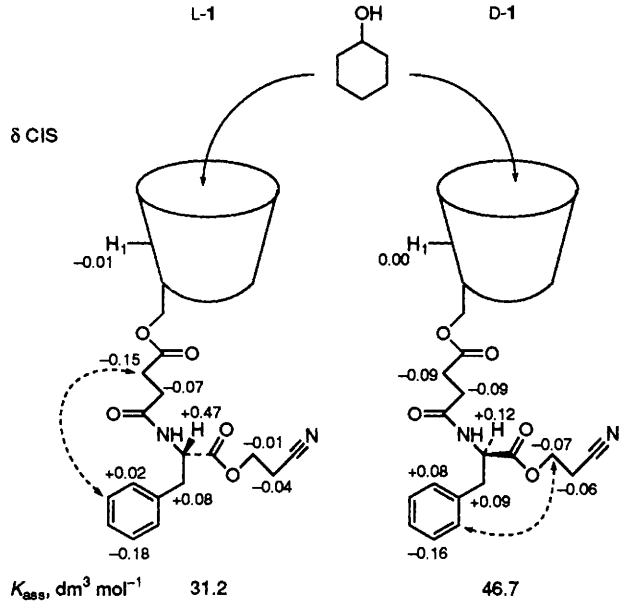


Fig. 1. ^1H NMR chemical shifts of **1** (CIS) induced by complexation with cyclohexanol, and the respective binding constants (K_{ass}). Dashed lines correspond to cross-peaks in the NOESY spectra of $2 \cdot 10^{-3}$ mol dm^{-3} **1** in D_2O.

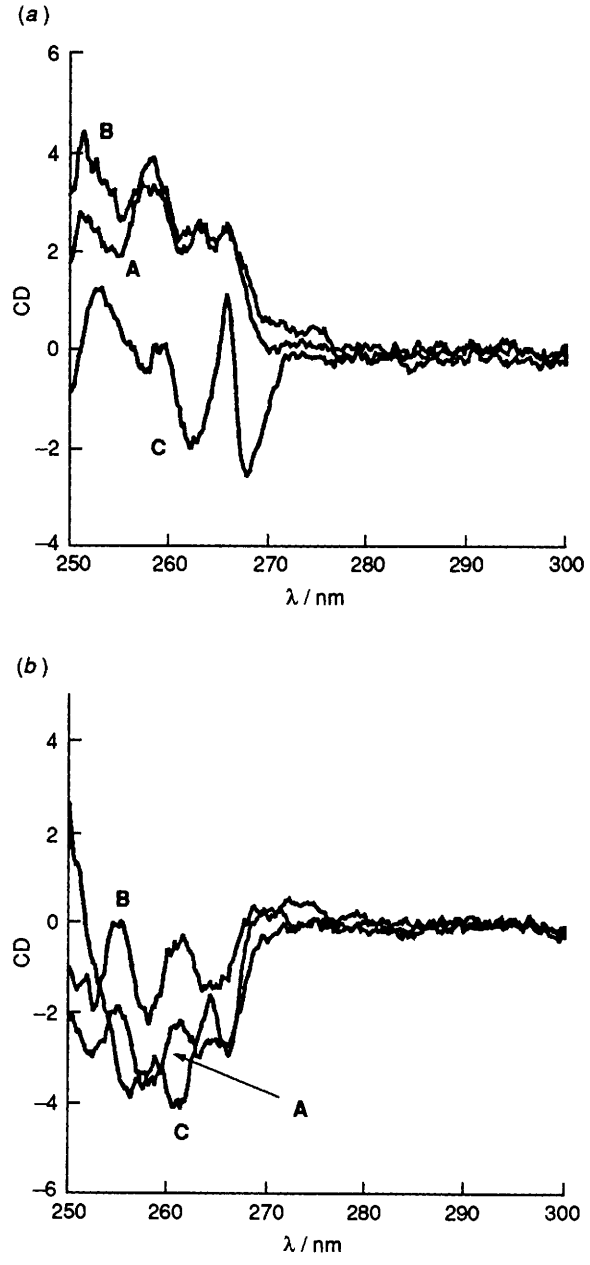
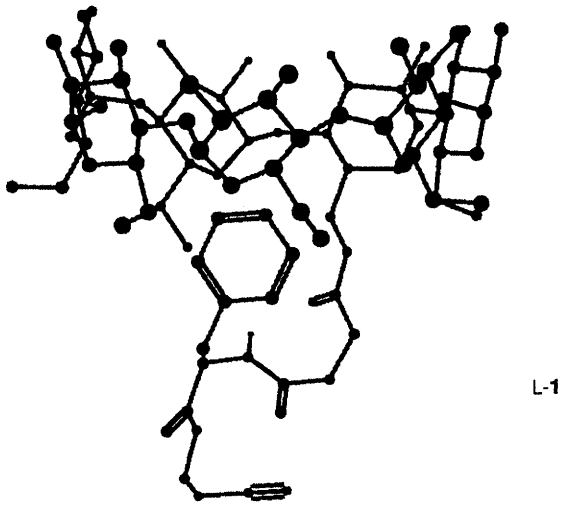


Fig. 2. CD spectra of (**A**) $3.5 \cdot 10^{-3}$ mol dm^{-3} phenylalanine cyanoethyl ester hydrochloride; (**B**) $3.5 \cdot 10^{-3}$ mol dm^{-3} phenylalanine cyanoethyl ester hydrochloride and 10^{-2} mol dm^{-3} mono-6^A-succinyl-β-CyD (ammonium salt); (**C**) $3.5 \cdot 10^{-3}$ mol dm^{-3} **1** in water. (*a*) L-isomers; (*b*) D-isomers.

of L- and D-**1** yielded cross-peaks within the side chains (Fig. 1, dashed arrows), in addition to cross-peaks typical for CyD.[4] At the same time, no cross-relaxation has been observed between intra-cavity 3-H and 5-H protons of the CyD and the aromatic protons which would be expected were an inclusion complex actually forming.

Another indication of the amino acid being located outside the cavity was obtained from circular dichroism (CD) spectra of **1** (Fig. 2). According to recent findings,[5] inclusion of an aromatic chromophore inside the CyD cavity should cause a positive shift in the CD spectrum. This effect we observed when 6^A-succinyl-β-CyD ('CyD part' of **1**) was mixed with phenylalanine cyanoethyl ester ('amino acid part' of **1**), owing to intermolecular complexation (*cf.* curves **A** and **B**, Fig. 2). The CD spectra of **1** (curves **C**, Fig. 2), however, are shifted towards negative values with respect to the pure amino acid esters, corresponding to the extra-cavity position of the aromatic rings.

Finally, despite the dependance of intramolecular complexation in **1** upon side-chain chirality, we found no measurable difference between L- and D-**1** in the rates of both alkaline hydrolysis of cyanoethyl esters (pH 9.5) (V_L/V_D = 1.01 ± 0.05) and the elimination of acrylonitrile (DBU, DMSO; V_L/V_D = 0.96 ± 0.05). Thus the ester group of phenylalanine, being external to the cavity, is kinetically unaffected by the host moiety.§



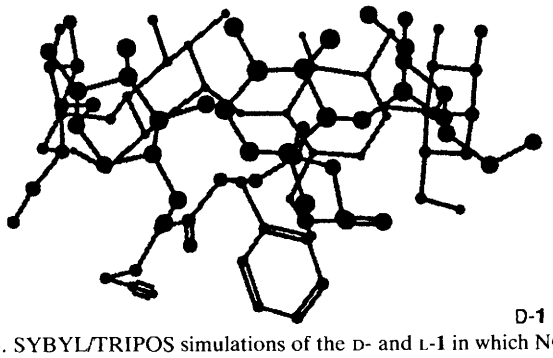


Fig. 3. SYBYL/TRIPOS simulations of the D- and L-**1** in which NOE constraints have been imposed

The spectroscopic and kinetic data suggest that in aqueous solutions of **1** the amino acid substituent is folded near the CyD moiety outside the host cavity. Force-field calculations shown in Fig. 3 are consistent with all physico-chemical properties of the complexes. A plausible driving force for these complexation modes could be multiple polar interactions (*e.g.* dipole–dipole and hydrogen bonds) between the CyD hydroxyls and C=O and NH groups of the side chain (similar to the way bilirubin[6] or nucleotides[7] associate with CyD). We believe this is the first example where a CyD 'prefers' surface interaction with the polar part of an amphiphilic species to possible inclusion complexation. These results seem to provide a new insight into the mechanism of chiral recognition by cyclodextrins, and they might assist in the design of new amino acid and peptide receptors.

This work was supported by the National Science Foundation.

Received, 9th June 1994; Com. 4/03498H

Footnotes

† β-CyD (Aldrich) was acylated by succinic anhydride in dry pyridine, and the mono-substituted derivative was isolated by ion-exchange chromatography (Sepharose-Q, NH_4^+-form, gradient of water–0.3 mol dm^{-3} NH_4HCO_3, yield 45%). CBZ-Phe (Sigma) was coupled with cyanoethanol (DCC–DMAP, yield 80%), deprotected by TFA (yield 30%) and transformed into the free base. 1 Equiv. of 6^A-succinyl-β-CyD (H-form) and 1 equiv. of Phe ester (free base) were heated in dry DMF under N_2 with 1.1 equiv. of EEDQ at 70 °C for 40 h. Precipitation from acetone and purification by reverse-phase chromatography (Whatman LRP-2, 8–20% ethanol–water) yielded 27% of **1**. The product gave correct mass and ^{13}C NMR spectra. ^1H NMR, (500 MHz, D_2O, assignments made by homonuclear COSY experiment): δ 7.4–7.0 (m, 5H, Ar), 5.02–4.90 [m, 7H, 1-H(CyD)], 4.30 (m, 2H, CH_2CN), 4.17 (br.s, 1H, CH asymm. Phe), 4.0–3.3 (m, other H), 3.03, 2.81 (dd, 2H, $PhCH_2$), 2.80 (t, 2H, CH_2O cyanoeth.), 2.57, 2.45 (m, 4H, CH_2 succ.); Found: C, 46.79; H, 6.22; N, 1.83. Calc. for **1**·$3H_2O$: C, 46.77; H, 6.23; N, 1.88%.

‡ According to force-field calculations (SYBYL/TRIPOS), gas-phase energy of the self-included form of L-**1** is 7.1 kcal mol^{-1} lower than that of the open form. (1 cal = 4.184 J.)

§ In the previously described examples of chiral intramolecular recognition in CyD derivatives[8,9] short spacers between the host and the guest seemed to force partial inclusion of the latter. The difference of our case is that the flexible succinyl chain provides proximity of the interacting moieties without restricting their possible conformations.

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