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Direct Correlation between Complex Conformation and Chiral Discrimination upon Inclusion of Amino Acid Derivatives by β- and γ-Cyclodextrins

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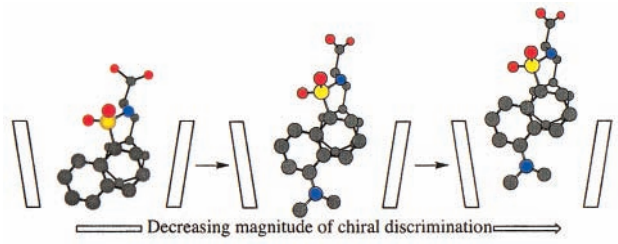
ABSTRACT



A correlation between the conformation and chiral recognition characteristics of a series of modified amino acid complexes with β- and γ-cyclodextrins has been determined, using titration microcalorimetry and ^{1}H NMR techniques. The enantiomeric discrimination (D or L) is found to be dependent on the adoption of one of two distinct conformations. With the magnitude of the chiral discrimination (K_D/K_L) arising from the guest's depth of penetration into the host's cavity.

Chiral discrimination is well-known to be a key factor in numerous bioprocesses such as drug−protein binding[1] and olfaction and is critical to the effective control and application of new chiral technologies.[2] The study of chiraly discriminating supramolecular systems offers a powerful tool for the detailed rationalization of this important process. Natural and modified cyclodextrins (CDs) have received much attention over recent years as aqueous-based hosts for studying the recognition of chiral organic guests[3] and have been successfully applied to chiral separation technologies.[4] In our recent study,[5] based on the thermodynamic parameters obtained for several families of structurally related chiral guests, a direct correlation between the mode of penetration and chiral recognition by β-CD has been established, with the isomeric preference depending on the position of the most hydrophobic group around the chiral carbon, which determines the direction of guest penetration, and thus the orientation of the interaction of the chiral group with β-CD. However because of the poor size complementarity between these compounds and γ-CD, it has not been possible to investigate this correlation for the larger cavity. Thus, to favor complexation with γ-CD, compounds with two aromatic groups linked by a chiral tether were investigated, (*N*-(1-naphthyl-

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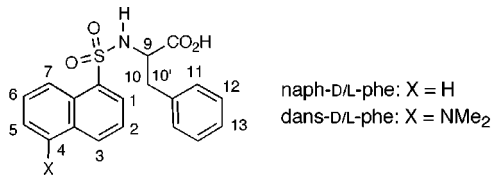
enesulfonyl-D/L-phenylalanine (naph-D/L-phe) and dansyl-D/L-phenylalanine (dans-D/L-phe)).



Guest molecules

In principle, when the aromatic rings of the guests are intramolecularly stacked, the size of the whole hydrophobic moiety becomes large enough to fill almost all the space inside of the γ-CD cavity resulting in a high degree of size/shape complementarity and thus is potentially able to form a stable host−guest complex. Here we report the differences in chiral discrimination and conformation found between these guests and β- and γ-CDs and how the rationalization of these leads to a detailed experimental understanding of the factors that govern both the enantiomeric discrimination (D versus L), and the magnitude of chiral discrimination (K_D/K_L).

We first consider the results of the microcalorimetric experiments.The treatment of the microcalorimetric titration data has been described previously.[5,6] All the equilibrium constants presented in Table 1 are based on a 1:1 binding model and a single binding site. Calculations were also performed to assess the possible occurrence of 1:2 binding. In these cases it was found that the additional parameters used to assess this possibility had large uncertainties comparable with the parameters themselves and that the quality of the overall fit was not improved. Thus, the assumption of the 1:1 binding model and a single binding site is the simplest choice, and more complex models are not justified by experimental microcalorimetric data. Initial concentrations of cyclodextrins and guests in the microcalorimetric experiments were 0.2−1.2 and 10−70 mM, respectively. Repetitions of the microcalorimetric experiments with different initial concentrations were performed to ensure reliability of the thermodynamic parameters obtained.

Table 1. Complex Stability Constant (K/M^{-1}), Enthalpy ($\Delta H°$/kJ mol^{-1}), and Entropy Changes ($T\Delta S°$/kJ mol^{-1} K^{-1}) for 1:1 Inclusion Complexation of dans-D/L-phe, naph-D/L-phe, and Related Compounds with β- and γ-CDs in 0.05 M Standard Phosphate Buffer (pH 6.9; T = 298.15 K)

host	guest	K	$\Delta H°$	$T\Delta S°$
β-CD	dans-D-phe	412 ± 10	−18.1 ± 0.3	−3.2 ± 0.3
	dans-L-phe	368 ± 14	−17.1 ± 0.5	−2.4 ± 0.5
	naph-D-phe	328 ± 20	−29.8 ± 1.5	−15.4 ± 1.5
	naph-L-phe	565 ± 20	−31.5 ± 1.0	−15.8 ± 1.0
γ-CD	dans-D-phe	3800 ± 150	−24.5 ± 0.4	−4.1 ± 0.4
	dans-L-phe	2600 ± 150	−20.8 ± 0.3	−1.3 ± 0.4
	naph-D-phe	1940 ± 50	−26.4 ± 0.4	−7.6 ± 0.4
	naph-L-phe	1200 ± 80	−25.6 ± 0.4	−8.0 ± 0.5

All complexation reactions under consideration are exclusively enthalpy driven, and in all cases complexation is associated with unfavorable entropy changes (see Table 1). This thermodynamic pattern suggests strong host−guest van der Waals interactions.[7] Values of the equilibrium constants are in accordance with the host−guest size complementarity and are always larger for γ-CD when compared with the corresponding β-CD complexation reaction. Complexation of naph-D/L-phe is always associated with more exothermic enthalpy and more negative entropy changes than the corresponding reaction involving dans-D/L-phe. This thermodynamic behavior is more pronounced for β-CD when compared with the corresponding γ-CD complexation reactions. It was found that the D-isomer of dans-phe is favored by both β- and γ-CDs, with the higher chiral discrimination in the latter case (K_D/K_L = 1.12 and 1.46 respectively); as well as for the naph-phe γ-CD complexes (K_D/K_L = 1.62). In contrast, the opposite isomer (L) of naph-phe is preferentially recognized by β-CD (K_D/K_L = 0.58). To understand the structural features of these complexes that lead to the observed enantiomeric discrimination, and the magnitude of chiral discrimination (K_D/K_L), the complexes were investigated by 1D and 2D ^{1}H NMR.[8]

Analysis of the NMR data shows that the aromatic rings of dans-D/L-phe and naph-D/L-phe in D_2O buffer solution are intramolecularly stacked, as revealed by the large upfield shifts (ca. $\Delta\delta$ = −0.2 ppm for dansyl/naphthyl and −0.5 ppm for phenyl) of the aromatic protons in comparison to reference compounds (*N*-dansylglycine, *N*-naphthylglycine, and acetyl-L-phenylalanine). Also, in the case of the dans-D/L-phe complexes, intramolecular NOEs are observed between the dimethylamino group and the phenyl ring. The stoichiometry of the dans-D-phe:γ-CD complex was found to be 1:1 by NMR Job plot analysis, which is in agreement with microcalorimetric analysis. Owing to the close structural similarity of the other host−guest systems, it is assumed that all the other complexes have the same host:guest ratio; indeed no other evidence from either NMR or microcalorimetry indicates otherwise.

The conformations of the complexes formed between naph-D/L-phe and dans-D/L-phe and γ-CD were determined by 1D and 2D NMR, with the aid of CPK molecular models for rationalizing experimental data. The complexation-induced upfield shifts of the guests phenyl protons of −0.12 to −0.38 ppm indicate restriction of the motion between the naphthyl and phenyl rings arising from the inclusion into the CD cavity. Furthermore, the NOEs between the naphthyl, dansyl, and phe rings with the H3/H5 protons of γ-CD ($H3_{CD}$ and $H5_{CD}$) show corresponding cross-peaks that indicate inclusion into the cavity. These data are consistent with the structure defined below as Conformation A (Figure 1).

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(8) All NMR experiments were performed in a 0.05 M potassium phosphate D_2O buffer using a JEOL JNM-EX400 NMR spectrometer.

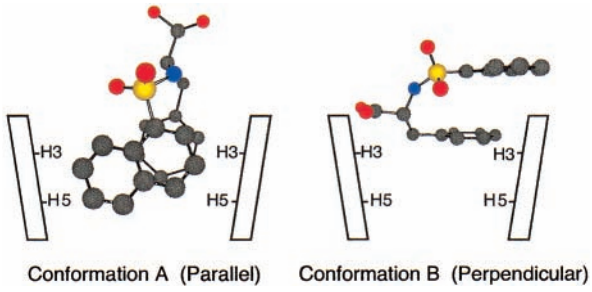


Figure 1. Complex Conformations A (for β- and γ-CDs) and B (for β-CD + naph-D/L-phe only).

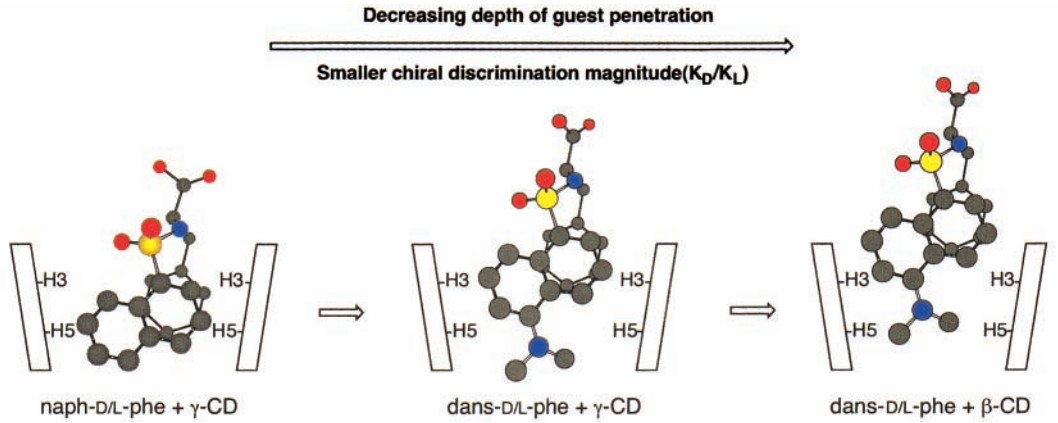
Additionally, the dans-D/L-phe and β-CD complexes were also shown to be in this conformation. All complexes in this conformation show enantiomeric discrimination in favor of the D isomer.

However, the complexes made between naph-D/L-phe and β-CD show an inverse enantiomeric selectivity in favor of the L isomer. Analysis of NMR data reveals the explanation for this being the formation of complexes in Conformation B (Figure 1). As expected for this conformation, strong NOE cross-peaks are observed between $H3_{CD}$ and H3, H4, and all the phenyl protons of the guests, while weaker NOEs are observed between H1, H5/6, and H7. No NOEs are found between $H5_{CD}$ and any naphthyl protons, with only those between the phenyl protons and $H5_{CD}$ observed. This conformation also results in greater contact between the guest and host, and this is reflected in the substantially more negative $\Delta H°$ and $\Delta S°$ values obtained for these two complexes from microcalorimetry (Table 1). The adoption of these two conformations accounts for the inversion in enantiomeric discrimination. In Conformation A (Figure 1) the host and guest are in a parallel orientation; however, Conformation B (Figure 1) brings these two molecules into a perpendicular orientation which affects the relative position/interaction of the host and guest chiral groups, resulting in the observed switch in enantiomeric chiral discrimination.

From detailed examination of the NMR and microcalorimetric data for the complexes in Conformation A, the chiral discrimination magnitudes (K_D/K_L) shown in Table 1 are found to be related to the average depth of penetration (and thus intermolecular contact between the chiral groups) of the guest into the cyclodextrin cavity. The data presented below show that the order of guest penetration depth into the host is γ-CD + naph-D/L-Phe > γ-CD + dans-D/L-Phe > β-CD + dans-D/L-Phe (Scheme 1). First, the $\Delta\delta$s of the phenyl group for the naph-D/L-phe and dans-D/L-phe γ-CD complexes on complexation are -0.12 to -0.38 for naph-D/L-phe and -0.11 to -0.27 for dans-D/L-phe. This shows that the phenyl groups of the naphthyl complexes are more restricted in their movements as a result of greater penetration. Second, in the ROESY spectra of the naphthyl complexes an NOE peak is observed between the guest's H10 and $H5_{CD}$ protons, whereas this is not observed for the dansyl complexes as a result of the greater interatomic distance. Third, from ROESY experiments, more intermolecular host−guest NOE peaks are observed for the naphthyl complexes, indicating deeper penetration. Fourth, the $\Delta H°$ values of the naphthyl complexes are more negative as a result of the greater intermolecular contact (even though the NMe_2 substituent of the dansyl group gives more potential for host−guest contact), with the correspondingly larger negative $\Delta S°$ values arising from the guest's more restricted motion inside the cyclodextrin cavity. dans-D/L-phe is not able to penetrate into the β-CD to the same degree as into the γ-CD as a result of the smaller β-CD cavity size, and this is reflected in the lower $\Delta H°$ values. Thus, it was found that small changes in the host/guest structure (and thus the complex conformation) result in substantial deviations in the magnitude of chiral discrimination (K_D/K_L). For the first time direct experimental evidence is obtained correlating the host−guest complex conformation with the magnitude of chiral discrimination (K_D/K_L), and not just for the enantiomeric preference (D or L).

To summarize, we have found that the mechanism of the observed enantiomeric discrimination (D versus L) is determined by the mode of guest penetration for these intramolecularly stacked compounds, with the magnitude of chiral

Scheme 1. Schematic Representation Relating Depth of Guest Penetration to K_D/K_L



discrimination (K_D/K_L) for complexes in Conformation A dependent upon the degree of intermolecular contact and thus the time averaged size chiral field experienced by the guest. The awareness and detailed rationalization of such conformational effects will be important in the understanding of a wide range of natural chiral recognition processes, such as the binding and competitive binding of chiral substrates to chiral receptors, and to the rational application of artificial chiral technologies that depend on chiral recognition processes for successful activity.

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Supporting Information Available: Additional 1D and 2D NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

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