Critical Difference in Chiral Recognition of *N*-Cbz-D/L-aspartic and -glutamic Acids by Mono- and Bis(Trimethylammonio)-β-cyclodextrins

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Aminated cyclodextrins, bearing positive charge(s) at neutral to acidic pHs, bind anionic guests stronger than native cyclodextrin (CD),[1−5] for which the attractive Coulombic interaction is, at least in part, responsible. In some cases, not only the binding but also the chiral recognition ability are enhanced by introducing amino group(s) to CDs.[1−6] However, this is not always the case and the cooperativity of several weak interactions is essential for the effective chiral recognition of anionic guests by native[7] and aminated CDs.[1]

To elucidate the cooperative weak interactions and mechanisms governing the chiral recognition by native and aminated CDs, we performed a comparative microcalorimetric and NMR spectral study on the complexation of homologous enantiomeric pairs of *N*-benzyloxycarbonyl-D/L-aspartic acid (Cbz-D/L-Asp) and Cbz-D/L-glutamic acid (Cbz-D/L-Glu) with mono- and *A*,*B*-, *A*,*C*-, and *A*,*D*-bis(6-trimethylammonio-6-deoxy)-β-CDs (TMA-β-CD[8] and TMA_2-β-CDs,[9] respectively). For comparison purposes, we also employed native β-CD and mono(6-ammonio-6-deoxy)-β-CD (Am-β-CD).[10]

Microcalorimetric Study. As amply demonstrated for a variety of homologous guest series,[3,7] Cbz-D/L-Glu gave more exothermic enthalpy changes ($-\Delta\Delta H° = 0.9-1.7$ kJ/mol) and less favorable entropy changes ($\Delta T\Delta S° = 0.5-1.3$ kJ/mol) than its lower "homologue" Cbz-D/L-Asp upon complexation with β-CD. Such compensating enthalpic and entropic changes are often observed and accounted for in terms of the stronger binding arising from the increased van der Waals contacts and the greater conformational fixation derived therefrom. As a consequence of the enthalpy−entropy compensation, the binding constants (K) for Cbz-D/L-Glu are only slightly larger (by 15−20%) than those for Cbz-D/L-Asp, and practically no chiral discrimination is observed for both guests. Compared with native β-CD, positively charged TMA-β-CD gave moderately enhanced K values (by 35−50%) for Cbz-D/L-Asp and -Glu, although no appreciable chiral discrimination was observed. Am-β-CD afforded almost doubled K values for both amino acids; this stronger binding is attributable to the less-bulky ammonio group that allows more intimate Coulombic interactions, as judged from the more exothermic $\Delta H°$.

Table 1. Thermodynamic Parameters for 1:1 Complexation of Native and Modified β-Cyclodextrins with Cbz-D/L-Glu and Cbz-D/L-Asp in Aqueous Buffer (pH 6.9) at 298.15 K

host	guest	K/M^{-1}	$\Delta H°$/ kJ mol^{-1}	$T\Delta S°$/ kJ mol^{-1}
β-CD	Cbz-L-Glu	86 ± 2	−10.49 ± 0.10	0.53 ± 0.15
	Cbz-D-Glu	84 ± 2	−11.20 ± 0.10	−0.22 ± 0.15
Am-β-CD	Cbz-L-Glu	162 ± 3	−10.51 ± 0.15	2.10 ± 0.15
	Cbz-D-Glu	168 ± 3	−11.32 ± 0.15	1.38 ± 0.15
TMA-β-CD	Cbz-L-Glu	119 ± 4	−10.9 ± 0.2	1.0 ± 0.2
	Cbz-D-Glu	114 ± 3	−10.11 ± 0.15	1.6 ± 0.2
A,*D*-TMA_2-β-CD	Cbz-L-Glu	107 ± 3	−7.80 ± 0.15	3.8 ± 0.2
	Cbz-D-Glu	94 ± 3	−7.52 ± 0.15	3.7 ± 0.2
A,*C*-TMA_2-β-CD	Cbz-L-Glu	133 ± 3	−8.90 ± 0.15	3.2 ± 0.2
	Cbz-D-Glu	113 ± 3	−6.85 ± 0.15	4.9 ± 0.2
A,*B*-TMA_2-β-CD	Cbz-L-Glu	189 ± 3	−12.79 ± 0.10	0.20 ± 0.15
	Cbz-D-Glu	135 ± 3	−10.00 ± 0.10	2.16 ± 0.15
β-CD	Cbz-L-Asp	74 ± 2	−9.59 ± 0.15	1.1 ± 0.2
	Cbz-D-Asp	71 ± 2	−9.50 ± 0.15	1.1 ± 0.2
Am-β-CD	Cbz-L-Asp	161 ± 2	−12.04 ± 0.15	0.54 ± 0.15
	Cbz-D-Asp	163 ± 2	−12.18 ± 0.15	0.45 ± 0.15
TMA-β-CD	Cbz-L-Asp	111 ± 3	−11.0 ± 0.2	0.7 ± 0.2
	Cbz-D-Asp	111 ± 4	−10.6 ± 0.2	1.1 ± 0.2
A,*D*-TMA_2-β-CD	Cbz-L-Asp	117 ± 3	−7.83 ± 0.15	4.0 ± 0.2
	Cbz-D-Asp	116 ± 4	−8.23 ± 0.15	3.6 ± 0.2
A,*C*-TMA_2-β-CD	Cbz-L-Asp	141 ± 5	−9.45 ± 0.15	2.8 ± 0.2
	Cbz-D-Asp	134 ± 4	−8.92 ± 0.15	3.2 ± 0.2
A,*B*-TMA_2-β-CD	Cbz-L-Asp	181 ± 3	−12.9 ± 0.2	0.0 ± 0.2
	Cbz-D-Asp	176 ± 4	−12.9 ± 0.2	−0.1 ± 0.2

Hence, the interaction of Cbz-D/L-Asp with Am-β-CD is an exception of the noncomplimentarity of multiple weak interactions, or a rare example of cooperative hydrophobic/van der Waals and Coulombic interactions.

On the other hand, D- and L-Cbz-Glu afforded appreciably different $\Delta H°$ and $T\Delta S°$ values, although the K values are exactly the same for both enantiomers. Thus, the two homologous amino acids exhibit distinctly different chiral recognition thermodynamics upon complexation with Am-β-CD. It should be emphasized that none of β-CD, Am-β-CD, and TMA-β-CD can differentiate the chirality of Cbz-Asp as a consequence of the same K, $\Delta H°$, and $T\Delta S°$ values for both enantiomers. In contrast, D- and L-Cbz-Glu are "virtually" differentiated by the same hosts, as judged from the distinctly different $\Delta H°$ and $T\Delta S°$ values, although "real" chiral discrimination is not observable in $\Delta G°$ due to the enthalpy−entropy compensation.

In an attempt to make the "hidden" chiral discrimination of Cbz-D/L-Glu visible, we synthesized a series of *A*,*X*-TMA_2-β-CD as bifunctional hosts. This is a logical choice for realizing higher chiral discrimination since, in principle, a more strict stereochemical fitting is required for dianionic guests such as Cbz-Glu to optimize the Coulombic interaction, while avoiding possible steric hindrance with the two bulky cationic TMA moieties located at the CD rim. Indeed, we have shown that the reduced positional/rotational freedom of the guest's penetrating group enhances the enantioselectivity,[7] although Smith et al. have shown that "real" discrimination of chiral phosphates is difficult to achieve even with *A*,*C*-diamino-β-CD.[11]

It has been demonstrated[3] that, when ion-paring interactions are significantly involved, the entropy factor plays a crucial role in the overall complexation thermodynamics. This is exactly the case with the diaminated *A*,*C*- and *A*,*D*-TMA_2-β-CD, which give positive $T\Delta S°$ (3−5 kJ/mol) upon complexation with both Cbz-Asp and -Glu, while native and monoaminated CDs (β-CD, Am-β-CD, and TMA-β-CD) give much smaller $T\Delta S°$ values (<1 kJ/mol). The introduction of two bulky TMAs, particularly at *A*,*C*-

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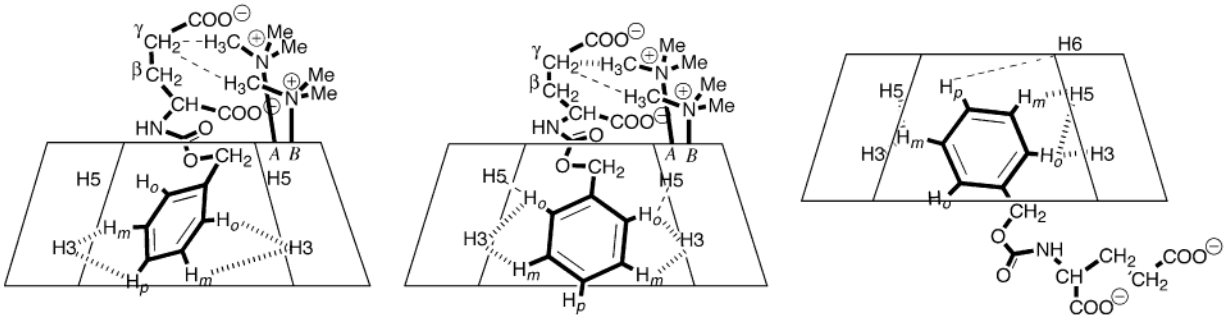

Figure 1. Plausible complex structures of (a) Cbz-L-Glu and (b) Cbz-D-Glu with *A,B*-TMA_2-*β*-CD and of (c) Cbz-L-Glu with native *β*-CD, elucidated from the ROESY experiments. Bold and thin hashes indicate strong and weak NOEs, respectively.

or *A,D*-glucose residues, appears to prevent the guest from enjoying the optimal van der Waals interactions, as judged from the reduced enthalpic gains of 7−9 kJ/mol. An extreme case is found in the affinity of *A,D*-TMA_2-*β*-CD toward Cbz-D/L-Glu (K = 94−107 M^{-1}), which is smaller than that of *A,D*-TMA_2-*β*-CD to the lower homologue Cbz-D/L-Asp (K = 116−117 M^{-1}) or even that of TMA-*β*-CD to Cbz-D/L-Glu (K = 114−119 M^{-1}). As the inter-substituent distance decreases in the order *A,D* > *A,C* > *A,B*, the "effective" combined steric hindrance is expected to decrease accordingly, which was demonstrated indeed by the gradually increasing enthalpic gain and complex stability for both Cbz-Glu and -Asp in the same order. The least-hindered cavity of the *A,B*-isomer allows the deepest guest penetration, which was directly observed by the 2-D NMR spectroscopy described below, to give exclusively enthalpy-driven complexation with the largest $-\Delta H°$ (13 kJ/mol) and K (189 M^{-1}) values.

We have found an intriguing chiral recognition behavior of *A,B*-TMA_2-*β*-CD, which is switched on just by adding one methylene to the guest Cbz-amino acid. Thus, *A,B*-TMA_2-*β*-CD does not recognize the chirality of Cbz-Asp at all, but can discriminate the enantiomers of Cbz-Glu with a $\Delta\Delta H°$ of 3 kJ/mol, which is canceled only in part by $T\Delta\Delta S°$ (2 kJ/mol), ultimately giving 40% difference in K. Similar phenomena are observed upon complexation of Cbz-D/L-Asp and -Glu with *A,C*- and *A,D*-TMA_2-*β*-CDs with smaller chiral discrimination. To the best of our knowledge,[3,7] this is the first example of the addition of one methylene group to the guest having such an extraordinary effect on the chiral recognition behavior of cyclodextrins.

NMR Spectral Study. To elucidate the structural features responsible for the high chiral recognition and unusual thermodynamic behavior of Cbz-D/L-Glu upon complexation with *A,B*-TMA_2-*β*-CD, 1-D and 2-D ^1H NMR (COSY, HOHAHA, and ROESY) studies were performed.

1-D NMR spectra indicate that chiral discrimination of Cbz-D/L-Glu (14 mM) is achieved by *A,B*-TMA_2-*β*-CD (14 mM) in aqueous phosphate buffer (pH 6.9) at 25 °C, as judged from the distinctly different chemical shift changes of the Cbz-Glu protons and particularly the H5 and H3 of TMA-appended glucose units of the host, as well as the TMA methyl protons.

On the basis of the ROESY spectra (see Supporting Information), we propose the complex structures of D- and D-Cbz-Glu with *A,B*-TMA_2-*β*-CD (Figure 1). From the NOEs between H_p and $H3_{C\ or\ D}$, the phenyl moiety of Cbz-L-Glu is inferred to be tilted and accommodated more tightly in the cavity (Figure 1a), while that of Cbz-D-Glu is positioned upright in the cavity (Figure 1b). Thus, the thermodynamic and NMR data are in excellent agreement with each other. Indeed, the closely packed, tilted Cbz-D-Glu in the cavity should lead to increased van der Waals interactions and reduced conformational freedom, affording higher enthalpic gain and lower entropic gain than those for Cbz-D-Glu.

It is interesting to compare the NMR spectral behavior of native *β*-CD with that of *A,B*-TMA_2-*β*-CD upon complexation with Cbz-Glu, since the former shows no chiral recognition owing to the "perfect" enthalpy−entropy compensation, while the latter shows appreciable chiral discrimination as a result of the "not-so-perfect" compensation. Such a comparative study may reveal the reason(s) for the often-encountered poor chiral recognition by native CDs and further the molecular basis of the enthalpy−entropy compensation effect.

In the ROESY spectra of the *β*-CD complexes of D- and L-Cbz-Glu, NOEs were found between *m*- and *o*-phenyl protons and H3 and/or H5 of CD. This and the NOE between H_p and H6 indicate that the penetration of the phenyl into the cavity occurs from the secondary side, as shown in Figure 1c. The inverted direction of guest penetration observed for *β*-CD and *A,B*-TMA_2-*β*-CD is attributable to the Coulombic interaction of the guest's carboxylates and the cationic TMAs at the primary side of *β*-CD, which enables the three-point interaction. The conformation of Cbz-Glu included in *A,B*-TMA_2-*β*-CD is obviously more "frozen" than that in *β*-CD. The lack of structural fixation in the cavity is thought to be the major reason for the ultimate enthalpy−entropy compensation in the complexation of Cbz-Glu with *β*-CD, where any enthalpic gain from the additional van der Waals contacts cannot survive the accompanying entropic loss arising from the reduced conformational freedom.

We have demonstrated that the degree of enantiomeric discrimination is determined by the location and position of penetrating hydrophobic moiety of the guest inside the *A,B*-TMA_2-*β*-CD cavity. The awareness and rationalization of such conformational effects is crucial in understanding various natural chiral recognition processes, including the binding of enantiomeric substrates to chiral receptors. These results have revealed the impressive ability of modified CDs as enantioselective binders and indicate at further promise for applications in chirotechnology.

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Supporting Information Available: ^1H NMR (COSY, HOHAHA, and ROESY) spectra of the Cbz-D/L-Glu:*A,B*-TMA_2-*β*-CD and Cbz-L-Glu:*β*-CD complexes (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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