1:1 and 1:2 Complexation Thermodynamics of γ-Cyclodextrin with *N*-Carbobenzyloxy Aromatic Amino Acids and ω-Phenylalkanoic Acids

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Abstract: The stability constants (K), standard free energies ($\Delta G°$), enthalpies ($\Delta H°$), and entropy changes ($T\Delta S°$) for the complexation of γ-cyclodextrin with *N*-carbobenzyloxy (CBZ)-D/L-phenylalanine, CBZ-D/L-tyrosine, CBZ-D/L-tryptophan, CBZ-D/L-histidine, CBZ-glycine, 3-phenylpropionic acid, 4-phenylbutyric acid, 5-phenylvaleric acid, and 6-phenylhexanoic acid have been determined in phosphate buffer solution (pH 6.9) at 298.15 K by titration microcalorimetry. The trends observed in the thermodynamic parameters for 1:1 and/or 2:1 host−guest complexation correlate with the systematic structural changes of the guest molecules. On the basis of the results obtained, we further discuss the possible thermodynamic implication for the entropic control of supramolecular systems.

Introduction

Natural and modified cyclodextrins (CDs) are widely recognized as excellent models for understanding general inclusion phenomena, as well as enzyme−substrate and receptor−ligand interactions.[1−3] Furthermore, due to their ability to form complexes with a variety of aliphatic and aromatic compounds, including biologically important metabolites and pharmaceuticals, CDs are considered to be important building blocks for the design of various supramolecular devices and materials which mimic biological activity and function.[4] Thus, to establish the correlations between structures and thermodynamic parameters of complexation is of great importance from both scientific and industrial perspectives.

In our recent review,[3] we showed that, in many cases, simple geometrical considerations can be used as a convenient, reliable tool to rationalize the trends of complexation thermodynamic parameters of one particular guest with different CDs. A typical example is the inclusion of adamantane derivatives. Although this guest is too big to be included in the α-CD cavity, it fits almost perfectly in the β-CD cavity but is somewhat smaller than the size of the γ-CD cavity. In accordance with this geometrical rationale, the affinity increases about 100 times from α-CD to β-CD and then decreases from β-CD to γ-CD. The inclusion of adamantane derivatives into β-CD is a predominantly enthalpy-driven process, with the entropy changes around zero depending on the particular derivative employed.[3] However, the complexation of bis-adamantane derivatives by modified bis-β-CDs shows approximately twice as much exothermic enthalpy (when compared with β-CD-adamantane complexes),[5] which is canceled to a large extent by negative entropy arising from the significant restriction of host/guest motions upon complexation. Other bis-CD/bis-guest combinations reveal a similar trend in the thermodynamic parameters.[1] Thus, in general, complexation of guests having several interacting sites with bis-CDs is associated with unfavorable entropy changes.

In this study, to extend our knowledge of the thermodynamics of "multiple" host/guest interactions, we have investigated the complexation processes in which two separate hydrophobic moieties of one guest are inserted into one CD cavity to form an appreciably stable complex. Furthermore, our goal is to compare thermodynamics of the 1:1 complexation with 2:1 complexation, in which two identical guest molecules with a hydrophobic moiety undergo insertion into one CD cavity. To succeed in such a study, the moiety should be relatively small with respect to the cavity size and thus, as a result of poor size complementarity with the CD cavity, should not be able to form a strong 1:1 complex. However, the combined size of two moieties should be highly comparable with the cavity size. It would also be beneficial for the moieties to interact not only with the cavity but also with each other, thus leading to the additional stabilization of the complex. In addition, the global shape of two guest moieties should be relatively symmetrical to fit into symmetrical CD cavity.

All of the above considerations led us to select γ-CD as a host and several amino and carboxylic acids possessing one or two phenyl rings as guests: *N*-carbobenzyloxy (CBZ)-D/L-phenylalanine (CBZ-D/L-phe), CBZ-D/L-tyrosine (CBZ-D/L-tyr), CBZ-D/L-tryptophan (CBZ-D/L-trp), CBZ-D/L-histidine (CBZ-D/L-his), CBZ-glycine (CBZ-gly), 3-phenylpropionic acid (Ph-C_3), 4-phenylbutyric acid (Ph-C_4), 5-phenylpentanoic acid (Ph-C_5), and 6-phenylhexanoic acid (Ph-C_6). This choice of guests allows us to compare the thermodynamics of 1:1 complexation of CBZ-phe (in which both phenyl rings of the same molecule should be included in the γ-CD cavity) with the thermodynamics of stepwise 2:1 complexation (where two phenyl rings from separate molecules (CBZ-gly, Ph-C_3, Ph-C_4, Ph-C_5, and Ph-C_6) participate in the complexation reaction). Such a comparison

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Table 1. Complex Stability Constant (K), Standard Free Energy ($\Delta G°$), Enthalpy ($\Delta H°$), and Entropy Changes ($T\Delta S°$) for 1:1 and 2:1 Inclusion Complexation of CBZ-Aromatic Amino Acids and Related Compounds with γ-Cyclodextrin at $T = 298.15$ K in Standard Phosphate Buffer (0.05 M, pH 6.9)

reaction	guest concn/mM	cyclodextrin concn/mM	N^a	K/M^{-1}	$\Delta G°$/kJ mol^{-1}	$\Delta H°$/kJ mol^{-1}	$T\Delta S°$/kJ mol^{-1}
Ac-L-phe^{-1} + γ-CD	137	1.89	1	*b*			
Ac -L-tyr^{-1} + γ-CD	197	1.89	1	*b*			
Ac -L-trp^{-1} + γ-CD	271	1.89	1	*b*			
CBZ-D-phe^{-1} + γ-CD	51	1.12	2	175 ± 3 (K_1)	-12.80 ± 0.04	-11.9 ± 0.2	0.9 ± 0.2
CBZ-L-phe^{-1} + γ-CD	52	0.89	2	185 ± 4 (K_1)	-12.94 ± 0.05	-12.8 ± 0.2	-0.1 ± 0.2
CBZ-D-tyr^{-1} + γ-CD	34	1.02	2	175 ± 4 (K_1)	-12.80 ± 0.05	-19.2 ± 0.3	-6.4 ± 0.3
CBZ-L-tyr^{-1} + γ-CD	44	0.86	2	192 ± 3 (K_1)	-13.03 ± 0.04	-20.4 ± 0.2	-7.4 ± 0.2
N-CBZ-D-his^{-1} + γ-CD	101	1.63−1.93	2	13 ± 2 (K_1)	-6.4 ± 0.5	-30 ± 3	-24 ± 3
N-CBZ-L-his^{-1} + γ-CD	75	1.63	2	16 ± 2 (K_1)	-6.9 ± 0.4	-28 ± 2	-21 ± 2
N-CBZ-D-trp^{-1} + γ-CD	47	0.51−1.40	2	53 ± 5 (K_1)	-9.8 ± 0.3	-31 ± 2	-21 ± 2
N-CBZ-L-trp^{-1} + γ-CD	31	0.51−1.40	2	57 ± 5 (K_1)	-10.0 ± 0.2	-31 ± 2	-21 ± 2
CBZ-gly^{-1} + γ-CD	150−700	1.89−5.77	6	19 ± 3 (K_1)	-7.3 ± 0.4	-0.7 ± 0.3	6.6 ± 0.5
[CBZ-gly• γ-CD]$^{-1}$ + CBZ-gly^{-1}	150−700	1.89−5.77	6	8.5 ± 1.5 (K_2)	-5.3 ± 0.5	-31 ± 3	-26 ± 3
3-Ph-C_3^{-1} + γ-CD	640	5.86	1	*c*			
[3-Ph-C_3• γ-CD]$^{-1}$ + 3-Ph-C_3^{-1}	640	5.86	1	*c*			
4-Ph-C_4^{-1} + γ-CD	209	2.92−5.54	2	15 ± 5 (K_1)	*d*	6 ± 3	*e*
[4-Ph-C_4• γ-CD]$^{-1}$ + 4-Ph-C_4^{-1}	209	2.92−5.54	2	10 ± 8 (K_2)	*d*	-45 ± 30	*e*
5-Ph-C_5^{-1} + γ-CD	185	2.90−5.54	2	50 ± 10 (K_1)	-9.7 ± 0.6	4.1 ± 0.8	14 ± 1
[5-Ph-C_5• γ-CD]$^{-1}$ + 5-Ph-C_5^{-1}	185	2.90−5.54	2	15 ± 8 (K_2)	-7 ± 2	-32 ± 6	-25 ± 6
6-Ph-C_6^{-1} + γ-CD	215−235	2.72−5.54	3	120 ± 30 (K_1)	-11.9 ± 0.7	4.0 ± 0.6	16 ± 1
[6-Ph-C_6• γ-CD]$^{-1}$ + 6-Ph-C_6^{-1}	215−235	2.72−5.54	3	32 ± 7 (K_2)	-8.6 ± 0.6	-26 ± 2	-17 ± 2

[a] Number of microcalorimetric titration experiments performed. [b] Equilibrium constant is too small to be determined by microcalorimetry (see discussion in sections Complexation Thermodynamics of ω-Phenylalkanoic Acids toward γ-CD and Complexation and Size Complementarity). [c] Meaningful fitting parameters cannot be derived from titration microcalorimetric data obtained. [d] Not determined since equilibrium constant is approximate value associated with the very large uncertainty. [e] Not determined since equilibrium constant and reaction enthalpy are approximate values associated with the very large uncertainties.

is useful in elucidating the thermodynamic stabilities of various supramolecular devices, in which two separate components are held together by noncovalent weak interactions. Varying the guests also allows us to gain insights into the contributions of various types of aromatic rings (CBZ-D/L-tyr, CBZ-D/L-trp, CBZ-D/L-his), and of CH_2 addition (Ph-C_3, Ph-C_4, Ph-C_5, and Ph-C_6) to the overall complexation thermodynamics with γ-CD. It should be noted that there are no data in the literature regarding reaction enthalpies and reaction entropies for any of the complexation reactions under consideration.

Experimental Section

Materials. The host and guest compounds used in this study, their Chemical Abstracts registry numbers, empirical formulas, formula weights, and suppliers (A = Aldrich, B = Bachem, S = Sigma, W = Wako) are as follows: γ-cyclodextrin, 17465-86-0, $C_{48}H_{80}O_{40}$, 1297.1, S; CBZ-D-phenylalanine, 2448-45-5, $C_{17}H_{17}NO_4$, 299.3, B; CBZ-L-phenylalanine, 1161-13-3, $C_{17}H_{17}NO_4$, 299.3, B; CBZ-D-tyrosine, 64205-12-5, $C_{17}H_{17}NO_5$, 315.3, B; CBZ-L-tyrosine, 1164-16-5, $C_{17}H_{17}NO_5$, 315.3, B; CBZ-D-tryptophan, 13058-16-7, $C_{19}H_{18}N_2O_4$, 338.4, B; CBZ-L-tryptophan, 7432-21-5, $C_{19}H_{18}N_2O_4$, 338.4, B; CBZ-D-histidine, 14997-58-1, $C_{14}H_{15}N_3O_4$, 289.3, B; CBZ-L-histidine, 14997-58-1, $C_{14}H_{15}N_3O_4$, 289.3, B; CBZ-glycine, 1138-80-3, $C_{10}H_{11}NO_4$, 209.2, S; 3-phenylpropionic acid, 501-52-0, $C_9H_{10}O_2$, 150.2, W; 4-phenylbutyric acid, 1821-12-1, $C_{10}H_{12}O_2$, 164.2, A; 5-phenylvaleric acid, 2270-20-4, $C_{11}H_{14}O_2$, 178.2, A; 6-phenylhexanoic acid, 5581-75-9, $C_{12}H_{16}O_2$, 192.3, A.

Commercially available samples of the highest purity were used in the microcalorimetric experiments without further purification. The vendors employed a variety of methods to determine and guarantee the purity of the guests as >98−99% (HPLC, LC, GC, titration, and/or elemental analysis). The γ-CD and some of the guest compounds contained water of hydration or crystallization, and appropriate corrections were made for this moisture content, based on values determined by the vendors and/or by us using the Karl-Fisher technique.

Microcalorimetric Measurements. An isothermal calorimetry (ITC) instrument, purchased from Microcal Inc., Northampton, MA, was used for all microcalorimetric experiments. The ITC instrument was periodically calibrated electrically using an internal electric heater. The instrument was also calibrated chemically by measurement of the neutralization enthalpy of the reaction of HCl with NaOH and the ionization enthalpy of TRIS buffer. These standard reactions gave excellent agreement (±1−2%) with the literature data.[6,7] The thermodynamic parameters of the complexation reaction of cyclohexanol with β-CD were also shown to be in good agreement with our previous results.[8,9]

Titration microcalorimetry allows us to determine simultaneously the enthalpy and equilibrium constant from a single titration curve. Each microcalorimetric titration experiment consisted of 20−40 successive injections: 20 successive injections were performed when 1:1 complexation (ΔH_1 and K_1) was expected, with 40 successive injections performed when a stepwise 2:1 complexation was expected (ΔH_1, K_1, ΔH_2, and K_2). In both cases, a constant volume (5 mL/injection) of guest solution was injected into the reaction cell (1.36 μL) charged with a CD solution in the same buffer; initial concentrations of guest and CD in each run are indicated in Table 1. Standard phosphate buffer at pH 6.9 [NaH_2PO_4 (0.025 M) + $NaHPO_4$ (0.025 M)] was used for all microcalorimetric experiments with the exception of CBZ-his, where standard carbonate buffer at pH 10.0 [$NaHCO_3$ (0.025 M) + Na_2CO_3 (0.025 M)] was used to satisfy the requirement $|pK_a(\text{guest}) - \text{pH}| > 2$.[10]

The heat of dilution of when the guest solution was added to the buffer solution in the absence of CD was determined in each run using the same number of injections and concentration of guest as used in the titration experiments. The dilution enthalpies determined in these control experiments were subtracted from the enthalpies measured in the titration experiments. It should be emphasized that the enthalpies of dilution obtained in all runs were of the same order of magnitude as the enthalpies of dilution of simple electrolytes such as NaCl at the

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same concentration. Thus, it was concluded that there is no significant self-association of any guest under the experimental conditions used. The basis for considering nonideality corrections as unnecessary in the experimental conditions employed was discussed previously.[10]

The ORIGIN software (Microcal Inc.), which was used to calculate the equilibrium constant and standard molar enthalpy of reaction from the titration curve in the cases of simple 1:1 and stepwise 2:1 complexations, gave a standard deviation based on the scatter of the data points in a single titration curve. As we reported previously,[10] the accuracy of the calculated thermodynamic quantities for 1:1 complexations was checked by performing several independent titration runs. The uncertainties in the observed thermodynamic quantities for 1:1 complexation (Table 1) are 2 standard deviations of the mean value unless otherwise stated. The basis for the estimation of uncertainties for the more complicated stepwise 2:1 complexation reactions (Table 1) is discussed below. Detailed descriptions of each step of 1:1 and 2:1 complexation processes are given in Table 1.

Results and Discussion

Complexation and Size Complementarity. In our recent study,[9] we found that affinities toward β-CD decreased in the order *N*-acetyl-L-tyrosine (Ac-tyr), *N*-acetyl-L-phenylalanine (Ac-phe), and *N*-acetyl-L-tryptophan (Ac-trp). Poor size complementarity of Ac-trp results in the lowest affinity, despite the fact that Ac-trp possesses the highest exothermic enthalpy of complexation (for other examples of thermodynamic consequences of poor CD−guest size complementarity, see refs 3 and 9). In this study, we have found no indication of any complexation of these guests with γ-CD. As the size of phenyl, phenol, and even indol rings are more complementary with the β-CD cavity than with γ-CD, it is reasonable that these guests have higher affinity toward β-CD[9] but show negligible affinity toward γ-CD (Table 1). In all titration microcalorimetric experiments performed with these guests, the presence of γ-CD in the reaction solution produced no appreciable heat, except for the heat of dilution. However, for CBZ-gly, small but steadily increasing differences were observed between the heat effect of reaction and the heat effect of dilution during microcalorimetric titration, when moderate concentrations of CBZ-gly (150 mM) and γ-CD (2 mM) were employed. The most likely explanation for the noticeable complexation of CBZ-gly with γ-CD is the larger distance between the phenyl ring and the charged carboxylate group as compared with the observations for the other guests under consideration. This is consistent with the fact that the longer the chain separating the aromatic ring from the charge, the higher the affinity toward CDs, as shown in our recent review.[3] This issue will be addressed later in more detail, and we intend to demonstrate that CBZ-gly forms 1:1 and 1:2 complexes with γ-CD.

Results of microcalorimetric experiments performed at our conventional host−guest concentrations of 150−200 mM CBZ-phe, CBZ-tyr, CBZ-trp, and CBZ-his and 2 mM γ-CD show significant deviations of data points from the theoretical curve based on a simple 1:1 model. The most logical explanation for these deviations is the coexistence of a 1:1 complex (two aromatic rings from one guest molecule are included into the same cavity) and various 2:1 complexes (two aromatic rings from two separate guest molecules are included into the same cavity) in the solution under the experimental conditions employed. Furthermore, we cannot logically exclude the presence of the even more complex 2:2 species (two aromatic rings from two separate guest molecules are included in the same cavity, and the two remaining aromatic rings from the same guest molecules are included in another cavity). To describe such a reaction mixture, one needs at least six parameters (they are reactions enthalpies and equilibrium constants): at least two parameters for each stoichiometry (1:1, 1:2, and 2:2). As is show below, even for a four-parameter fit (CBZ-Gly + γ-CD), the issue of uncertainties in the thermodynamic data obtained becomes very complicated. Thus, we decided to simplify the reaction mixture rather than attempting to perform the six-parameter fitting procedure. Indeed, the deviations from a simple 1:1 model were gradually reduced and finally completely vanished as more and more diluted solutions of host/guest were used in the microcalorimetric experiments. As discussed previously,[3,9] the dilution test may be considered as a standard general procedure when the involvement of species more complex than 1:1 are suspected, as the contribution from these can be reduced by lowering the concentrations of the guest or/and host. It should be emphasized that only such sets of experimental data that show excellent agreement with the theoretical curve based on the simple 1:1 model were used for determination of thermodynamic parameters presented in Table 1.

The difference in enthalpy between CBZ-phe and CBZ-tyr complex formation is −7 to −8 kJ mol^{-1} and is probably attributable to intermolecular hydrogen bond formation for CBZ-tyr. The same tendency has been reported previously[8] for the complexation enthalpy obtained with tyramine, 3-(4-hydroxyphenyl)propionic acid, and 3-(2-hydroxyphenyl)propionic acid upon complexation with β-CD, when compared to the relevant guests lacking a hydroxyl group. In addition to the above examples, a similar difference was found for the pair of Ac-phe and Ac-tyr upon complexation with β-CD.[9] Typically, in the cases of the β-CD complexes of tyramine, 3-(4-hydroxyphenyl)propionic acid, and 3-(2-hydroxyphenyl)propionic acid, we have revealed the presence of the hydrogen bond spectroscopically.[8]

It is interesting to compare the affinities of CBZ-phe and CBZ-tyr toward γ-CD with those of Ac-phe and Ac-tyr toward β-CD. Ac-tyr shows twice the affinity toward β-CD when compared with Ac-phe. The enthalpy gain for Ac-tyr due to the presence of the hydroxyl group is canceled, in part, by the entropy losses which probably arise from additional restriction of the motional freedom of the Ac-tyr molecule upon hydrogen bond formation. In contrast, CBZ-phe and CBZ-tyr possess virtually the same affinities toward γ-CD. Thus, the entropy losses are larger for the complexation of the two aromatic rings of CBZ-tyr with γ-CD than for the complexation of Ac-tyr with β-CD.

The very low affinity of CBZ-his toward γ-CD, despite the relatively large reaction enthalpy, is probably due to the highly hydrophilic nature of the imidazole residue.[11] Steric effect or size incomplementarity is the most likely reason for the lower affinity found for CBZ-Trp as compared with CBZ-phe and CBZ-tyr. Again as in the case of Ac-trp•β-CD complex formation,[9] despite a large reaction enthalpy, CBZ-Trp (possessing an indole ring) is unable to fit properly into the γ-CD cavity, which leads to large losses in entropy.

Chiral Discrimination of *N*-Carbobenzyloxy Aromatic Amino Acids upon Complexation with γ-CD. L-Isomers of CBZ-phe ($K_L/K_D = 1.06$) and CBZ-tyr ($K_L/K_D = 1.10$) show a higher affinity toward γ-CD (Table 1). In both cases, the enhancement of affinity is enthalpically driven, but the enthalpic gain is offset to a great extent by the accompanying entropic loss. It seems likely that the L- rather than the D-isomers of CBZ-trp and CBZ-his are bound more strongly with γ-CD (Table 1), but we cannot unambiguously claim the L-preference due to the large uncertainties involved (Table 1). Such large

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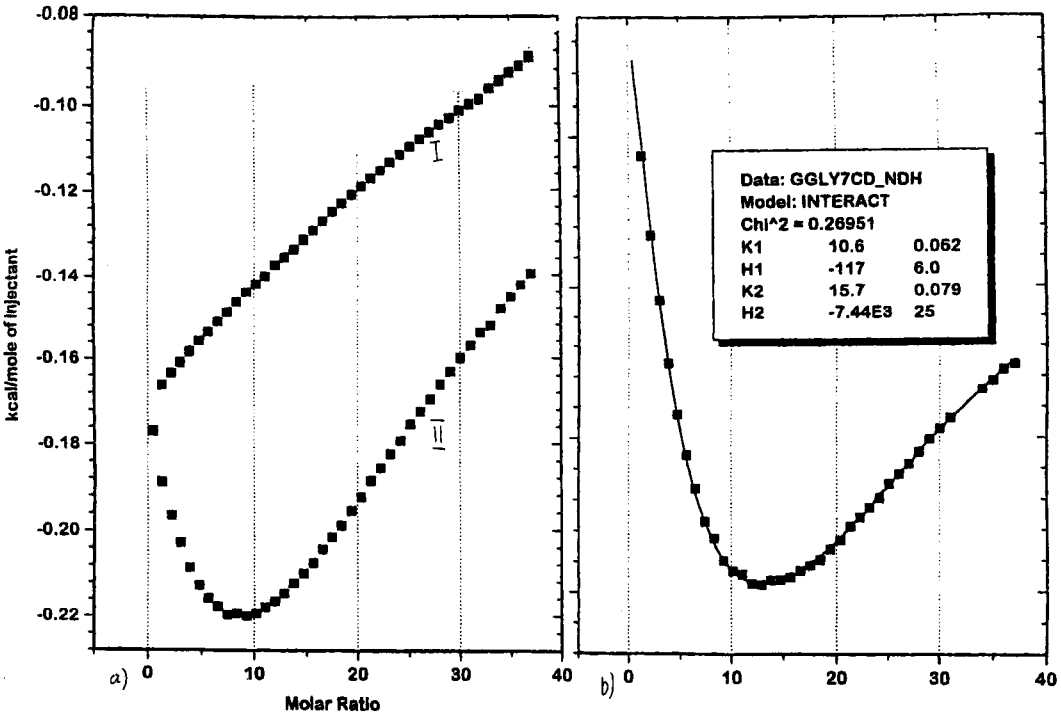

Figure 1. (a) Heat effects of the dilution (I) and of the complexation reactions (II) of CBZ-gly with γ-CD for each injection during titration microcalorimetric experiment. (b) "Net" heat effects of complexation of CBZ-gly with γ-CD for each injection, obtained by subtracting the heat of dilution from the heat of reaction, both of which are fitted to the theoretical curve obtained by computer simulation using the identical interacting sites model.

uncertainties are often encountered when microcalorimetric experiments were performed under circumstances where only a low guest affinity is obtained and/or a very low concentration is used. Experiments under improved conditions, using higher concentrations, could not be performed due to the intervention of more complicated complex species (see above discussion). Previously[9] we established a direct correlation between the mode of guest penetration into the CD cavity and the chiral discrimination. For instance, the L-isomers of Ac-phe, Ac-trp, Ac-tyr, glycidylphenylalanine (gly phe), and phenylalanine amide (phe-am) always show higher affinities toward β-CD than the corresponding D-isomers. In contrast, D-isomers of *N*-Boc-alanine, *N*-Boc-serine, and *N*-Boc-alanine methyl ester are better bound to β-CD. Indeed, the introduction of a Boc group to alanine or serine at the amino terminus switches the hydrophobicity order through the conversion of the originally most hydrophilic group (NH_3^+) to the most hydrophobic group (*t*-BuOCON). Consequently, the guests with the same absolute configuration are consistently preferred, as long as the hydrophobicity order of the substituents around the asymmetric center is preserved throughout the enantiomeric guest pairs. Thus, if one alters the position of the hydrophobic substituent around the asymmetric center, the antipodal guest is favored. In the case of CBZ-phe and CBZ-tyr, the two aromatic rings attached to the different atoms around the chiral carbon atom of the amino acid are simultaneously included in the same γ-CD cavity upon complexation. Hence, the mode of penetration of the CBZ-aromatic amino acids into the γ-CD cavity cannot be compared with those of other amino acid derivatives possessing only one aromatic ring. At present, we do not have enough data for elaborating a general rule for the prediction of chiral discrimination upon complexation with γ-CD for these guests.

Complexation of CBZ-Gly with γ-CD. As noted above, the heat production upon calorimetric titration using a moderate concentration of CBZ-gly (150 mM) was too small to allow precise determination of thermodynamic parameters. Hence, the complexation of CBZ-gly with γ-CD was studied using a high concentration of the guest (750 mM) as well as a high guest:host ratio of up to 40. Representative results obtained in a titration microcalorimetric experiment performed under such conditions are shown in Figure 1. Obviously, the experimental curve (Figure 1) does not fit to the simple 1:1 model, since the gradually increasing heat production is observed in the initial part of the curve. Similarly, the simultaneous 2:1 binding model failed to fit to the experimental data, since that model can fit only the data which pass through the origin of the axis. Thus, the simplest choice to give a satisfactory fit is the stepwise 2:1 complexation model.

A set of thermodynamic quantities obtained by the four-parameter fit using the identical interacting site model is shown in Figure 1. It should be mentioned that K_1 and K_2 in Figure 1 are intrinsic constants of the identical interacting site model, which allow easy comparison with non-interacting expectations.[12] In the case of stepwise 2:1 complexation (Figure 1), the value of the uncertainty given by the ORIGIN program does not seem to be a good criterion for judging the accuracy of the thermodynamic parameters obtained, as several considerably different sets of parameters (K_1^{int}, ΔH_1, K_2^{int}, ΔH_2) can often give similarly good fits to the experimental curve. The potential surface of the five-dimensional fit (χ^2 as a function of four parameters K_1^{int}, ΔH_1; K_2^{int}, ΔH_2) is probably very "flat" (if this word is applicable for description of a fifth-dimensional numerical universe), and therefore the scattering of the experimental data points does not allow the ORIGIN program to find an absolute minimum.[12,13] We repeated computer simulations

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many times with various initial sets of the four parameters, using the constancy of χ^2 as a criterion of the results of calculations, and finally we selected the following thermodynamic parameters for the complexation between CBZ-gly and γ-CD: $K_1 = 19 \pm 3$, $\Delta H_1 = -0.7 \pm 0.3$ kJ mol^{-1}, $K_2 = 8.5 \pm 1.5$, and $\Delta H_2 = -31 \pm 3$ kJ mol^{-1}. Practically, no other sets of the parameters could better describe the experimental data without increasing the χ^2 value by 2−3 times.

It is interesting to compare the thermodynamic parameters obtained for the complexation reaction of CBZ-gly with γ-CD with those reported for β-CD, which also could be taken as an independent assessment of the reliability of the above four-parameter fit. The equilibrium constant for the 1:1 complex formation of CBZ-gly with γ-CD is about one-tenth that for the CBZ-gly·β-CD complex,[9] and the heat effect obtained for the γ-CD complex is about one-third that for the β-CD complex. It is reasonable to expect less pronounced van der Waals interactions and therefore a smaller exothermic heat effect when the cavity is too large to comfortably accommodate the guest molecule, as demonstrated previously for the complexation of aliphatic alcohols with α- and β-CDs,[14] where the reaction enthalpy is always more exothermic for α-CD than for β-CD.

Insertion of the second CBZ-gly molecule into the same, half-occupied, γ-CD cavity is expected to be accompanied by a large exothermic heat effect due to the more effective van der Waals contacts, and also by a large unfavorable entropy change due to the tight association of two CBZ-gly molecules within the γ-CD cavity. The experimental thermodynamic data obtained agree nicely with those expected from such a prediction.

In principle, the ternary complex formation is less favorable from the entropic point of view and consequently is accompanied by a more negative entropy change than that observed for the binary counterpart. In this context, it is reasonable to assume that the entropic loss associated with the inclusion of two separate aromatic guest molecules (such as CBZ-gly) into the same cavity, giving a 2:1 complex [γ-CD·(CBZ-gly)$_2$], becomes larger than that caused by the simultaneous inclusion of two aromatic moieties of the same guest molecule (such as CBZ-phe), forming a 1:1 complex with γ-CD. This prediction is in a good agreement with the experimental data. Thus, the inclusion of the second CBZ-gly molecule in the γ-CD cavity is accompanied by a very large entropy loss ($T\Delta S^\circ = -26$ kJ mol^{-1}), which is in sharp contrast with the positive entropy change ($T\Delta S^\circ = 6.6$ kJ mol^{-1}) upon inclusion of the first CBZ-gly molecule (Table 1). This negative entropy change cancels the large exothermic enthalpy change ($\Delta H^\circ = -31$ kJ mol^{-1}) to a great extent, giving a smaller equilibrium constant for the second inclusion step, i.e., $K_1 = 19$ and $K_2 = 8.5$.

Complexation Thermodynamics of ω-Phenylalkanoic Acids with γ-CD. As demonstrated above, two types of *N*-protected amino acids, i.e., CBZ-gly versus Ac-phe, Ac-tyr, and Ac-trp, exhibit distinctly different thermodynamic behavior upon complexation with γ-CD. Thus, CBZ-gly gives moderately stable 1:1 and 2:1 complexes with γ-CD, while the latter three *N*-acetyl aromatic amino acids show no indication of complex formation. Since these two series of guest molecules possess a hydrophobic aromatic ring and a hydrophilic carboxylate moiety in common, the contrasting complexation behavior observed is most probably attributable to the difference in distance between aromatic and carboxylate group. To test this hypothesis, we have chosen a series of ω-phenylalkanoic acids as homologous guests with varying distance between the aromatic and negatively charged groups: 3-phenylpropionic acid (Ph-C$_3$), 4-phenylbutyric acid (Ph-C$_4$), 5-phenylpentanoic acid (Ph-C$_5$), and 6-phenylhexanoic acid (Ph-C$_6$).

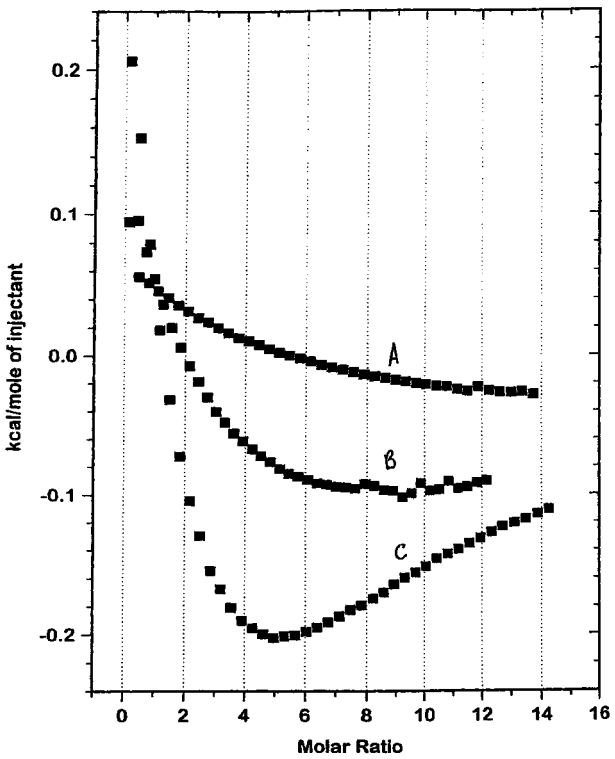

Figure 2. Patterns of the heat effects (after correction for the heat of dilution) of each injection during titration microcalorimetric experiments in the case of complexation reactions of γ-CD with 4-Ph-C$_4$ (A), 5-Ph-C$_5$ (B), and 6-Ph-C$_6$ (C) under comparable experimental conditions (2.7−2.9 mM γ-CD and 190−230 mM guest in the initial solutions).

The heat evolution patterns upon injection of the guest solution in the titration microcalorimetric experiments are shown in Figure 2 for the complexation reactions of γ-CD with Ph-C$_4$, Ph-C$_5$, and Ph-C$_6$ under the comparable experimental conditions (2.7−2.9 mM γ-CD and 190−230 mM guest in the initial solutions). Although the shape of each titration curve would appear to be qualitatively similar to that observed for CBZ-gly, a close examination readily reveals a clear difference: the curves for Ph-C$_4$, Ph-C$_5$, and Ph-C$_6$ cross the horizontal line at a different molar ratio in each case. The inversion of the heat evolution curve during the titration means that, in contrast to the CBZ-gly case, the heat effect at the initial stage of the titration (formation of the 1:1 complex) has a sign opposite to that in the second stage (formation of the 2:1 complex); i.e., the first stage is endothermic and the second exothermic.

As was the case with CBZ-gly, the identical interacting sites model (stepwise 2:1 complexation) was used as the simplest, most reasonable choice for the treatment of the microcalorimetric titration data obtained for the complexation of Ph-C$_4$, Ph-C$_5$, and Ph-C$_6$ with γ-CD, affording a satisfactory match between the experimental and theoretical curves, as exemplified in Figure 3. The thermodynamic parameters for 1:1 and 2:1 complexation of Ph-C$_4$, Ph-C$_5$, and Ph-C$_6$ with γ-CD are collected in Table 1. The basis for the estimation of the uncertainties reported in Table 1 has been discussed above for the 2:1 complexation of CBZ-gly with γ-CD.

It should be noted that the microcalorimetric experiments with Ph-C$_3$ performed under the same conditions as above exhibited

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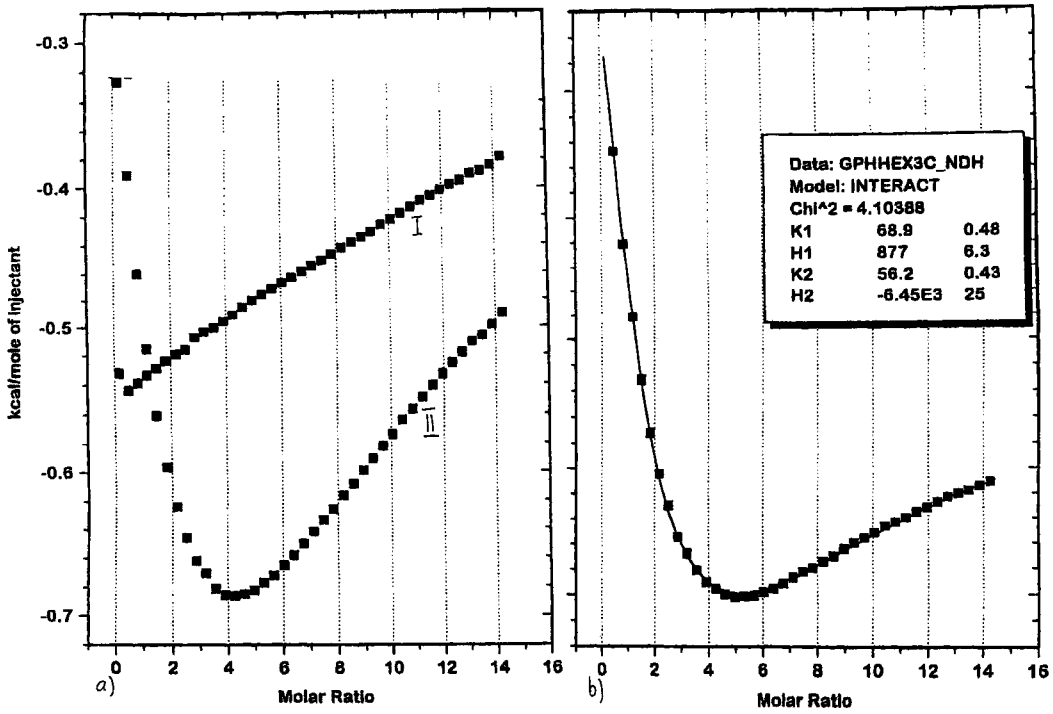

Figure 3. (a) Heat effects of the dilution (I) and of the complexation reactions (II) of 6-Ph-C_6 with γ-CD for each injection during titration microcalorimetric experiment. (b) "Net" heat effects of complexation of 6-Ph-C_6 with γ-CD for each injection after subtraction of the heat of dilution from the heat of reaction, which are fitted to the theoretical curve obtained by computer simulation using the identical interacting sites model.

no appreciable net heat effect ascribable to complex formation, thus giving practically the same heat effects upon dilution and titration with γ-CD. Hence, the experiments were repeated with much higher host/guest concentrations, as indicated in Table 1 (640 mM Ph-C_3 and 5.8 mM γ-CD). Only under high concentration conditions were small but steadily increasing differences observed between the heat effect of dilution and those of complexation during the microcalorimetric titration, with the developing pattern of the heat effect during titration very similar to that observed for the titration of CBZ-Gly with γ-CD at moderate concentrations of 150 and 2 mM, respectively. However, the small heat effects and the large scattering of the data points from the theoretical curve do not allow us to determine a meaningful set of fitting parameters (K_1^{int}, ΔH_1, K_2^{int}, and ΔH_2).

As expected, the K_1 values obtained for the 1:1 complexation of ω-phenylalkanoic acids steadily increase with increasing methylene chain length connecting the phenyl and carboxyl groups: i.e., Ph-C_4 < Ph-C_5 < Ph-C_6. It is also reasonable that the K_2 values for 2:1 complexation show a similar trend. Although the large uncertainties associated with the ΔH and ΔS values for Ph-C_4 do not allow us to fully discuss the quantitative trend of these parameters, the thermodynamic parameters obtained for Ph-C_5 and Ph-C_6 reveal that the enhancement of affinity upon expanding the methylene chain is entropically driven. The ΔH_1 values are comparable to each other, while the $T\Delta S_1$ value is more positive for Ph-C_6. This observation is in good agreement with our previous conclusion derived from the examination of the thermodynamic parameters for the complexation of several homologous aromatic carboxylic acids and amines with β-CD, where the affinity enhancements arise exclusively from the entropic contributions.[3,9] As proposed previously,[3,9] a relatively small methylene group added to a phenyl guest is hindered and therefore prevents full direct contact with the CD walls, minimizing the enthalpic contribution. But the additional methylene group can entropically contribute to the overall complexation thermodynamics through the rearrangement of the water molecules inside the cavity and/or the loss of host/guest solvation. The same explanation is applicable for the rationalization of the trend of thermodynamic parameters for 1:1 and 2:1 complexation of the ω-phenylalkanoic acids with γ-CD, since the direct van der Waals contact of the added methylene with CD walls appears to be more difficult for γ-CD with a larger cavity than for β-CD.

It is interesting to compare the thermodynamic parameters for 1:1 and 2:1 complexations of ω-phenylalkanoic acids with the corresponding values obtained for CBZ-gly. The 1:1 complexation of CBZ-gly with γ-CD is slightly exothermic ($\Delta H_1 = -0.7$ kJ mol^{-1}), while the relevant reactions of Ph-C_4, Ph-C_5, or Ph-C_6 with γ-CD are moderately endothermic ($\Delta H_1 = 4-6$ kJ mol^{-1}). This may be attributed to the bulkier tether between carboxylate and phenyl groups of CBZ-gly as compared with those for the "simple" ω-phenylalkanoic acids. The bulkier group is expected to enhance the van der Waals interactions with CD walls, giving a more exothermic heat effect. Comparison of the 1:1 and 2:1 complex stabilities of CBZ-gly with those of Ph-C_5 is also interesting. Although the distance between the phenyl and carboxyl groups is about the same for the two guests, both the K_1 and K_2 values are 2 times larger for Ph-C_5 than for CBZ-gly. This difference is readily attributed to the hydrophobicity of the tether group, i.e., $OCONHCH_2$ versus $(CH_2)_4$, and such thermodynamic behavior is frequently found in the literature data.[3] For instance, the same trend is found in the complexation of Ac-phe and Ph-C_3 with β-CD, where the latter guest gives a 2 times larger K value than the former one.[8-10]

Although the direct microcalorimetric determination of the binding constants of Ac-phe and Ph-C_3 toward γ-CD was not successful due to the negligible heat effects upon complexation, the thermodynamic data collected in Table 1 are useful in

estimating K values for these guests. If the trend of log K_1 for the homologous guest series, i.e., Ph-C_6, Ph-C_5, and Ph-C_4, is linearly extrapolated to the shorter chain length, the estimated K_1 value for the complexation of Ph-C_3 with γ-CD should be in the range 4−6 M^{-1}. Certainly, this is only an approximate value of K_1, and no ΔH_1, K_2, or ΔH_2 can be determined or estimated under such circumstances, yet the estimated K value would be useful for comparison with the relevant data. In this context, it would be of some value to roughly estimate the binding constant of Ac-phe with γ-CD. As mentioned above, Ac-phe has half the affinity toward β-CD in comparison to Ph-C_3. If the same trend is kept upon complexation with γ-CD, the K_1 value estimated for the complexation of Ac-phe with γ-CD would be in the range 2−3 M^{-1}.

Implications for the Design of Supramolecular Materials. In his recent "conceptual" article, Lehn has defined that "supramolecular materials are by nature dynamic and they rely on the explicit manipulation of the non-covalent forces that hold the components together and of the recognition processes that they underlie, for their controlled and reversible build-up from suitable units by self-assembly".[15] From this definition, it is obvious that such supramolecular materials can exist only in the presence of a certain amount of "raw" monomeric materials which are enough to maintain the supramolecular assembly upon equilibration. If the concentration of monomers is close to zero, any supramolecular assembly disappears simply due to the second law of thermodynamics. It is well known that relatively small enthalpy changes associated with noncovalent weak interactions by themselves are not sufficient to thermodynamically stabilize such supramolecular materials, and thus the entropy factor also plays a crucial role. As the number of components in a supramolecular system increases, the contribution of entropy to its stability is expected to increase. A thermodynamic comparison between two- and three-component association processes, in which two phenyl groups are involved in the complexation with γ-CD, is interesting. For instance, the two-component system [CBZ-D/L-phe + γ-CD] gives a smaller exothermic enthalpy change ($\Delta H° \approx -12$ kJ mol^{-1}) than the three component system [2(Ph-C_6) + γ-CD] ($\Delta H° = -22$ kJ mol^{-1}), while both systems have a quite similar overall entropy, close to zero ($T\Delta S° \approx \pm 1$ kJ mol^{-1}) from the corresponding "monomeric" components. Thus, the second system is thermodynamically more stable under the standard conditions ($c = 1$ M). However, if one fixes the concentration of the "monomers" to 1 mM, ca. 15% of the original "monomers" undergo complexation in the first system, but only less than 0.5% forms a supramolecular assembly in the second case. Therefore, the thermodynamic stability is inverted.

Micelles might be considered as a supramolecular assembly, association of which is driven by weak interactions with serious contribution of the entropic factor and is therefore easily destroyed by diluting the aqueous micellar solution. However, the intentional control of supramolecular composition and structure is difficult to achieve in general for conventional micelles or supramolecular assemblies. Thus, a new methodology, which can realize a highly structured yet freely controllable supramolecular assembly, should help in designing advanced supramolecular materials. To realize the desired structure and property as well as controllable function in supramolecular materials, one usually introduces additional structural regularity and/or functional group(s) to the monomeric components. Such a strategy, using built-in structure/function, is inevitably accompanied by a loss of entropy, which often cancels the enthalpic gain obtained. Hence, we have to find a way to compensate or even overwhelm the entropy losses, giving a positive $\Delta S°$. Although such an apparently contradicting requirement does not appear to be fulfilled, we would propose the control of solvation/desolvation processes as one of the possible strategies. As demonstrated above, the methylene groups of the ω-phenylalkanoic acid series (Ph-C_3, Ph-C_4, Ph-C_5, and Ph-C_6) contribute to complex formation exclusively through the rearrangement of the water molecules inside of the CD cavity; the increasing affinity toward γ-CD with increasing tether length in the guests is a good example of the entropic control of the overall complexation thermodynamics through the solvation/desolvation processes. Since the physical nature and thermodynamic behavior of noncovalent weak interactions are common to a wide variety of supramolecular assemblies, these examples and the subsequent considerations would open a channel to the entropic control of the ultimate thermodynamic stability of supramolecular system working in aqueous solutions. To generalize, we would like to emphasize that, since enthalpic gains arising from the noncovalent interactions are not sufficiently large to stabilize supramolecular assemblies, the "ordering entropy" [16,17] associated with the rearrangement of solvent molecules is the main thermodynamic driving force which enables highly ordered supramolecular materials to exist.

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