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Calorimetric and NMR studies on the direct correlation between supramolecular complex structure and the thermodynamics of *N*-benzyloxycarbonyl-L-aspartic and -glutamic acid inclusion complexes with mono- and bis(trimethylammonio)-β-cyclodextrins[†]

Hatsuo Yamamura,[1] Mikhail Rekharsky,[2] Asako Akasaki,[1] Schuki Araki,[1] Masao Kawai[1] and Yoshihisa Inoue[2]*

[1]Department of Applied Chemistry, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya 466-8555, Japan
[2]Inoue Photochirogenesis Project, ERATO, JST, 4-6-3 Kamishinden, Toyonaka 560-0085, Japan

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ABSTRACT: The stability constants and standard Gibbs energy, enthalpy and entropy changes for the 1:1 inclusion complexation of *N*-benzyloxycarbonyl-L-aspartic and -glutamic acids with native β-cyclodextrin (β-CD), mono(6-amino-6-deoxy)-β-CD, mono(6-trimethylammonio-6-deoxy)-β-CD and A,B-, A,C- and A,D-bis(6-trimethylammonio-6-deoxy)-β-CDs were determined in aqueous phosphate buffer (pH 6.9) at 298.15 K by titration microcalorimetry. The supramolecular complex structures were elucidated from 1D and 2D NMR spectra, including ROESY, COSY and HOHAHA techniques. Combining the results obtained from the microcalorimetric and NMR studies, we elucidated not only the detailed complex structures and the thermodynamic consequences, but also the mutual dependence between the complex structure and the overall complexation thermodynamics. Copyright © 2001 John Wiley & Sons, Ltd.

KEYWORDS: microcalorimetry; thermodynamics; NMR; β-cyclodextrin derivatives; amino acids

INTRODUCTION

The structure–property relationship is currently one of the most intriguing and intensively investigated topics in supramolecular chemistry.[1–3] It is also widely recognized that the host–guest complexation thermodynamic parameters such as the enthalpy and entropy changes, not only the complex stability, are essential physicochemical properties which critically govern the complexation behavior, consequently determining the inherent ability, and possible application, of various supramolecular systems[4] such as cyclodextrin (CD).[5,6] Hence the close examination and unambiguous elucidation of the interdependence between the complex structure and complexation thermodynamics of CDs are of primary importance from both scientific (theoretical) and practical (industrial) viewpoints.[7–10] There have been significant investigations of the complexation of amino acids and their various derivatives by natural and modified CDs.[5–10] However, many of the studies were limited to only one experimental method, which certainly hinders elucidation of the structure–property relationship.

In our recent comparative NMR and microcalorimetric study on the complexation of amino acid derivatives such as dansyl-D/L-phenylalanine (Dans-Phe) and naphthalenesulfonyl-D/L-phenylalanine (Naph-Phe) with β- and γ-CDs,[11] we demonstrated that the complexation thermodynamic parameters critically reflect, or directly correlate with, the penetration mode (direction and orientation) and depth (location) of the two intramolecularly stacked aromatic moieties in the CD cavity. In particular, Naph-Phe and Dans-Phe give different complex structures and thermodynamic parameters upon inclusion by β-CD. The NMR study revealed that the phenyl group of Naph-Phe is preferentially accommodated from the secondary side of β-CD with the phenyl face parallel with the wider rim of β-CD, over which the naphthyl moiety is stacked capping the cavity; the microcalorimetric study indicated that this relatively rare mode of complexation leads to a highly exothermic enthalpy change. In contrast, the dansyl and phenyl groups in Dans-Phe are simultaneously accommodated and stacked in β-CD, where the aromatic

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**Correspondence to:* Y. Inoue, Department of Molecular Chemistry, Faculty of Engineering, Osaka University, Yamadaoka, Suita 565-0871, Japan.
Email: inoue@chem.eng.osaka-u.ac.jp

[†]This paper is dedicated to Professor Dr Hans-Jörg Schneider (University of Saarland) on the occasion of his 65th birthday.

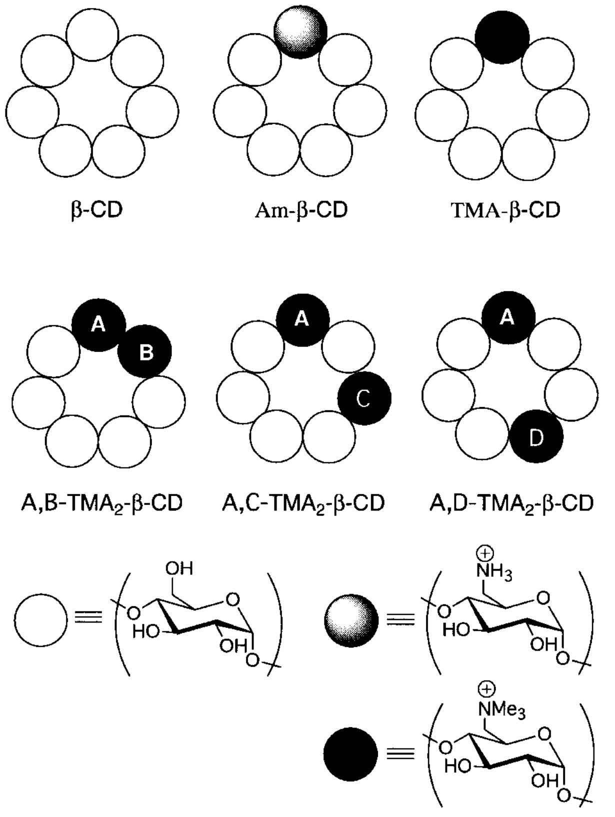

Chart 1. Nature, ammonio- and mono- and bis(trimethylammonio)-β-cyclodextrins

moieties are oriented parallel to the cavity axis. In this case, the complexation enthalpy becomes less exothermic than that of Naph-Phe, probably as a result of inclusion of the dimethylamino group and the subsequent shallower penetration of the aromatic moieties. This NMR study clearly demonstrated that the penetration of both Naph-Phe and Dans-Phe occurs exclusively from the wider secondary side of β- and γ-CD. As far as aromatic amino acid derivatives are concerned, the preference for secondary-side penetration seems natural. Indeed, if the penetration occurs from the primary side the accompanying conformation changes of the seven or eight primary hydroxymethyl groups in β- or γ-CD will be disadvantageous from an energetic point of view. Hence, in order to attempt to invert the penetration direction from the secondary to primary side, some modification that results in an attractive interaction with the guest should be introduced at the primary side.

In the present study, we introduced one or two amino (Am) or trimethylammonio (TMA) group(s), these are protonated at neutral pH and thus positively charged at the primary side of β-CD to afford Am-β-CD, TMA-β-CD and A,B-, A,C-, and A,D-TMA$_2$-β-CD (Chart 1), therefore expecting additional electrostatic interactions with dicarboxylic amino acid guests, i.e. *N*-benzyloxycarbonyl-L-aspartic acid [Cbz-L-Asp: $C_6H_5OCONHCH(CH_2COOH)CO_2H$] and *N*-benzyloxycarbonyl-L-glutamic acid [Cbz-L-Glu: $C_6H_5OCONHCH(CH_2CH_2COOH)CO_2H$]. The choice of hosts allows us not only to explore the effects of the number of positive charge on the CD rim (+1 for Am- and TMA-β-CD versus +2 for TMA$_2$-β-CD) and of the bulkiness of the charged group (Am versus TMA), but also to elucidate the influence of varying the position of TMA groups around the CD rim (A,B-, A,C- and A,D-TMA$_2$-β-CD) upon the supramolecular complex structures and their thermodynamics. The choice of dicarboxylic amino acid guests is also rational, since Asp and Glu both possess −2 charges but with a different number of methylenes, which means that they are well suited for assessing contributions from the electrostatic interaction with the +1 and +2 charged CD hosts, and also from the van der Waals interactions of the extra methylene in the latter guest.

EXPERIMENTAL

Materials. Commercially available Cbz-L-Asp, Cbz-L-Glu and β-CD of the highest purities available were used in the microcalorimetric and NMR experiments without further purification. Am-β-CD, TMA-β-CD and A,B-, A,C- and A,D-TMA$_2$-β-CD were synthesized, according to the procedures reported previously[12,13] (H. Yamamura, Y. Yamada, S Araki and M. Kawai, to be published). All CDs contained water of hydration, and appropriate corrections were made for the moisture contents, which were determined by the vendor (β-CD) or by us (Am-β-CD, TMA-β-CD and A,B-, A,C- and A,D-TMA$_2$-β-CD) using the Karl Fisher technique.

Microcalorimetric measurements. An isothermal calorimetry (ITC) instrument, purchased from Microcal (North ampton, MA, USA) 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% errors) with the literature data.[14,15] The thermodynamic parameters obtained for the complexation of cyclohexanol with β-CD showed good agreement with our previous results.[16,17]

Titration microcalorimetry allows us to determine simultaneously the reaction enthalpy and equilibrium constant from a single titration run. Each microcalorimetric titration experiment consisted of 20 successive injections, where a constant volume (5 μl per injection) of guest solution was injected stepwise into the reaction cell (1.36 ml) charged with a CD solution in the same buffer; the initial concentrations of guest and CD used in each run are indicated in Table 1. It should be mentioned that the concentrations cited in Table 1 refer to the concentrations of the host in the sample cell and of the

Table 1. Complex stability constant (K) and standard Gibbs energy ($\Delta G°$), enthalpy ($\Delta H°$) and entropy changes ($T\Delta S°$) for 1:1 inclusion complexation of amino-, trimethylammonio- and A,X-bis(trimethylammonio)-β-cyclodextrins with Cbz-L-Asp-OH and Cbz-L-Glu-OH in aqueous buffer (pH 6.9) at 298.15 K

Guest (charge) + host (charge)	Guest concentration[a] (mM)	Cyclodextrin concentration[a] (mM)	No. of runs	K(M^{-1})	$\Delta G°$(kJ mol^{-1})	$\Delta H°$(kJ mol^{-1})	$T\Delta S°$(kJ mol^{-1})
Cbz-L-Asp-OH (−2) + β-CD (0)	152	2.23	1	74.6 ± 1.5	−10.69 ± 0.05	−9.76 ± 0.15	0.9 ± 0.15
Cbz-L-Asp-OH (−2) + Am-β-CD (+1)	123	1.17	2	161 ± 2	−12.60 ± 0.03	−12.04 ± 0.15	0.54 ± 0.15
Cbz-L-Asp-OH (−2) + TMA-β-CD (+1)	163	0.91	2	111 ± 3	−11.67 ± 0.07	−11.0 ± 0.2	0.7 ± 0.2
Cbz-L-Asp-OH (−2) + A,B-TMA$_2$-β-CD (+2)	190	1.02	2	181 ± 3	−12.89 ± 0.05	−12.9 ± 0.2	0.0 ± 0.2
Cbz-L-Asp-OH (−2) + A,C-TMA$_2$-β-CD (+2)	188	0.91	2	141 ± 5	−12.27 ± 0.09	−9.45 ± 0.15	2.8 ± 0.2
Cbz-L-Asp-OH (−2) + A,D-TMA$_2$-β-CD (+2)	152	1.19	2	117 ± 3	−11.81 ± 0.06	−7.83 ± 0.15	4.0 ± 0.2
Cbz-L-Glu-OH (−2) + β-CD (0)	157	2.16	1	87 ± 2	−11.06 ± 0.06	−10.82 ± 0.15	0.24 ± 0.15
Cbz-L-Glu-OH (−2) + Am-β-CD (+1)	115	1.00	2	168 ± 3	−12.70 ± 0.04	−11.32 ± 0.15	1.38 ± 0.15
Cbz-L-Glu-OH (−2) + TMA-β-CD (+1)	144	1.06	2	119 ± 4	−11.85 ± 0.09	−10.9 ± 0.2	1.0 ± 0.2
Cbz-L-Glu-OH (−2) + A,B-TMA$_2$-β-CD (+2)	168	1.07	2	189 ± 3	−12.99 ± 0.04	−12.79 ± 0.10	0.20 ± 0.15
Cbz-L-Glu-OH (−2) + A,C-TMA$_2$-β-CD (+2)	157	1.11	2	133 ± 3	−12.12 ± 0.06	−8.90 ± 0.15	3.2 ± 0.2
Cbz-L-Glu-OH (−2) + A,D-TMA$_2$-β-CD (+2)	98	0.91	2	107 ± 3	−11.58 ± 0.07	−7.80 ± 0.15	3.8 ± 0.2

[a] It should be pointed out that the concentrations cited refer to the concentrations of the host in the sample cell and of the guest in the injected aliquots.

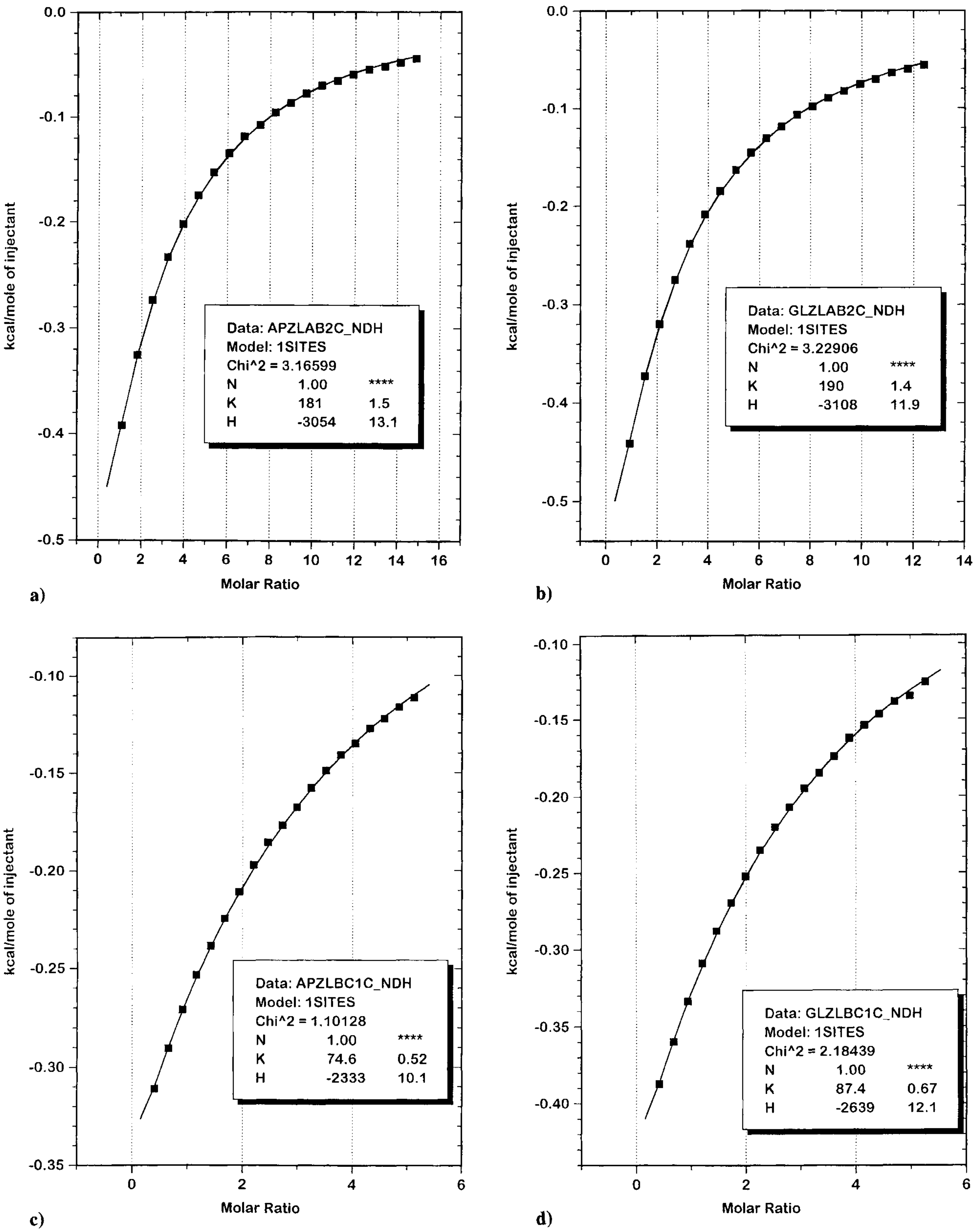

Figure 1. (a)–(d) Representative microcalorimetric titration curves for the complexation reactions of Cbz-L-Asp and Cbz-L-Glu with A,B-TMA$_2$-β-CD and β-CD, respectively

guest in the injected aliquots. Standard phosphate buffer of pH 6.9 [NaH_2PO_4 (0.025 M) + $NaHPO_4$ (0.025 M)] was used for all microcalorimetric experiments to satisfy the requirement $|pK_a(\text{guest}) - \text{pH}| > 2$.[18] Corrections for non-ideality were not made under the experimental conditions employed, the reason for which was discussed previously.[18]

The heat of dilution of the guest solution upon addition 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 runs were subtracted from the apparent enthalpies measured in the titration experiments.

The ORIGIN program (Microcal.), which was used to calculate the equilibrium constant and standard molar enthalpy of reaction from the titration curve, gave a standard deviation based on the scatter of the data points in a single titration curve. As we reported previously,[18] the accuracy of the calculated thermodynamic quantities for 1:1 complexations were checked by performing several independent titration runs. The uncertainties in the thermodynamic quantities obtained for 1:1 complexation (shown in Table 1) are two standard deviations of the mean value, unless stated otherwise.

Representative microcalorimetric titration curves for the complexation reactions of Cbz-L-Asp and Cbz-L-Glu towards A,B-TMA_2-β-CD (highest affinities; see Table 1) and for the complexation reactions of the same guests toward β-CD (smallest affinities; see Table 1) are shown in Fig. 1(a)–(d), respectively. In the case of reaction of Cbz-L-Asp and Cbz-L-Glu with A,B-TMA_2-β-CD, the total charge of the 1:1 complex is zero, hence there is no electrostatic repulsion with the second guest on interaction with the complex. To evaluate this possibility, microcalorimetric titration experiments were performed with a high excess of the guests up to a molar ratio of 14–16 [Fig. 1(a) and (b)]. However, no significant deviation of the data points from the 1:1 model were observed even at a high excess of the guests. This is an additional experimental confirmation of the applicability of a simple 1:1 model for the systems under consideration. It should be mentioned that the numerical values of the reaction enthalpies presented in Table 1 and Fig. 1(a)–(d) can deviate from each other (2–3%) since the data in Fig. 1(a)–(d) are results of computer simulation without correction for electrical calibration of the microcalorimeter. It should be also noted that the uncertainties presented in Table 1 are generally larger than these shown at Fig. 1(a)–(d) since the former reflect not only scattering of the data points from the theoretical fit but also uncertainties in purity determination of host and guest, accuracy in preparation of the solutions and accuracy of the calibration parameters.

NMR measurements. ^1H NMR spectra at 600 MHz were measured at 25 °C in D_2O. A Bruker Avance 600 spectrometer was used to obtain 1D and 2D NMR spectra, including ROESY, COSY and HOHAHA. HOHAHA experiments with the MLEV-17 pulse sequence were performed with a mixing time of 120 ms. ROESY spectra were recorded with a mixing time of 750 ms.

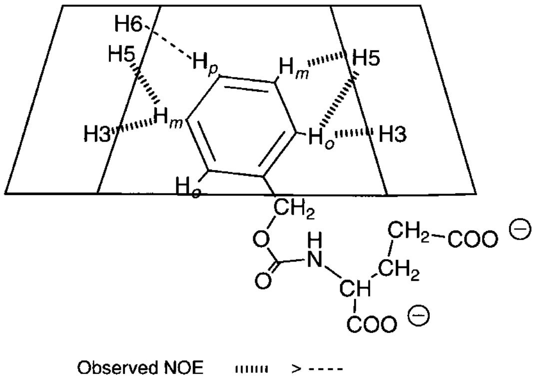

Figure 2. Observed intermolecular NOEs between the host and guest protons and a schematic drawing of the plausible complex structure of Cbz-L-Glu (and Cbz-L-Asp) with β-CD

RESULTS AND DISCUSSION

Native β-CD

The observation of NOEs is very informative in the determination of the structure of inclusion complexes.[19–21] As was reported previously for the complexation of Naph-Phe and Dans-Phe with β- and γ-CDs,[11] it was shown from the NMR NOE experiments that both Cbz-L-Asp and Cbz-L-Glu penetrate into the cavity from the secondary side of native β-CD. Weak-to-moderate NOE cross peaks were clearly observed between the *ortho* and *meta* protons (H_o and H_m) of Cbz and the H3/H5 protons of β-CD. This and additional NOE between the *para* proton (H_p) of Cbz and H6 proton of β-CD confirm the penetration of phenyl into the cavity from the secondary side as illustrated in Fig. 2, along with the plausible structure of complex formed between Cbz-L-Glu and β-CD.

Additionally, Cbz-L-Asp and Cbz-L-Glu afforded almost indistinguishable NOE signals upon complexation with β-CD, indicating that the modes, depths and orientations of penetration are very similar for both guests, irrespective of the different methylene numbers. In both cases we observed appreciable NOEs for the Cbz phenyl protons at δ 7.39–7.43 with the CD H3 protons at δ 3.94 and H5 at δ 3.74; a weaker, but appreciable, NOE was observed even for Cbz H_o and CD H6 at δ 3.86. It is concluded therefore that the phenyl moiety of both Cbz-L-Asp and Cbz-L-Glu is fairly deeply included in the β-CD cavity.

The complex structure shown in Fig. 2 is reasonable in view of the optimal van der Waals contacts between the phenyl group and the cavity, leading to the exclusively enthalpy-driven complexation as confirmed by the thermodynamic data. The analogous complex structures proposed for Cbz-L-Asp and Cbz-L-Glu nicely coincide with the fact that both guests give comparable complexation thermodynamic parameters, shown in Table 1. Indeed, the two guests exhibit only slight differences of 15% in their complex stability constants (*K*) or 0.36 kJ mol^{-1} in Gibbs energy ($\Delta G°$), 0.9 kJ mol^{-1} in enthalpy ($\Delta H°$) and 0.6 kJ mol^{-1} in entropy ($T\Delta S°$) (Table 1). Judging from the almost identical NOE patterns observed for both guests, the small but appreciable enhancement of $\Delta H°$ by 0.9 kJ mol^{-1} for Cbz-L-Glu is attributable not to the increased van der Waals contacts of the extra methylene, but rather to fine conformational adjustments (or slightly deeper penetration) of the guest molecule within the cavity, which are, however, not explicitly detected by the NOE experiments. This rationalization appears reasonable since the other hosts did not always give more exothermic $\Delta H°$ values for Cbz-L-Glu.

Am- and TMA-*β*-CDs

The cationic moiety introduced to the primary rim of *β*-CD has a striking effect on the mode of guest inclusion; with the penetration direction switched from the secondary side to the primary side upon complexation of dianionic Cbz-L-Asp and Cbz-L-Glu guests with Am-*β*-CD and TMA-*β*-CD, as illustrated in Fig. 3.

NMR studies on the complexation of Cbz-L-Glu with Am-*β*-CD and TMA-*β*-CD gave very similar NOE spectra, indicating similar complex structures for both hosts. In the ROESY spectrum of the Cbz-L-Glu complex with Am- *β*-CD, all of the phenyl protons (*o, m* and *p*) gave NOE cross peaks with CD H3 at δ 3.92. The complex with TMA-*β*-CD gave appreciable NOEs for the phenyl protons H_o and H_m with H3 at around δ 3.91–3.94 and 3.82–3.85, and a weak NOE for H_o with H5 at δ 3.73. All of the above NMR data clearly indicate that the guest insertion occurs from the primary side (Fig. 3), while the predominant NOEs of the phenyl protons with H3 indicate that the phenyl moiety is located near the secondary rim, in a somewhat tilted conformation as shown in Fig. 3. This conformation is suitable for obtaining the pronounced van der Waals contacts between the phenyl and the cavity walls, and is compatible with the exclusively enthalpy-driven complex formation (Table 1).

Neutral *β*-CD, in contrast to cationic Am- and TMA-*β*-CDs, accommodates the same guest but with penetration from the opposite side of the cavity, giving different complex structures, yet exhibiting similar thermodynamic parameters upon complexation with the dicationic guests Cbz-L-Asp or Cbz-L-Glu, i.e. highly exothermic $\Delta H°$ and small positive $\Delta S°$ values. Therefore, the consistent predominantly enthalpy-driven complexation is most likely attributable to a tight-fitting inclusion of the aromatic moiety into the *β*-CD cavity in both cases. This view is consistent with our previous observation[11] that the intramolecularly stacked aromatic moieties of Dans-Phe and Naph-Phe are included much more tightly in *β*-CD, with the accompanying greater van der Waals contacts and greater conformational restriction, which in turn results in much greater exothermic $\Delta H°$ and unfavorable negative $\Delta S°$.

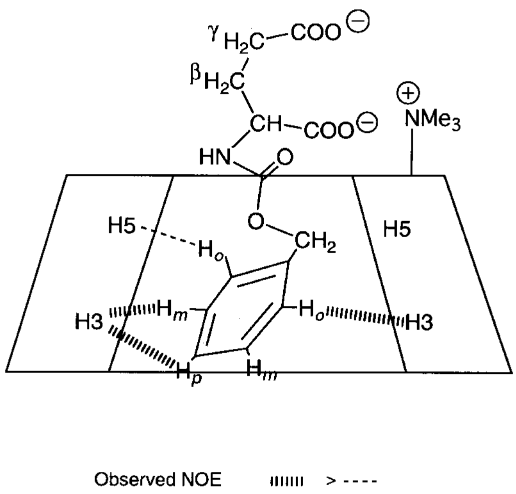

Figure 3. Schematic drawing of the plausible complex structure of Cbz-L-Glu with TMA-*β*-CD and Am-*β*-CD

Even though Am- and TMA-*β*-CDs possesses the same +1 charge, they behave very differently, with the former host giving much higher affinities for both Cbz-L-Asp and Cbz-L-Glu than the latter. Since the Coulombic attraction is sensitive to the intercharge distance, the less bulky ammonio group in Am-*β*-CD is expected to interact more strongly with the guest's carboxylate anion than the bulkier trimethylammonio substituent in TMA-*β*-CD. Indeed, Am-*β*-CD gives larger $\Delta G°$ and $\Delta H°$ values by ca 1 kJ mol^{-1} upon complexation with Cbz-L-Asp and Cbz-L-Glu compared with TMA-*β*-CD. It is therefore inferred that the resulting complex structure, or more strictly the degree of molecular restriction, does not differ greatly in the two cases, as the reaction entropy shows only a negligible difference. The charge-shielding effect of TMA seems significant in general, judging from the fact that the dicationic TMA_2-*β*-CDs gives only slightly larger (for the A,B-isomer) or even smaller $\Delta G°$ and $\Delta H°$ (for the A,C- and A,D-isomers) values than those for monocationic Am-*β*-CD.

A,B-, A,C- and A,D-TMA$_2$-*β*-CDs

Trends in the thermodynamic parameters obtained for the complexation of diaminated CDs (A,B-, A,C- and A,D-TMA_2-*β*-CDs) are also reasonably accounted for in terms of the depth of phenyl penetration into the cavity. However, before going to a detailed discussion, we wish to consider several contributions that make a direct comparison of the thermodynamic parameters for neutral, mono- and diaminated CD hosts difficult. Indeed, the real reason(s) are not immediately clear why more exothermic complexation enthalpies are obtained for the monocationic Am- and TMA-β-CDs rather than the neutral β-CD. One possibility would be a better shape/size complimentarity upon inclusion of a phenyl group from the primary side of β-CD. However, it is more likely that the larger exothermic $\Delta H°$s for Cbz-L-Glu or -Asp complexes with Am- and TMA-β-CDs originate from the greater electrostatic interactions in the cationic CD cases. The more exothermic $\Delta H°$s for Am-β-CD compared with TMA-β-CD clearly rule out the possible greater van der Waals contributions or hydrophobic interactions of TMA, but are readily attributable to the stronger electrostatic interactions as a result of the smaller steric hindrance of Am, as mentioned above. This idea may be supported by the fact that dicationic A,B-TMA$_2$-β-CD gives even higher affinities for both guests. The less favorable $T\Delta S°$ values for A,B-TMA$_2$-β-CD than those for Am- and TMA-β-CDs are readily ascribed to the additional restriction of guest conformation through the fixation of two carboxylates in the guest.

It is puzzling that, as demonstrated above for β-CD versus Am- and TMA-β-CDs, the distinctly different original host structures and resulting complex structures can display unexpectedly similar thermodynamic behavior. However, in the following experiments using A,B-, A,C- and A,D-TMA$_2$-β-CDs as hosts, the systematic variation in host structure leads to consistent and meaningful changes in both complex structure and thermodynamic parameters.

In the NMR studies on the Cbz-L-Glu complexes of isomeric A,B-, A,C- and A,D-TMA$_2$-β-CDs, we first assigned all of the relevant glucose protons by using the inter-residual ROESY, COSY and HOHAHA spectra. We then elucidated the plausible structures of the Cbz-L-Glu complexes with isomeric A,B-, A,C- and A,D-TMA$_2$-β-CDs from the ROESY experiments. The ROESY spectrum of the A,B-TMA$_2$-β-CD complex is shown in Fig. 4(a), where appreciable NOE cross peaks are found for the phenyl *ortho* proton (H_o) with CD $H3_A$ at δ 4.01, with $H3_C$, $H3_{D/E}$ and $H3_G$ at δ3.94–3.99 and with $H3_{E/D}$ and $H3_F$ at δ 3.91–3.93. Also, weak cross peaks with H5 were observed around δ 3.70–3.76 and 3.65. The *meta* proton (H_m) gave cross peaks with $H3_{C,D/E,G}$ at δ 3.96–3.99 and with $H3_{E/D,F}$ at δ 3.90–3.93, and the *para* proton (H_p) with $H3_{C,D/E,G}$ at δ 3.96–3.98.

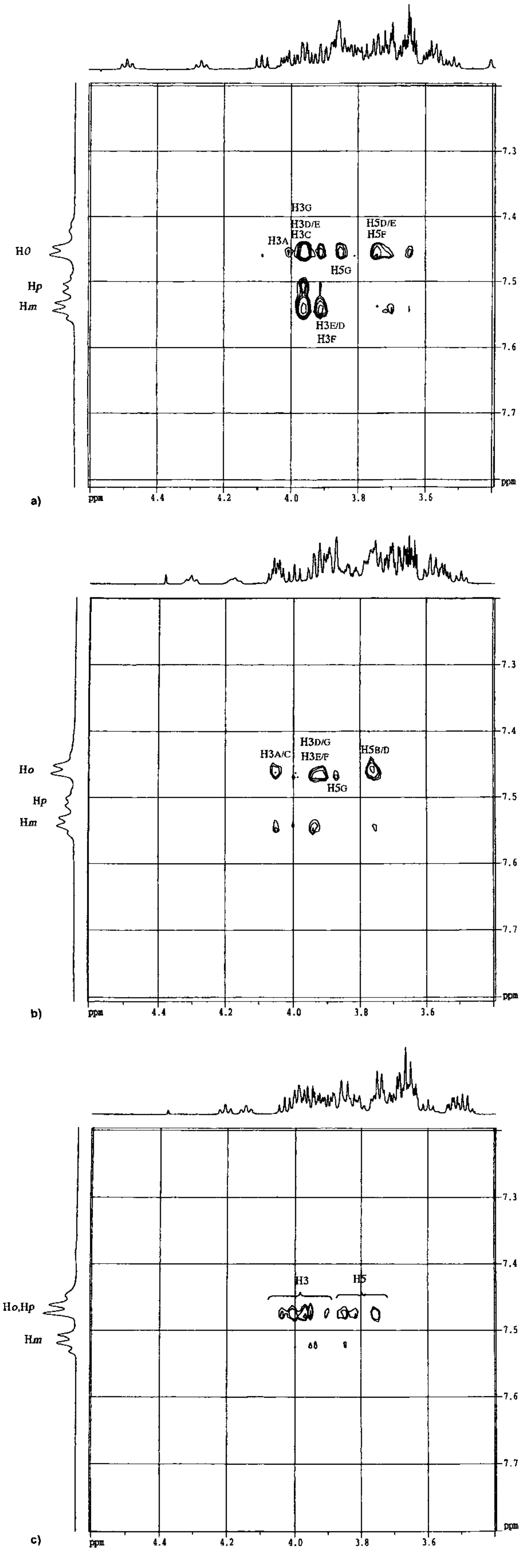

Figure 4. ROESY spectrum of the mixture of Cbz-L-Glu with (a) A,B-TMA$_2$-β-CD, (b) A,C-TMA$_2$-β-CD and (c) A,D-TMA$_2$-β-CD in D_2O. The concentrations of Cbz-L-Glu and TMA$_2$-CDs are 20.4 mM each

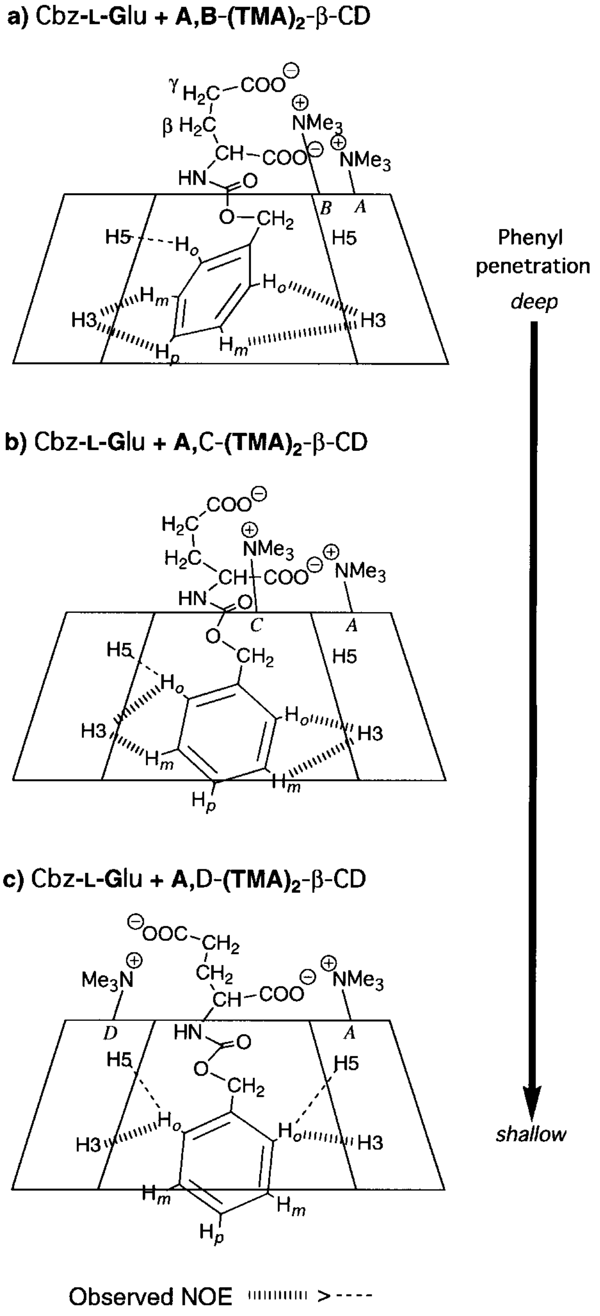

Figure 5. Schematic drawing of the plausible complex structures of Cbz-L-Glu with (a) A,B-, (b) A,C- and (c) A,D-TMA_2-β-CDs

These ROESY results clearly indicate that the Cbz phenyl is fully accommodated in a tilted conformation near the wider rim of the cavity, as illustrated in Fig. 5(a).

For the A,C-TMA_2-β-CD complex of Cbz-L-Glu, the phenyl H_o gave NOE cross peaks with CD $H3_{A/C}$ at δ 4.04–4.07 and $H3_{D/G,E/F}$ at δ 3.90–3.95, and also with $H5_G$ at δ 3.88 and $H5_{B/D}$ at δ 3.67, as shown in Fig. 4(b). H_m gave NOEs with $H3_{A/C}$ at δ 4.06 and with $H3_{D/G}$ at δ 3.93–3.95, whereas H_p showed no appreciable cross peaks with any of the glucose protons. These observations led us to the complex structure illustrated in Fig. 5(b), where the Cbz phenyl is accommodated fairly deeply in the cavity with a slight tilt along the CD axis. For the A,D-TMA_2-β-CD complex, the Cbz H_o gave NOE cross peaks with H3 protons around δ 3.94–4.03 and 3.90 and weaker cross peaks with H5 at δ 3.82–3.86 and 3.77, whereas H_m showed no appreciable NOEs with the interior CD protons, as shown in Fig. 4(c). The lack of NOE for H_m indicates a shallower inclusion of the Cbz phenyl in the cavity, as shown in Fig. 5(c).

It is intriguing that the complex structure is critically affected by the relative position of the two TMA groups introduced to the rim of β-CD. We now discuss the thermodynamic consequences of such structural variations by examining the thermodynamic quantities obtained for the inclusion complexation of Cbz-L-Glu with isomeric A,B-, A,C- and A,D-TMA_2-β-CDs. We would emphasize that the same discussion and conclusions will also be applicable to the corresponding Cbz-L-Asp complexes, since these two guests, differring by just one methylene, give very similar thermodynamic parameters and tendencies upon complexation with the native and modified β-CDs.

The complex structure of A,B-TMA_2-β-CD with Cbz-L-Glu as elucidated from ROESY experiments is similar to that of Am-β-CD and TMA-β-CD. As shown in Figs 4(a) and 5(a), the Cbz phenyl is fully accommodated and somewhat tilted in the cavity, maximizing the van der Waals contacts of the aromatic protons with the CD H3 protons. Such a mode of complexation leads to the exclusively enthalpy-driven complexation with slight entropy assistance. The increased electrostatic interactions of the two adjacent TMA groups in A,B-TMA_2-β-CD with the dianionic Cbz-L-Glu guest do not greatly alter the complex structure, but certainly enhance the complexation enthalpy.

As the distance between two TMAs on the CD rim increases from the A,B- to the A,C-position, the tether between the Cbz and Glu moieties appears to become more constrained. This additional restriction in tether conformation does not allow the adoption of the tilted phenyl conformation, driving part of the phenyl out of the cavity and into the bulk water [Fig. 5(b)]. This shallower inclusion should lead to a less exothermic $\Delta H°$ as a result of weaker van der Waals interactions, and also to a more positive $T\Delta S°$ arising from increased positional and rotational freedoms, both of which were confirmed experimentally by thermodynamic experiments (Table 1).

Further increase in the distance between two TMAs up to the A,D-position induces additional constraints to the tether, forcing greater phenyl moiety exposure to the bulk water [Fig. 5(c)]. The shallowest inclusion amongst the TMA_2-β-CDs is nicely correlated with the smallest complexation $\Delta H°$ and the largest positive $T\Delta S°$ for A,D-TMA_2-β-CD.

Thus we have found a direct correlation between the increasing depth of guest penetration into the cavity (in the order A,D-TMA$_2$-β-CD < A,C-TMA$_2$-β-CD < A,B-TMA$_2$-β-CD) and an increasing reaction enthalpy, accompanied by a decreasing reaction entropy. To the best of our knowledge, this is the first direct and detailed experimental confirmation of the postulated, but never proved, correlation between supramolecular complex structure and complexation thermodynamic parameters.

CONCLUSIONS

Cbz-L-Asp and Cbz-L-Glu penetrate into the native β-CD cavity from the secondary side as was the case with the complexation of Naph-Phe and Dans-Phe.

Introduction of positive charge(s) on the primary rim of β-CD can alter the direction of guest penetration through the electrostatic interaction between positively charged group(s) of the host and the negatively charged groups of the guest. Thus, Cbz-L-Asp and Cbz-L-Glu consistently penetrate from the primary side upon complexation with Am-β-CD, TMA-β-CD, A,B-TMA$_2$-β-CD, A,C-TMA$_2$-β-CD and A,D-TMA$_2$-β-CD.

The additional electrostatic interactions enhance the complex stability through the increased exothermic $\Delta H°$ without significantly changing $T\Delta S°$.

Increasing depth of guest penetration in the order A,D-TMA$_2$-β-CD <A,C-TMA$_2$-β-CD <A,B-TMA$_2$-β-CD is nicely correlated with the gradually increasing exothermic $\Delta H°$ and corresponding decreasing $T\Delta S°$.

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REFERENCES

1. Schneider H-J., Mohammad-Ali AK. In *Comprehensive Supramolecular Chemistry*, vol. 2, Vögtle F (ed). Pergamon Press: Oxford, 1996; 69–101.
2. (a) Stites WE. *Chem. Rev.* 1997; **97**: 1233–1251; (b) Bender SL, Babine RE. *Chem. Rev.* 1997; **97**: 1359–1473; (c) Zeng F, Zimmerman SC. *Chem. Rev.* 1997; **97**: 1681–1712; (d) Zeng A, Shinkai S. *Chem. Rev.* 1997; **97**: 1713–1734.
3. Echegoyen L., Kaifer AE (eds). *Physical Supramolecular Chemistry.* Kluwer: Dordrecht, 1996.
4. Lehn JM (ed). *Comprehensive Supramolecular Chemistry*, vols 1–4. Pergamon Press: Oxford, 1996.
5. Szejtli J., Osa T (eds). *Comprehensive Supramolecular Chemistry*, vol. 3, *Cyclodextrins.* Pergamon Press: Oxford, 1996.
6. Connors KA. *Chem. Rev.* 1997; **97**: 1325–1358.
7. Schneider H-J, Hacket F, Rudiger V, Ikeda H. *Chem. Rev.* 1998; **98**: 1755–1786.
8. Breslow R, Dong SD. *Chem. Rev.* 1998; **98**: 1997–2012.
9. Rekharsky MV, Inoue Y. *Chem. Rev.* 1998; **98**: 1875–1917.
10. Uekama K, Hirayama F, Irie T. *Chem. Rev.* 1998; **98**: 2045–2076.
11. Hembury G, Rekharsky M, Nakamura A, Inoue Y. *Org. Lett.* 2000; **2**: 3257–3260.
12. Yamamura H, Akasaki A, Yamada Y, Kano K, Katsuhara T, Araki S, Kawai M, Tsuda T. *Electrophoresis* in press.
13. Hamasaki K, Ikeda H, Nakamura A, Ueno A, Toda F, Suzuki I, Osa T. *J. Am. Chem. Soc.* 1993; **115**: 5035–5040.
14. Chen X, Oscarson JL, Gillespie SE, Cao H, Izatt RM. *J. Solution Chem.* 1994; **23**: 747–768.
15. Ojelund G, Wadso I. *Acta Chem. Scand.* 1968; **22**: 2691–2695.
16. Ross PD, Rekharsky MV. *Biophys. J.* 1996; **71**: 2144–2154.
17. Rekharsky MV, Inoue Y. *J. Am. Chem. Soc.* 2000; **122**: 4418–4435.
18. Rekharsky MV, Goldberg RN, Schwarz FP, Tewari YB, Ross PD, Yamashoji Y, Inoue Y. *J. Am. Chem. Soc.* 1995; **117**: 8830–8840.
19. Simova S, Schneider H-J. *J. Chem. Soc., Perkin Trans 2* 2000; 1717–1722.
20. Rüdiger V, Eliseev A, Simova S, Schneider H-J, Blandamer MJ, Cullis PM, Meyer AJ. *J. Chem. Soc., Perkin Trans. 2* 1996; 2119–2123.
21. Schneider H-J, Blatter T, Simova S. *J. Am. Chem. Soc.* 1991; **113**: 1996–2000.