DIFFERENCE IN ORIENTATION OF A GUEST WITHIN A CAVITY OF β-CYCLODEXTRINS BEARING AN *N*-ACETYLAMINOACYL GROUP

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Abstract-. ^{1}H-NMR studies revealed that either the benzene or the naphthalene ring of 8-anilinonaphthalene-1-sulfonate (ANS) was accommodated by β-cyclodextrin (β-CyD) bearing an *N*-Ac-L-Leu residue, while β-CyDs bearing an *N*-Ac-L-Ile and *N*-Ac-L-Val residues accommodated the naphthalene ring of ANS.

Cyclodextrins (CyDs) are typical host compounds capable of binding hydrophobic guests in aqueous solution.[1] In addition, since CyDs are composed only of glucopyranose, they show extremely low toxicity against living systems. These features make them a class of drug carriers in the pharmaceutical field by improving solubility, stability, and bioavailability of drugs.[2] If a residue having site-specific biological activity is attached to the CyDs, the resulting CyD derivatives can be used as a sophisticated drug carrier with which drugs can be transported to a target site (cell).[3] In line with this idea, we chose amino acids as the modification groups because some amino acids are known to be transported by amino acid-binding proteins.[3] In addition, chemical interest exists in CyD modified with amino acids because the hydrophobicity of the CyD cavity would be close to that in proteins.[4] This implies that information on the intermolecular interactions among the side chains of amino acids in proteins and bound molecules may be drawn from an analysis of the host-guest interaction of the CyD derivatives. Here, we report the binding behavior, focused on the complexation with 8-anilinonaphthalene-1-sulfonate (ANS), of β-CyD derivatives having *N*-acetyl-L-leucyl (**1**), *N*-acetyl-L-isoleucyl (**2**), and *N*-acetyl-L-valyl (**3**) moieties (Figure 1). Compounds **1-3** were synthesized from β-CyD monotosylate and the sodium salts of the corresponding *N*-acetylamino acids in DMSO. Purification of **1-3** was performed by successive column chromatography using Sephadex G-15 (water) and DEAE Sephadex A-25 (HCO_3^- form,

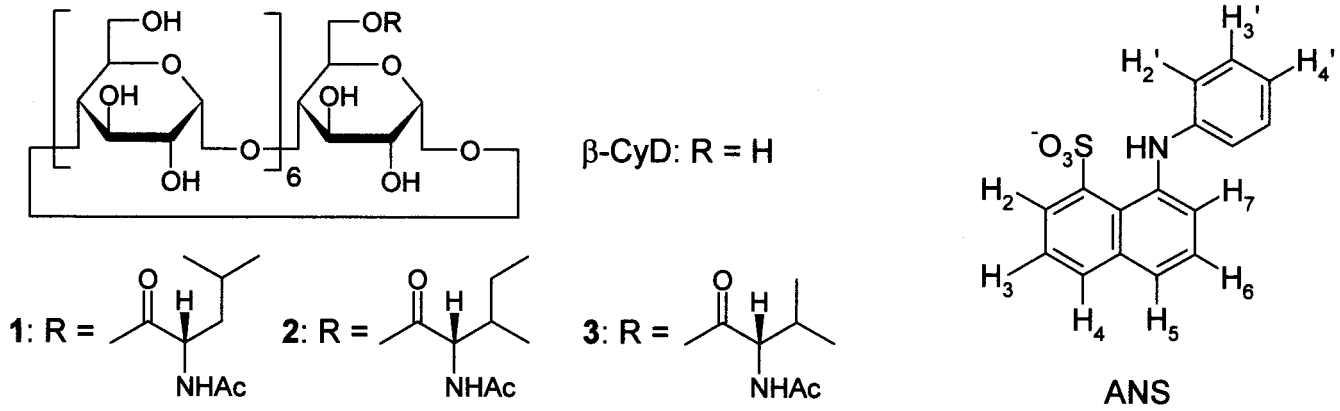

Figure 1. Structures of β-CyD, **1-3**, and ANS

water).[5]

In 10% aqueous DMSO solutions (pH 7.4),[6] **1-3** bound ANS had association constants (K) of 142 ± 3, 144 ± 5, and 77 ± 3 M^{-1} for **1-3**, respectively, which were determined from ANS fluorescence enhancement caused by **1-3**. These values are larger than the K value of β-CyD for ANS under the same condition (25.9 ± 2.1 M^{-1}), indicating that the hydrophobic residue promoted the ANS binding by increasing the hydrophobicity of the CyD cavity.

Regarding the binding conformations, ^{1}H-NMR studies were made and the resulting CIS (complexation-induced chemical shift change) values are summarized in Table 1. It is known that chemical shifts for guest protons shift either toward lower or higher field upon complexation with CyD.[10,11] From this Table, we observed several interesting features. The naphthyl protons of ANS shifted to a lower field, and the phenyl protons caused a negligible shift upon complexation with **2** and **3** except for the H_2' protons residing next to the naphthalene ring. This suggests that **2** and **3** preferentially bind the naphthalene ring of ANS, contrary to a β-CyD-ANS complex in aqueous solution where β-CyD accommodates the benzene ring of ANS as suggested by 1D- and 2D-NMR spectroscopy.[7-10] This change in bound moieties may be due to the presence of the hydrophobic moiety on the primary hydroxyl side of β-CyD, because when β-CyD binds ANS, the benzene ring was reported to be shallowly bound by β-CyD from the secondary hydroxyl side.[10] In the complexes of **2** and **3**, the hydrophobic moiety should prohibit ANS from penetrating into the CyD cavity from the primary hydroxyl side. Thus, when ANS is accommodated into the cavity of **2** or **3**, ANS should be inserted from the wider secondary hydroxyl side, and the larger naphthalene ring would preferably be accommodated into the cavity to maximize contact, namely the van der Waals interaction, among the naphthalene ring of ANS, CyD framework, and hydrophobic residue in the cavity. In contrast to **2** and **3**, **1** caused medium upfield shifts of the phenyl protons (H_3' and H_4')

Table 1. CIS Values (ppm) for ANS Protons in 1-3 Complexes[a)]

Host	H_2	H_3	H_4	H_5	H_6	H_7	H_2'	H_3'	H_4'
1	–[b)]	+0.06	-0.04	–[b)]	–[b)]	–[b)]	–[b)]	-0.07	-0.07
2	+0.07	+0.10	–[b)]	–[b)]	+0.08	+0.08	+0.06	–[b)]	–[b)]
3	+0.10	+0.19	+0.06	+0.09	+0.15	+0.17	+0.12	–[b)]	–[b)]

a) CIS = $\delta_{\text{pure complex}} - \delta_{\text{free}}$; +, downfield shift; –, upfield shift. $\delta_{\text{pure complex}}$ was calculated on the basis of the binding constants obtained from fluorescence measurements.

b) CIS values were not determined due to small shifts upon complexation (CIS $>\pm 0.03$ ppm).

in addition to medium shifts of a few naphthyl protons (H_3 and H_4) of ANS. These CIS values may indicate that both the benzene and naphthalene rings were accommodated by the cavity of 1. However, the size of the cavity of 1 is not sufficient to accommodate them both simultaneously based on CPK model studies. Considering the steric restriction, two conformers may exist with respect to a 1-ANS complex, one of which is a "phenyl-in" conformer and the other is a "naphthyl-in" conformer.[9,12]

The difference between 1 and 2 is the position of the methyl group; it exists at C_γ and C_β in 1 and 2, respectively. Presumably, the bulkier C_γ dimethyl group of 1 decreases the probability of penetration of the ANS naphthalene ring into the β-CyD cavity, narrowing the actual volume of the β-CyD cavity. The observation of a large negative CIS value (-0.23 ppm) for the C_γ dimethyl group upon complexation with ANS indicated that the dimethyl group interacted with the aromatic rings of ANS, and existed near the aromatic π surface so as to undergo a magnetic anisotropic effect. The lack of a methylene group in the side chain (1 vs 3) also resulted in a change of the accommodated part of ANS. A CIS value for the dimethyl group of 3 was negligible ($>$ +0.02 ppm), indicating that the dimethyl group did not exist close to the aromatic rings of ANS. The methyl groups of 2 participate in stabilizing the ANS complex, as suggested by the negative CIS values (-0.09 ppm). In addition, a signal assigned to the C_γ methylene group also caused an upfield shift (-0.23 ppm). This indicates that the methylene group as well as the methyl groups of 2 existed close to the naphthalene ring of ANS. It is noteworthy that the binding constants of 1 and 2 were close and trends in the CIS values of 2 and 3 were similar. These results suggest that the bulkiness of the aminoacyl residues governs the binding strength, whereas the small difference in the aminoacyl residues controls the binding conformations. To obtain more straightforward evidence on the binding conformations, 2D-NMR measurements will be needed, which are now underway.

Our results presented here demonstrated that the trivial difference in the side chain structure of

amino acids possibly changes the molecular recognition behavior of the CyDs, and this feature may be expanded to the molecular recognition by point-mutated proteins, such as a β-amyloid precursor protein of the familiar Alzheimer's disease.[13]

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5. Selected data for **1**-**4**: **1**: MS (FAB) *m/z* 1312 ($[M+Na]^+$); Anal. Calcd for $C_{50}H_{83}NO_{37}\cdot 8H_2O$: C, 41.85; H, 6.96; N, 0.98; Found: C, 41.76; H, 6.87; N, 1.21. **2**: MS(FAB) *m/z* 1290 ($[M+H]^+$); Anal. Calcd for $C_{50}H_{83}NO_{37}\cdot 5H_2O$: C, 43.49; H, 6.79; N, 1.02; Found: C, 43.29; H, 6.80; N, 1.17; . **3**: MS (FAB) *m/z* 1276 ($[M+H]^+$); Anal. Calcd for $C_{49}H_{81}NO_{37}\cdot 5H_2O$: C, 43.06; H, 6.72; N, 1.03; Found C, 43.35; H, 6.66; N, 1.25.
6. We used 10% aqueous DMSO solutions for the measurements due to the low solubility of **1** and **2** toward water. The presence of 10% DMSO impaired the stability of the β-CyD complexes (cf. refs. 7-9).
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