^{1}H NMR Study of Conformation of Formyl-L-phenylalanyl-6-deoxy-6-amino-cyclomaltoheptaose That Has Excellent Ability of Chiral Recognition

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N-(*N*-Formyl-L-phenylalanyl)-6-deoxy-6-aminocyclomaltoheptaose (f-L-Phe-β-CD) is one of CD derivatives with excellent ability of chiral recognition. In order to make clear the mechanism of chiral recognition, the conformation of free f-L-Phe-β-CD has been studied by using ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy. From the measurement of ^{1}H homonuclear Overhauser enhancement on the rotating frame, it is concluded that f-L-Phe- residue of the f-L-Phe-β-CD is inserted into its cavity from the upside of primary hydroxyl groups of the macrocyclic ring. Analysis of ^{1}H and ^{13}C spectra of f-L-Phe-β-CD and model compounds of f-L-Phe- residue also support this conclusion. The macrocyclic ring is found to be distorted.

Utilization of cyclodextrins (CDs) for chiral recognition of optically active substances, that is, separation of respective components from mixtures of enantiomers, diastereomers, or stereo isomers by using CDs is one of the most interesting topics in CD chemistry and has been greatly studied recently.[1–4] CDs have already been used for mobile phase or stationary phase of high-performance liquid chromatography and several successful cases of chiral recognition and separation have been reported.[5] In these cases, the CD molecule should recognize and separate one of chiral isomers on the basis of difference in stability of the host–guest inclusion complexes between the host CD and the guest chiral compounds. As the abilities of unmodified CDs to recognize chiral isomers are not, in general, very excellent,[6] several attempts have been made to improve their abilities of chiral recognition.[4,7] One of such attempts is a selective modification of CDs by amino acids.

N-(*N*-Formyl-L-phenylalanyl)-6-deoxy-6-aminocyclomaltoheptaose (f-L-Phe-β-CD) (Fig. 1) is one of CD derivatives with improved ability of chiral recognition. Hattori et al. have reported that the values of association constant k_a for complexation of f-L-Phe-β-CD with L-tryptophan (k_a=510 M^{-1}) is about seven times higher than that with D-tryptophan (k_a=70 M^{-1}).[8] Although this enhanced ability of D,L-recognition comes from amino acid moiety, details of interactions between host f-L-Phe-β-CD and guest tryptophan molecules have not been yet characterized. It seems very important to make clear the concrete host–guest interactions and the chiral recognition mechanism, especially, the role of modifying amino acid residue in the chiral recognition, from the viewpoint of designing the CD derivatives that have more excellent ability of chiral recognition. For this purpose, it needs to study the conformations of host molecule in the free state and in the host–guest complexes and the relative stability of complexes with guests of different chiralities.

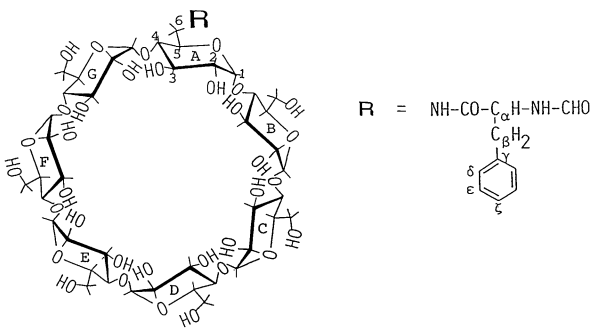

Fig. 1. The structure of f-L-Phe-β-CD, viewed from secondary face, illustrating the ring-labelling sequence. Assignments of carbon atoms are also shown.

Several modern techniques of high-resolution nuclear magnetic resonance (NMR) spectroscopy are expected to provide information useful to elucidate the conformation of CD derivatives.[9,10] Especially, measurements of ^{1}H NMR signal enhancement due to ^{1}H nuclear Overhauser effect (NOE) are powerful means to obtain information about short-range distances among proton nuclei.[11]

We now report on the results of conformational analysis of f-L-Phe-β-CD in aqueous solution by 500 MHz ^{1}H and 125 MHz ^{13}C NMR studies. Since ^{1}H NMR spectrum of f-L-Phe-β-CD consists of too severely overlapped resonances, it seemed not easy to obtain unambiguous resonance assignments of all peaks without the combined use of various two-dimensional (2D) NMR techniques.

Experimental

The ^{1}H and ^{13}C NMR spectra were recorded at 500 MHz

and 125 MHz, respectively, at 40 or 25 °C on a JEOL GX-500 spectrometer. ^1H and ^{13}C chemical shifts δ are given in parts per million (ppm) downfield from the resonance of Me_4Si. Digital resolutions of ^1H and ^{13}C shifts are 0.000488 and 0.0107 ppm, respectively.

f-L-Phe-β-CD was prepared from 6-deoxy-6-aminocyclomaltoheptaose (6-deoxy-6-amino-β-CD) and *N*-formyl-L-phenylalanine in the presence of dicyclohexylcarbodiimide as follows: 0.5 g of *N*-formyl-L-phenylalanine and 3 g of 6-deoxy-6-amino-β-CD were dissolved in 10 ml of dry DMF, treated with dicyclohexylcarbodiimide at 5 °C for 3 h. After removing *N,N'*-dicyclohexylurea, the reaction mixture was evaporated to dryness. The precipitate was washed by acetone and recrystallized by water. 1.2 g of f-L-Phe-β-CD was formed, yield (35%); (Found: C, 45.88; H, 6.49; N, 2.08%. Calcd for $C_{52}H_{80}O_{36}N_2 \cdot 3H_2O$: C, 45.81; H, 6.36; N, 2.06%).

Results and Discussion

Assignments of ^1H Resonances of f-L-Phe-β-CD.

The 500 MHz ^1H NMR spectrum of f-L-Phe-β-CD, and for comparison, those of 6-deoxy-6-aminocyclomaltoheptaose (amino-β-CD) and cyclomaltoheptaose (β-CD) are shown in Figs. 2C, 2B, and 2A, respectively. f-L-Phe-β-CD exhibits severely overlapped ^1H spectrum even though observed at 500 MHz. Although ^1H NMR resonances of f-L-Phe-β-CD are too complex to be assigned completely by using only ^1H-^1H-COSY

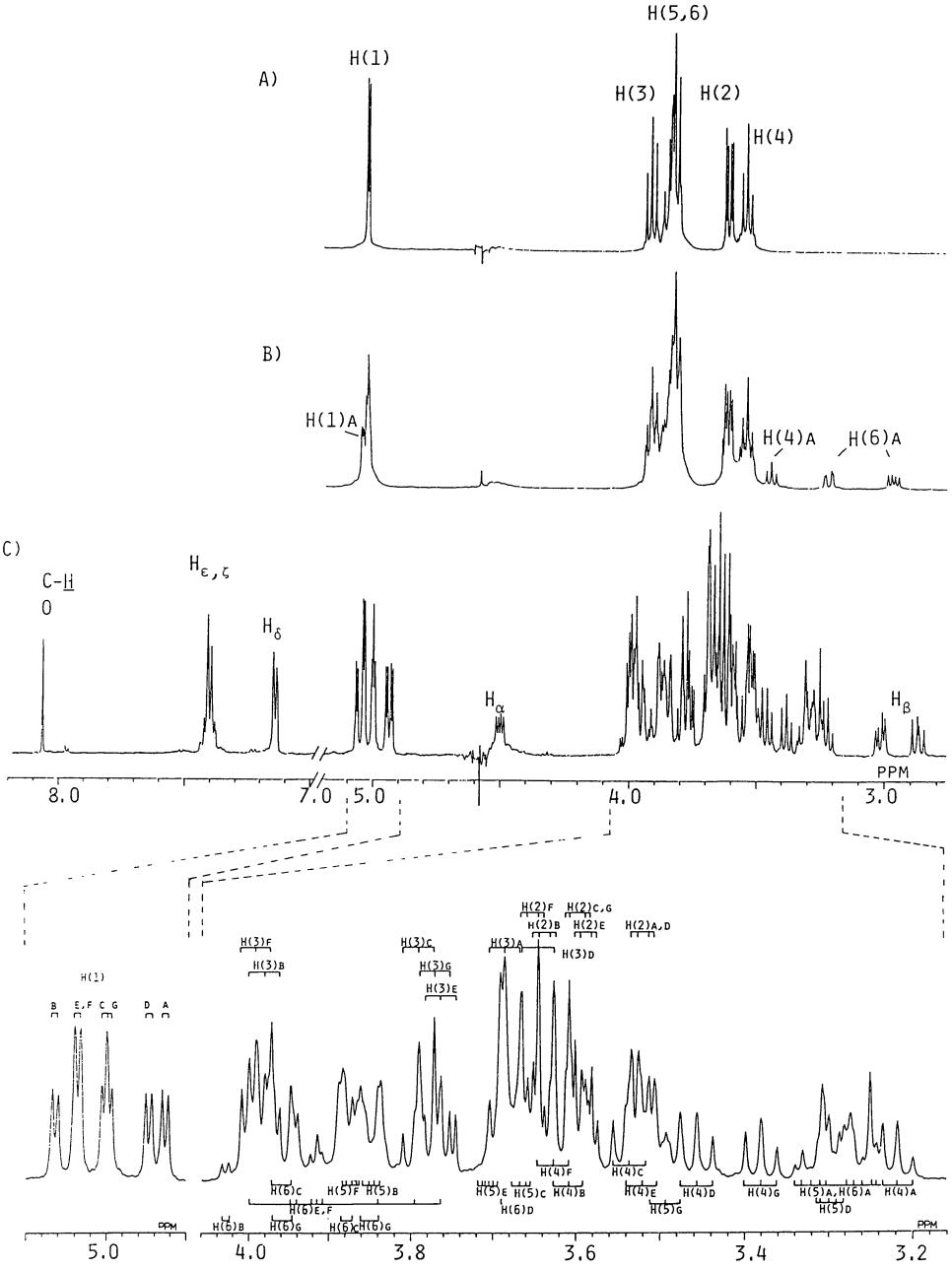

Fig. 2. 500 MHz ^1H NMR spectra of β-CD (A), amino-β-CD (B) and f-L-Phe-β-CD (C) in 1.2×10^{-3} M solution in D_2O at 40 °C.

that is very useful technique to assign "normal" one-dimensional (1D) spectrum, the combined use of DQF-COSY and HOHAHA[12] etc. makes possible to assign all the resonances. Firstly, the detailed procedures of the assignments of ^{1}H resonances will be shown below.

Generally, in the case of sugars, those anomeric protons exhibit characteristic lowfield shifts and so the assignment is started from the anomeric protons. In the case of f-L-Phe-β-CD, the resonances that exhibit similar characteristic lowfield shifts are assigned as anomeric protons. Although anomeric protons of β-CD exhibit only one degenerated resonance, those of f-L-Phe-β-CD exhibit six separated resonances (Fig. 2A) and are labeled from A to G (E and F peaks are overlapped).

By using ^{1}H-^{1}H-COSY that is very useful technique to assign spectrum, only H(2), H(3), and a part of H(4) resonances of f-L-Phe-β-CD can be assigned and, what was worse, the assignments of H(3) and H(4) resonances are quite ambiguous because these resonances are severely overlapped. Even by using phase sensitive COSY, DQF-COSY, the improvement in ambiguity of assignments is only a little, and H(5) and H(6) resonances can not be assigned by these methods. It seems that the assignments of H(5) and H(6) resonances are very important to determine the orientation of f-L-Phe- residue. By using 2D-HOHAHA and 2D-NOESY(ROESY) methods, all the resonances can be fully assigned.

A part of 2D-HOHAHA spectrum of f-L-Phe-β-CD covering the anomeric proton region in F_1 axis is shown in Fig. 3A, and an example of cross-section data

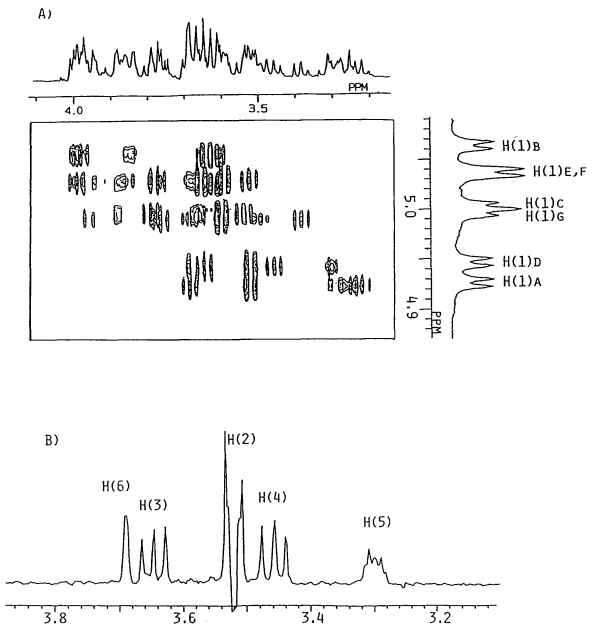

Fig. 3. (A) A part of 2D-HOHAHA spectrum of f-L-Phe-β-CD covering the anomeric proton region in F_1 axis. (B) An example of cross-section data along F_2 axis of (A) sliced at δ=4.947 of F_1 axis.

along F_2 of this experiment are also shown in Fig. 3B. Each anomeric proton (H(1)) resonance has crosspeaks with H(2) to H(6) resonances belonging to the same glucopyranose residue. However, it is difficult to assign each crosspeaks to each protons by the single 2D experiment. In the HOHAHA experiment, changing a mixing time τ_m, the degree of magnetization transfer form H(1) to another protons along the bond is able to control: at the short mixing time, the crosspeaks can be observed with only H(2), and becoming longer the mixing time, the crosspeaks are observed with more distant protons, that is, with H(3), H(4), H(5), and finally H(6).[12] Due to degeneracy of anomeric proton resonances of E and F units, their H(6) proton resonances can not be distinguished, whereas the other proton resonances can be distinguished by the combined use of several 2D methods such as HOHAHA, DQF-COSY and so on.

To confirm validity of assignements, we reinvestigated whether the assignments include all peaks appeared in 1D spectrum and all crosspeaks in 2D spectra, whether they consist with peak relative intensities and whether the coupling constants are not different unreasonably between the nuclei of the same numbering.

Determination of the Sequence of Glucopyranose Units. At the beginning of determination of the sequence of glucopyranose units along the CD macrocycle, a spin system of which H(6) resonance shifts largely to the upperfield is identified as the glucopyranosyl residue modified by the f-L-Phe-residue, that is, the A unit. In amino-CD, its spin systems are divided into two groups, one is a modified residue and the other is unmodified residues. The H(6) and C(6) resonances of modified residue shift very largely to the upperfield and their chemical shifts are very close to those of f-L-Phe-β-CD.

The sequence of specific assignments of resonances of glucopyranose units along the macrocycle can not be determined by correlation experiments that based on the J-coupling such as COSY and HOHAHA, because each spin system of glucopyranose unit is bonded through the α-(1—4)-glucosidic linkage and so each forms an independent spin network.

In the CDs, the distance between an H(1) of one glucopyranose unit and an H(4) of its neighbouring unit across the α-(1—4) glucosidic linkage is close enough to give rise to through-space NOE enhancement[13] which allows the sequence-specific assignments of resonances of glucopyranose units along the CD macrocycle. In Fig. 4 is shown a part of H(1) in F_1 axis of ROESY[14–17] spectrum of f-L-Phe-β-CD, indicating the NOE connectivities between the H(1) and H(4) resonances. A spin network of glucopyranose unit of which H(4) resonance has a negative crosspeak with the H(1) resonance of the A unit is identified as that of the B unit, and in a similar way all spin

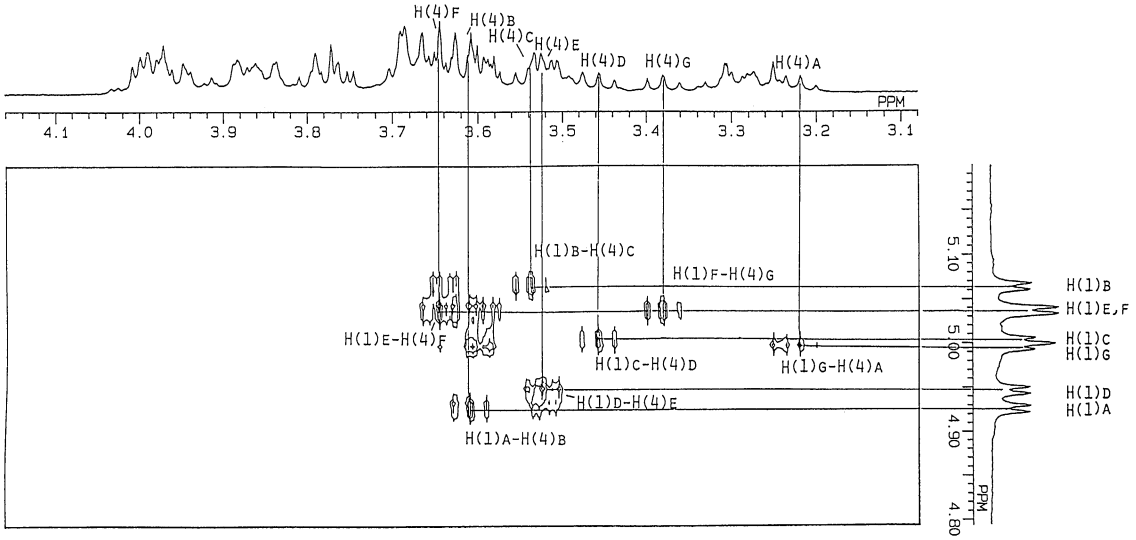

Fig. 4. A part of ROESY spectrum of f-L-Phe-β-CD covering the H(1) region in F_1 axis, indicating the NOE connectivities between H(1) and H(4) resonance of A unit has negative crosspeak with H(4) resonance of B unit, and in a similar way, labelling of all glucopyranose units are determined. As the H(1) resonance of G unit finally has NOE cross peak with H(4) resonance of A unit, the validity of sequential assignments were verified.

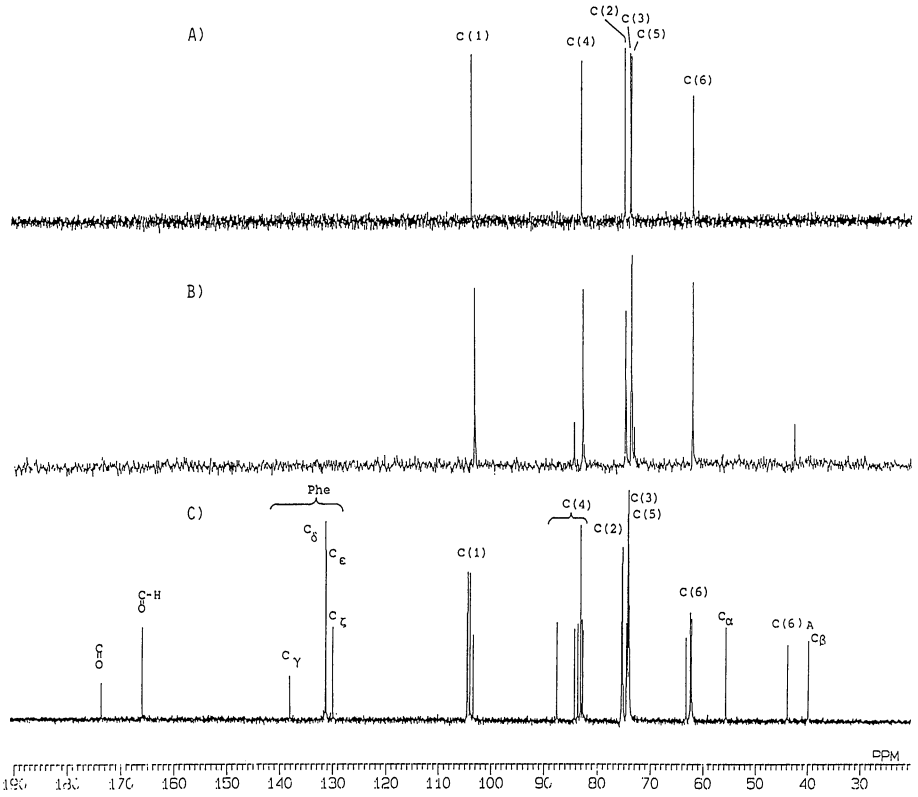

Fig. 5. 125 MHz ^{13}C NMR spectra of β-CD (A), amino-β-CD (B) and f-L-Phe-β-CD in 1.2×10^{-3} M solution in D_2O at 40°C. Assignments are also shown.

networks of glucopyranose units up to the G unit are successively determined. The validity of sequential assignments was confirmed, as the H(1) resonance of the G unit has an NOE crosspeak with the H(4) resonance of the A unit.

Interpretation of ^1H Resonances. In the case of β-

CD, because all seven glucopyranose units are magnetically equivalent due to the presence of the C_7 symmetry axis on its molecule in solution, a single set of NMR resonances is observed as if there were only one glucopyranosyl residue.[18]

On the other hand, for f-L-Phe-β-CD and amino-β-CD, due to the existence of the symmetry-breaking constituent, i.e., f-L-Phe- and amino residues respectively, the C_7 symmetry of the macrocyclic ring is perturbed. In the spectrum of amino-β-CD the resonances are divided into only two independent sets of spin network systems, the one is a set of resonances corresponding to a glucopyranose unit modified by an amino group and the other is the resonances corresponding to the magnetically equivalent unmodifed six glucopyranose units.

Furthermore, in the spectrum of f-L-Phe-β-CD, seven sets of NMR resonances with equal intensity are well discriminated. This is clearly seen in the H(1) resonances (Fig. 2A), and is indicating that all glucopyranose units are magnetically nonequivalent with each other. Those magnetical nonequivalence can be observed on ^{13}C NMR spectra. In Figs. 5C, 5B, and 5A are shown the 125 MHz ^{13}C NMR spectra of f-L-Phe-β-CD, amino-β-CD, and β-CD, respectively, with their assignments (for these assignments, ^{1}H-^{13}C-COSY spectra with assignments of ^{1}H resonances were used). In agreement with ^{1}H resonances, removing the degeneracy among the glycosyl residue in β-CD, there are two and seven sets of resonances for amino-β-CD and f-L-Phe-β-CD,respectively. It seems that the ring current effect from the phenyl groups of f-L-Phe-residue is possible to induce such nonequivalence of H(5) and H(6) protons, but cannot cause that of H(1) and H(4) protons of each glucopyranose unit because they are far from the phenyl group of f-L-Phe- residue wherever it would orient. Since the chemical shifts of H(1) and H(4) protons and C(1) and C(4) carbons are known to reflect the conformation around the glucosidic bond of CD,[19,20] so distortion of the α-(1—4)-linkage of macrocyclic ring is suggested to be a possible cause of H(1) nonequivalence.

From the fact that the unmodified grucopyranose units of amino-β-CD exhibit only one set of ^{1}H NMR resonance, it seems that the effect of amino group substitution does not extend to the six unmodified glucopyranose units.

Furthermore, it has been reported[21] that the branched glycosyl residue of 6-*O*-α-D-glucopyranosyl-cyclomaltohaxaose (G_1-α-CD) orients away from the macrocyclic ring in aqueous solution and does not significantly interact with the macrocylic ring, resulting in only two sets of spin networks for ^{1}H resonances of glycosyl residues of its macrocyclic ring. Accordingly, although number of glucopyranose units is different, the distortion of the (1—4)-α-linkage of macrocyclic ring of f-L-Phe-β-CD should be caused by some significant affection of f-L-Phe- residue.

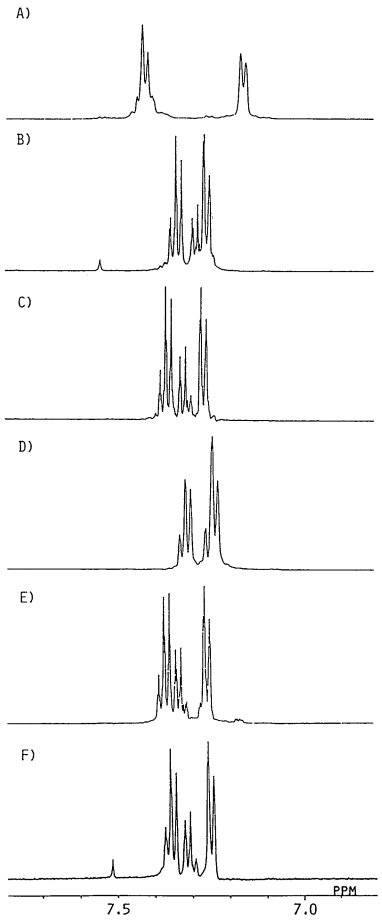

Fig. 6. Phenyl ring proton region of 500 MHz ^{1}H NMR spectra of f-L-Phe-β-CD (A), f-L-Phe (B), L-Phe (C), Gly-Phe (D), Phe-Gly (E) and complex of f-L-Phe and amino-β-CD (F) in 1.2×10^{-3} M solution in D_2O at 25 °C.

In Fig. 6A, 6B, 6C, 6D, 6E, and 6F are shown ^{1}H resonances of phenyl ring protons of f-L-Phe-β-CD, f-L-Phe, L-phenylalanine (L-Phe), glycyl-L-phenylalanine (Gly-Phe), L-phenylalanylglycine (Phe-Gly) and f-L-Phe complexed with amino-β-CD. Assignments of these resonances are also shown. The ^{1}H resonances of phenyl ring protons of f-L-Phe-β-CD exhibit two broad peaks, whereas those of others exhibit well resolved many sharp peaks. The peaks of H_δ, H_ε, and H_ζ protons of phenyl ring of f-L-Phe-β-CD shift about 0.11 ppm to the uperfield, 0.05 ppm to the lowerfield and 0.11 ppm to the lowerfield, respectively, by comparison with those of L-Phe. The chemical shift changes of phenyl-ring ^{1}H resonances of f-L-Phe, Gly-Phe, and Phe-Gly reference to L-Phe are much smaller than those of f-L-Phe-β-CD, suggesting that the effect induced by substitution does not cause the large chemical shift change of phenyl ring proton resonances of f-L-Phe-β-CD. The ^{1}H resonances of phenyl ring protons of f-L-Phe complexed with amino-β-CD

do not also change largely compared with those of f-L-Phe-β-CD. Furthermore, no concentration-dependent changes were observed in the appearance of resonances of f-L-Phe-β-CD at the f-L-Phe-β-CD concentration of 1.2×10^{-2}, 1.2×10^{-3} and 1.2×10^{-4}, indicating the absence of meaningful intermolecular interaction between the phenyl ring of one f-L-Phe-β-CD molecule and the cavity of another f-L-Phe-β-CD molecule (results are not shown). Therefore, the large shift and breadth of resonances of phenyl ring protons of f-L-Phe-β-CD must be caused by the some intramolecular interaction between f-L-Phe- residue and the upper side of CD macrocyclic ring.

Determination of Orientation of f-L-Phe- Residue against Macrocyclic Ring by ROESY. The ^1H homonuclear NOE measurement is one of the most effective techniques in order to obtain informations concerned with through-space distances among proton nuclei within 5 Å. But, practically, no effective NOE can be observed in f-L-Phe-β-CD by the conventional 2D-method, that is NOESY. In some molecules with molecular weight of about 1000 such as CDs, NOE is not always observed with effective intensity, because its NOE enhancement is nearly equal to zero under the condition $\tau_c\omega\approx1$ where τ_c is a motional correlation time related with molecular

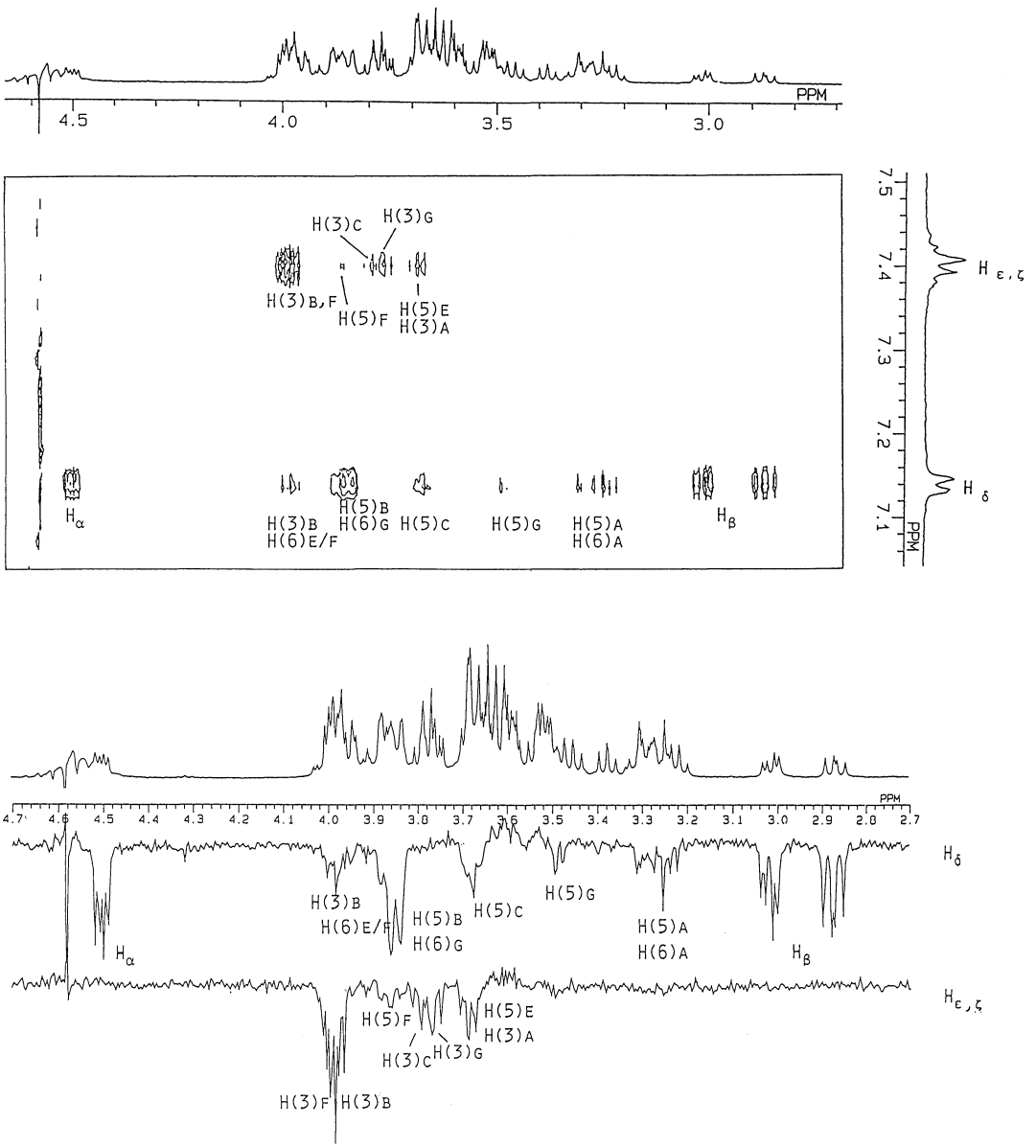

Fig. 7. (A) A part of ROESY spectrum of f-L-Phe-β-CD covering the phenyl ring proton region in F_1 axis. (B) Cross-section data along F_2 axis of (A). Assignments of crosspeaks are also shown. Resonances of H_ε and H_ζ can not be distinguished.

weight and ω is the angular Larmor frequency. We tried to measure NOE using ROESY method[22] that measures NOE in a rotating frame and overcomes a shortcoming of NOESY method. According to the ROESY method, NOE is quite large and always positive for any values. Under the conditions used for ROESY experiments, J cross-peaks due to the first-order spin diffusion pathways and chemical exchange between scalar coupled spins can also invade the ROESY spectrum. However, NOE and J cross-peaks in the 2D-ROESY spectra can be differentiated by their relative signs[17]; Hartmann–Hahn magnetization transfer[23] leads to positive but NOE leads to negative cross-peaks relative to the diagonal peaks. Therefore it is possible to distinguish the NOE and J cross-peaks in ROESY spectrum when complicated J cross peaks are observed.

In Fig. 7 is shown a part of ROESY spectrum of f-L-Phe-β-CD ¹H spectra along F_1 and F_2 axis. ε and/or ζ protons of f-L-Phe- residue have NOE crosspeaks with H(3) protons of A, B, C, G, and F units. On the other hand, δ of f-L-Phe- residue have NOE crosspeaks with H(5) protons of A, B, C, and G units, with H(6) of A and E or F units and with H(3) proton of B unit. The results provide a direct evidence of the orientation of the f-L-Phe- residue of f-L-Phe-β-CD with respect to the CD macrocyclic ring. Considering with these experimental facts, we can conclude that the f-L-Phe- residue is inserted into the CD cavity from the primary-hydroxyl group side of macrocyclic ring (Fig. 8). As the line-widths of L-Phe aromatic proton resonances are significantly broad, it seems that this orientation is fairly static, differing from the case of δ-CD and Phe system, although measurements of spin-lattice relaxation times are needed to discuss the molecular dynamics. If the aromatic ring of the guest molecule is included into the cavity of δ-CD, the H(3) and H(5) resonances are known to shift to upperfield or lowerfield respond to the degree of the depth of inclusion[18,24] due to the ring-current effect of aromatic ring. It is expected from the orientation of f-L-Phe- residue in Fig. 8 that $H(5)_A$, $H(5)_G$ and $H(5)_D$ should shift to upperfield. In Fig. 9 is shown each proton shift as compared with corresponding shift of unmodified β-CD. This result is consistent with expectation from the orientation of f-L-Phe- residue.

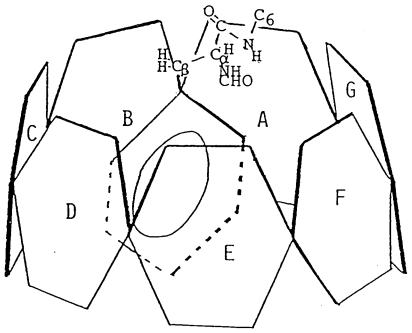

Fig. 8. Schematic diagram of the most possible averaged conformation of f-L-Phe-β-CD in D_2O at 40°C. Its f-L-Phe-residue is inserted into the CD cavity from the primary-hydroxyl group side of own macrocyclic ring.

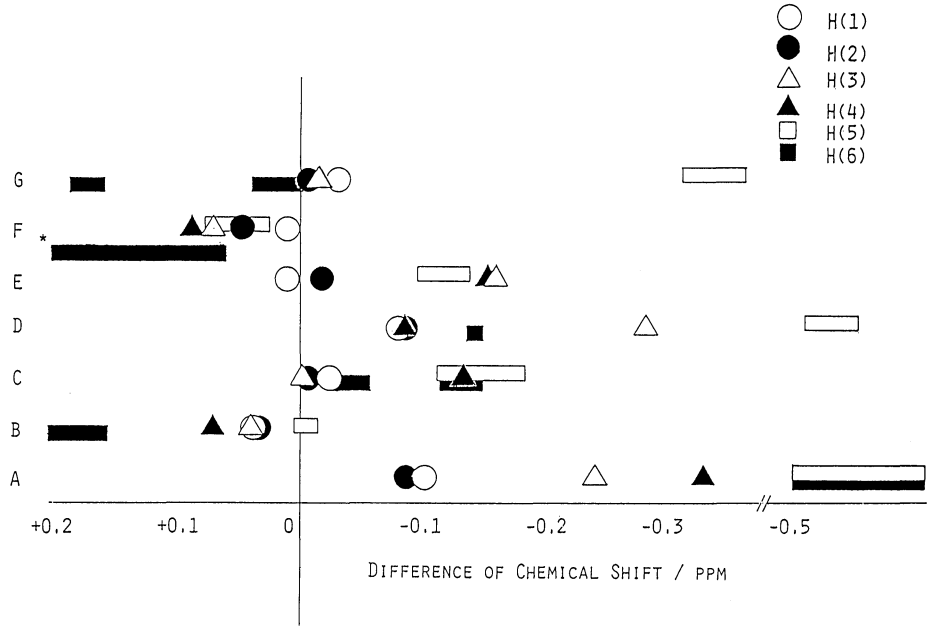

Fig. 9. Proton chemical shifts differences of each glucopyranose residue of f-L-Phe-β-CD with respect to those of β-CD. H(6) resonances of E and F units can not be distinguished.

As the reason of this orientation, it is possible to suggest that the hydrophobic phenyl group of f-L-Phe-β-CD moiety runs away from bulk water and orients to the relatively hydrophobic cavity of f-L-Phe-β-CD and/or hydrogen bond is formed between H(6) and f-L-Phe- residue. But nothing has not been concretely clear yet to make decisive explanation of this orientation.

Although the mechanism of chiral recognition is not clear at present, it seems that there is no room for doubt that the distortion of macrocyclic ring and the existense of optically active residue on the rim of the cavity give f-L-Phe-δ-CD the potential ability of chiral recognition. The NMR study is in progress for the complexes of f-L-Phe-β-CD with L- and D-Trp.

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