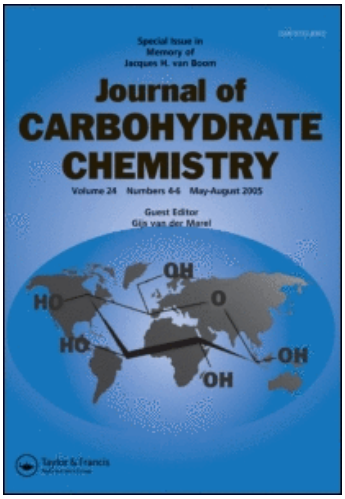

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NMR Studies of Cyclodextrin Inclusion Complex

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REVIEW ARTICLE

NMR STUDIES OF CYCLODEXTRIN INCLUSION COMPLEX

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I. INTRODUCTION

Cyclodextrins[1,2](CDs) are cyclic oligosaccharides composed of six or more (1→4) linked α-D-glucopyranosyl residues(see FIG. 1). The common CDs are called α-, β-, and γ-CD and are constructed of six, seven, and eight α-D-glucopyranosyl units, respectively. The CD molecule is a toroidal, hollow, truncated cone with primary and secondary hydroxyl groups crowning the narrower and the wider rims, respectively. Methine protons and glucosidic oxygens are oriented in its interior and the space inside the hollow, called the cavity, is relatively hydrophobic. The internal diameter of the cavity is about 5~9 Å for the common CDs.

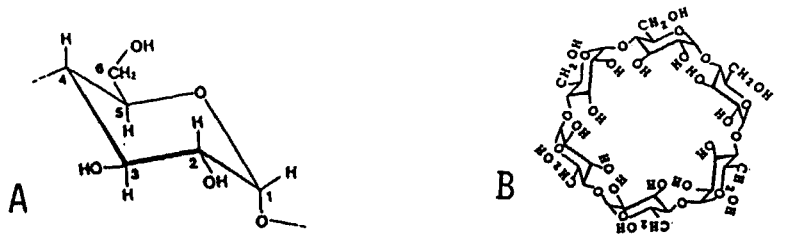


FIG. 1. Structure of the CD constituent glucopyranosyl residue(A) and α-CD(B).

One of the striking features of CD is its ability to form inclusion complexes with a variety of compounds, i.e., the trapping of various external molecules(guest molecules) inside the cavity of CD. Because of this property, CDs have received a great deal of attention from diverse fields. CD is also of interest as an enzyme model because it catalyzes numerous chemical reactions, possibly via inclusion complexation as a reaction intermediate, analogous to an enzyme-substrate complex.[3] Considerable effort has been devoted to determining the mechanism of the CD inclusion complexation process and to provide a detailed description of the interaction of CD and guest molecules. To characterize the inclusion phenomena of CD on the molecular level, accurate structures of inclusion complexes in solution have to be determined.

In this article, we describe the potential usefulness of various NMR techniques for the studies of the CD inclusion complexes in solution and in the solid state. The insertion of a guest molecule into the cavity of CD is clearly reflected by changes in NMR chemical shift values, and structural alteration of the host and guest molecules induced upon complexation leads to changes on various NMR parameters.[4,5] The procedures for NMR determination of the solution structure of CD inclusion complexes are discussed in some detail.

II. SOLUTION STRUCTURE OF CD

The 500 MHz ^{1}H NMR spectrum of α-CD is illustrated in trace A of FIG. 2. The hexagonal symmetry of α-CD molecule results in the appearance of single set of resonances arising from one kind of glucopyranosyl residue. The spectrum of the ^{1}H-^{1}H homonuclear shift correlated spectroscopy(COSY) shown in FIG. 3 provides most unambiguous signal assignment.[6] Information on the coupling constants can be obtained using J coupling

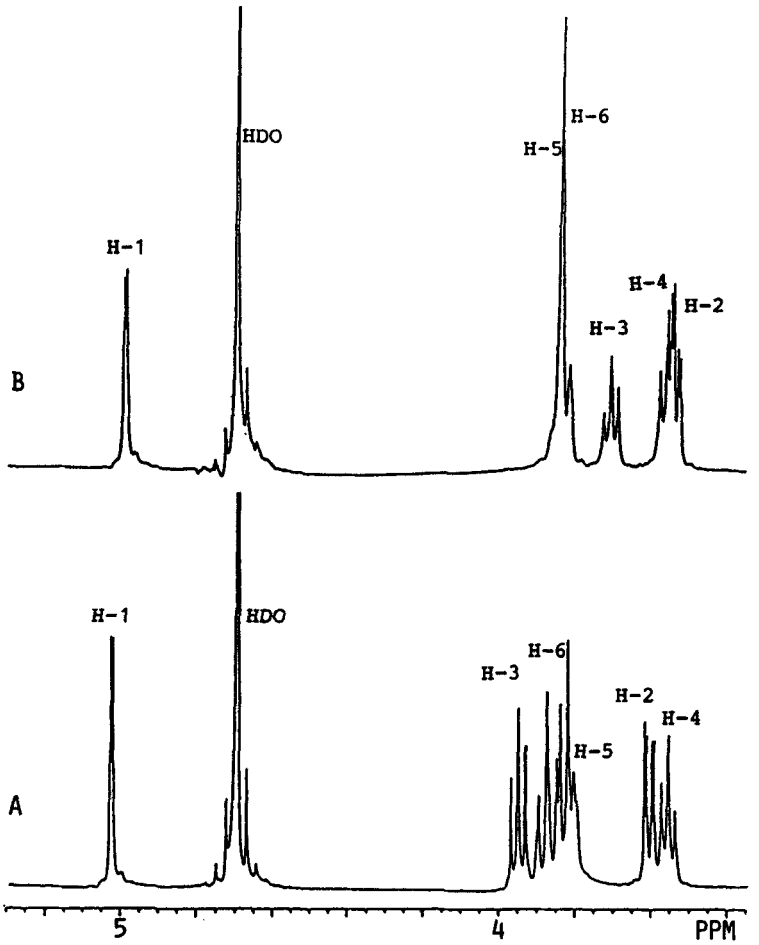


FIG. 2. 500 MHz ^{1}H NMR spectra of α-CD(A) and α-CD-pNP complex(B) in D_2O at pD 7 and 25 °C. In B, only α-CD resonances are shown.

resolved spectroscopy(2DJ) as shown in FIG. 4. Although the coupling constants among H-5, H-6_a, and H-6_b protons provide the conformation around the C-5-C-6 bond(see FIG. 5), their spin coupling system, AA'B, is not easily analyzed even by means of 2DJ, unless stereospecific labeling on H-6 protons is used. Wood et al.[7] used a computer simulation technique to obtain coupling constants and reported the predominance of the gg conformer in CD.

The ^{1}H-^{13}C heteronuclear shift correlated(^{1}H-^{13}C COSY) spectrum of α-CD is shown in FIG. 6. The ^{13}C signal assignments of carbohydrates have been largely determined by model studies or laborious heteronuclear

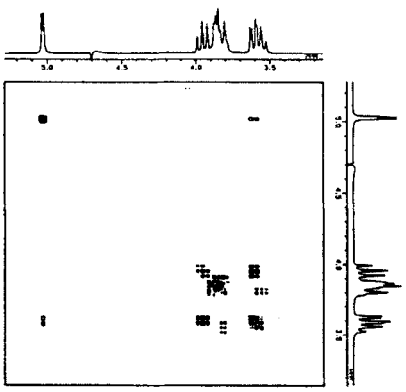


FIG. 3. ^{1}H COSY spectrum of α-CD in D_2O at pD 7 and 25°C.

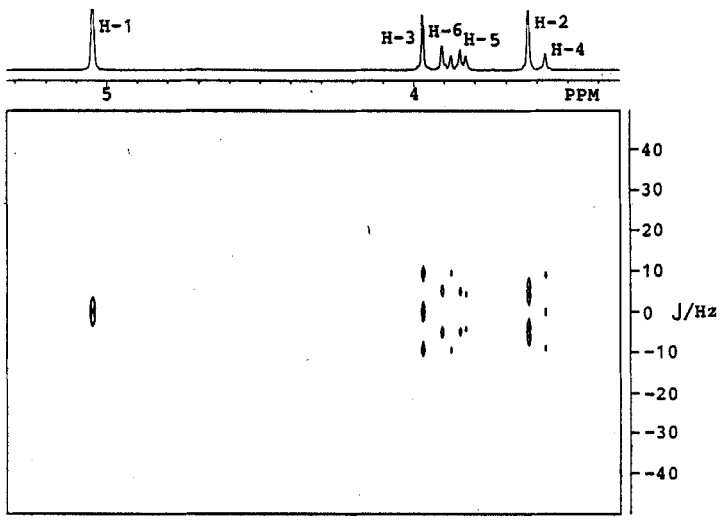
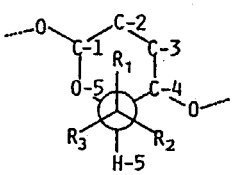


FIG. 4. ^{1}H 2DJ spectrum of α-CD in D_2O at pD 7 and 25 °C.



	R_1	R_2	R_3
Gauche-gauche (gg):	OH	$H\text{-}6_a$	$H\text{-}6_b$
Gauche-trans (gt):	$H\text{-}6_a$	$H\text{-}6_b$	OH
Trans-gauche (tg):	$H\text{-}6_b$	OH	$H\text{-}6_a$

FIG. 5. Stable conformations around C-5-C-6 bond of glucopyranosyl residue.

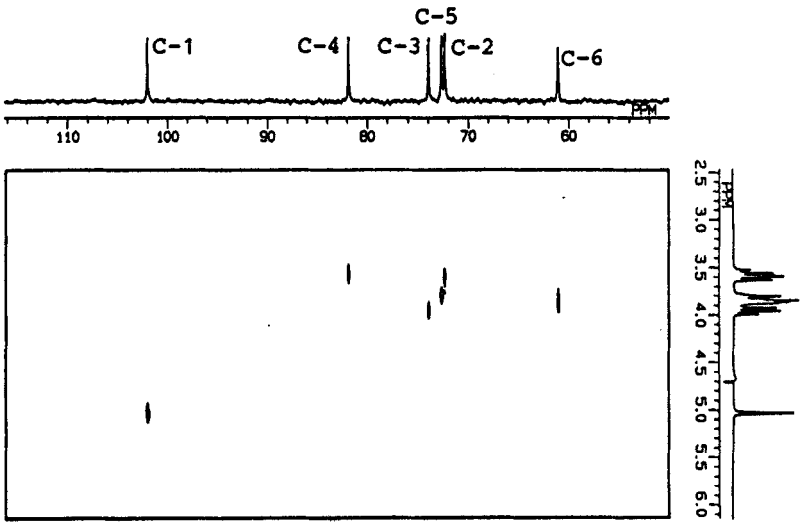


FIG. 6. ^{1}H-^{13}C COSY spectrum of α-CD in D_2O at pD 7 and 25 °C. ^{1}H and ^{13}C spectra are attached along the F_2 and F_1 axes, respectively.

selective decoupling.[8] By using the ^{1}H-^{13}C COSY experiment, ^{13}C assignments can be easily and unambiguously obtained from the known ^{1}H assignment.[9] The ^{13}C NMR studies provide useful information about the conformation and dynamics of the CD molecule and those aspects will be discussed later.

The 270 MHz ^{1}H NMR spectrum of α-CD in DMSO is illustrated in FIG. 7. In addition to the resonances observed in trace A of FIG. 2, three hydroxyl proton resonances appear in the spectrum. These hydroxyl proton resonances shift towards upfield with increasing temperature and the plots of these observed shifts against temperature are shown in FIG. 8. The proposed interglucosidic hydrogen bonds(see FIG. 9) between adjacent hydroxyls at C-2 and C-3 carbons (OH-2 and OH-3, respectively) in solution can be examined from a temperature dependence study.[10,11] The $d\delta/dT$ values (the temperature dependence of the observed chemical shifts) are 4.36, 2.67, and 5.21×10^{-3} ppm deg^{-1} for the OH-2, OH-3, and OH-6 protons of α-CD, respectively. The smaller $d\delta/dT$ value indicates that the hydroxyl proton is more strongly shielded from the solvent and/or involved in an intramolecular interaction such as the intramolecular hydrogen bond. Although the possibility of the flip-flop type hydrogen bond is not completely ruled out, the fact that the $d\delta/dT$ value of the OH-3 proton is smaller than that of the OH-2 proton indicates that the former is a predominant proton donor

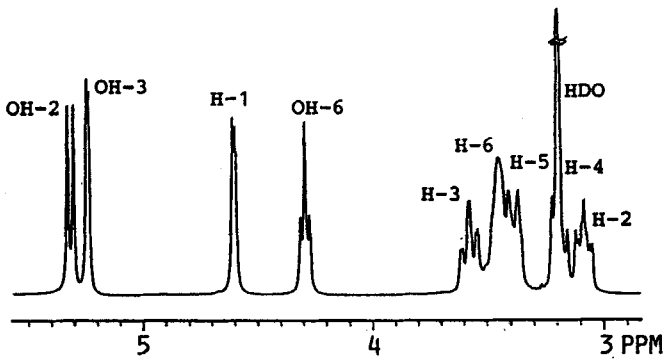


FIG. 7. 270 MHz ^{1}H NMR spectrum of α-CD in DMSO-d_6 at 25 °C. Three hydroxyl proton resonances are observed in the spectrum.

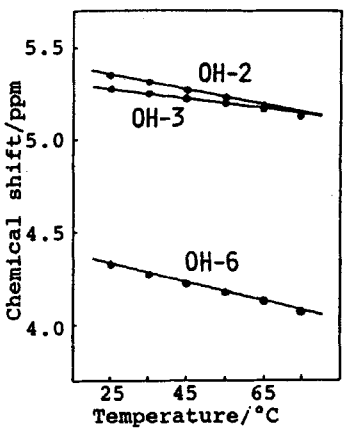


FIG. 8. Plot of the observed chemical shifts of the hydroxyl proton resonances of α-CD in DMSO-d_6 vs. temperature. The $d\delta/dT$ values are 4.36, 2.67, and 5.21 × 10^{-3} ppm deg^{-1} for the OH-2, OH-3, and OH-6 protons, respectively.

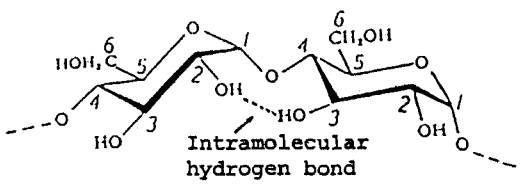


FIG. 9. Interglucosidic hydrogen bond in CD.

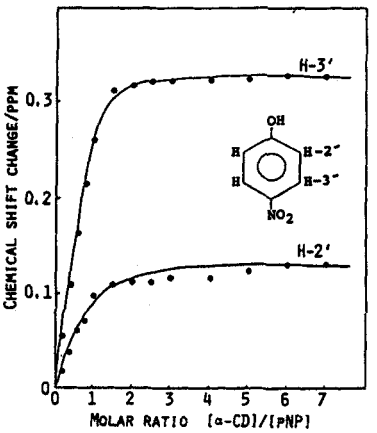


FIG. 10. Plot of induced chemical shift change versus molar ratio ([α-CD]/[pNP]).

for the interglucosidic hydrogen bond in α-CD. Additionally, the dihedral angle calculated from the coupling constant($^3J_{HCOH}$) supports the above interpretation.[11] These interglucosidic hydrogen bonds significantly contribute to the stabilization of a specific macrocyclic conformation of CD in solution as well as in the solid state[12]. The permethylation of the hydroxyl groups of CD leads to an alteration of its molecular structure that is monitored by ^1H and ^{13}C NMR.[9]

III. NMR STUDY OF COMPLEX FORMATION PROCESS

The spectrum of α-CD with guest para-nitrophenol(pNP) in D_2O, pD 7, is illustrated in trace B of FIG. 2. The resonances of the H-3 and H-5 protons located inside the cavity generally shift more than those of the other protons on the outer surface by the addition of aromatic guest molecule and these chemical shift changes are interpreted in terms of the magnetic anisotropy effect arising from the aromatic ring of the guest molecule.[5,7,13,14] The larger shift change of the H-3 resonance not only presents direct evidence to indicate that pNP is incorporated into the cavity of α-CD but also provides a clue for determining the solution structure of α-CD-pNP inclusion complex. The resonances arising from the guest are also perturbed by complexation with CD and the analyses of those resonances provide the kinetic parameters for the inclusion complexation reaction.[7,13] The chemical shift changes of the guest proton resonances are plotted against the ratio of [α-CD] to [pNP] in FIG. 10. The curve

Table I. Dissociation Constants(Kd's) of Some CD-pNP Inclusion Complexes

	pD = 3	pD = 10
α-CD	5.8×10^{-3}	6.1×10^{-4}
α-TMCD	5.5×10^{-4}	8.0×10^{-5}
β-CD	n.d.	1.5×10^{-3}
β-TMCD	9.9×10^{-3}	6.9×10^{-3}

Kd's are given in M at room temperature.
TMCD: Trimethylcyclodextrin. n.d.: Not determined.

indicates a predominant 1:1 complexation, and using the Benesi-Hildebrand equation[15], the dissociation constant(Kd) of the inclusion complex can be calculated. The Kd values for some CD-pNP inclusion complexes are listed in Table I. The results agree well with those obtained from the optical method.[14] The stability of the CD-pNP inclusion complex seems to depend on the ionization state of pNP. Considering the pKa value of 7.2 for pNP, pNP^- ion appears to be more stable than neutral pNP inside the cavity of CD.[14] The orientation of guest molecule with respect to the cavity of CD can be also deduced from the chemical shift changes induced on the resonances of guest molecule.[16,17]

IV. DETERMINATION OF HOST-GUEST GEOMETRY

Quantitative description of the host-guest geometrical relationship[18] between the guest and CD in solution requires the determination of both the orientation of the guest molecule with respect to the CD molecule and the depth of the guest insertion into the CD cavity. The former can be carried out by either measurements of the induced chemical shift changes on the resonances of the guest upon complexation[16,17] or intermolecular ^{1}H NOE[19,20]. The intermolecular NOE information on the inclusion complexes in solution can be obtained by either one-dimensional NOE difference spectrum[19] or two-dimensional ^{1}H-^{1}H NOE correlated spectroscopy (NOESY)[20]. A section of the NOESY spectrum of α-CD-pNP complex in D_2O at pD 7 is shown in FIG. 11. In the NOESY spectrum, the cross-peaks connecting the H-3 resonance of α-CD to both H-2' and H-3' proton resonances of pNP and the H-5 to only H-3' indicate that pNP is preferentially inserted into the cavity of α-CD as shown in the same figure and the result is completely consistent with the orientation of pNP molecule in solid α-CD-pNP complex determined by an X-ray study[21]. Similar NOESY

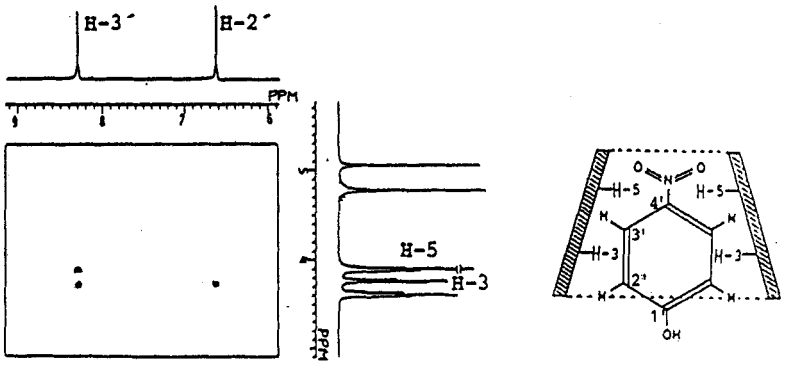


FIG. 11. A section of NOESY spectrum of α-CD-pNP complex in D_2O at pD 7 and 30 °C. The cross-peaks connecting H-3 and H-5 proton resonances of α-CD to both H-2' and H-3' and H-3' proton resonances of pNP, respectively, indicate that the structure of this complex is as illustrated in the inset.

experiments should be possible for other CD inclusion complexes and useful information about the orientation of the guest molecule with respect to the CD cavity in solution can be obtained.

^{13}C NMR studies of α-CD inclusion complexes with a series of para-disubstituted benzene derivatives in aqueous solution have demonstrated that the orientation of the guest molecule in the cavity of α-CD is reflected in the chemical shift change of the ^{13}C resonances of the guest. The resonance of the most deeply inserted quaternary carbon (in the case of α-CD-pNP complex, the C-4' carbon) is largely upfield shifted, compared with the resonance of the other quaternary carbon (the C-1' carbon for pNP).[16] These characteristic results are used to determine the orientation of the guest in the inclusion complex and can be interpreted on the basis of the electrical effects on carbon nuclei. Those shifts are considered to arise from transferring the guest molecule in a free state, surrounded by polar water molecules, to the cavity of CD with a relatively non-polar environment. Neglecting a steric perturbation on the molecular structure of the guest inside the cavity, the results can be explained in terms of solvent effects. The non-polar environmental effects by the CD cavity can be evaluated by the so-called "double-layer" model.[22] It was assumed that the guest molecule inside the cavity is in the environment of dielectric constant ε_1, and the outer part of the molecule is exposed to the solvent with dielectric constant of ε_2 ($\varepsilon_1 << \varepsilon_2$). Calculation was carried out with the borderline as a variable parameter and, in the case of the α-CD-pNP complex, this calculation precisely predicted the depth of

the guest insertion into the cavity which agrees well with the X-ray result.

The depth of the guest molecule insertion into the CD cavity is also determined by rather classic calculation from the induced ring-current chemical shift changes on the H-3 and H-5 resonances.[13,14,23-26] The shift changes of those proton resonances are interpreted in terms of the geometric factors of the corresponding protons with respect to the center of the aromatic ring of the guest molecule using the Johnson-Bovey theory.[27] The theory predicts the magnitude of the magnetic shielding effect induced by the dipolar magnetic field arising from the ring-current of the benzene on a proton of interest if the geometric factor of that proton nucleus, with respect to the benzene ring, is known.

The Johnson-Bovey curves for the α-CD-pNP inclusion complex are calculated with the model shown in A of FIG. 12 and the results are given in B. In the case of the α-CD-pNP complex at pD 10, the induced shift changes of the H-3 and H-5 protons are -0.27 and 0.00 ppm, respectively, indicating that the center of the aromatic ring is located at 0.8 ± 0.1 Å inside the cavity relative to the plane comprised of the H-3 protons. Although this procedure does not provide information regarding the guest orientation, the position of the guest molecule inside the CD cavity can be determined in a quantitative sense.

The complexation between guest and CD molecules in solution leads to an alteration of the nature of the molecular dynamics of both molecules. The host-guest dynamic coupling can be estimated from spin-lattice relaxation time(T_1) analyses.[28-31] The ^{13}C T_1 values of both the host and the guest molecules in the α-CD inclusion complexes with some peptides have been observed and the correlation times for the overall molecular reorientation(τ_o) and the anisotropic internal rotation(τ_i) determined from the T_1 values are given in Table II. The τ_o values for the guest peptides increases by a factor of about 2 due to complexation, suggesting a relatively weak host-guest dynamic coupling in these complexes. On the other hand, the τ_i values of the guests are increased by a factor of up to 8 upon complexation with α-CD, clearly indicating the insertion of the phenyl ring moiety of the guest molecule into the CD cavity. The magnitude of the host-guest molecular dynamic coupling depends on the system, and among the α-CD, β-CD, and γ-CD-Phe inclusion complexes, the strongest coupling was observed for the β-CD complex, in which the phenyl ring moiety of the guest is deeply inserted and is tightly trapped.

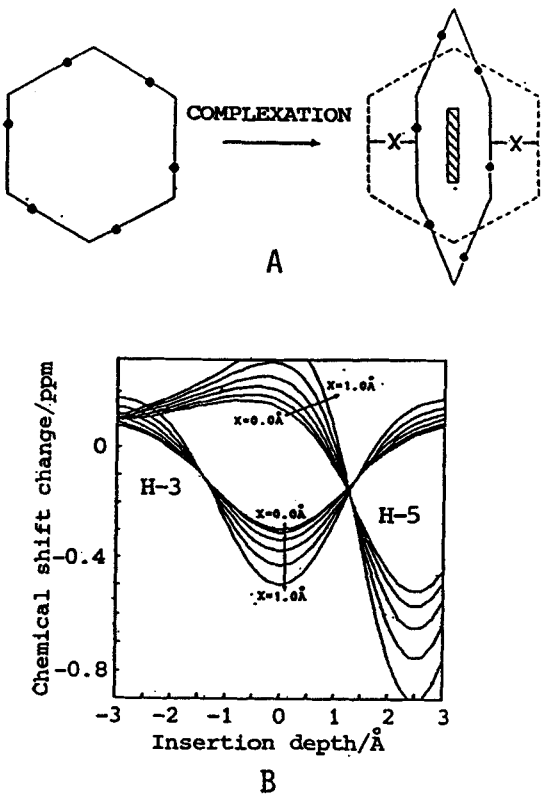


FIG. 12. A: Model for α-CD inclusion complex. The position of H-3 (or H-5) protons are indicated by ● and the shaded rectangle represents the plane of the benzene ring of pNP. Displacement of X (in Å) on the macrocyclic structure of α-CD upon complexation with pNP is considered. B: The Johnson-Bovey curves calculated using the model shown in A. Insertion depth is defined as the distance between the center of the benzene ring of pNP and the plane comprised of the H-3 protons of α-CD. The positive sign indicates that the center of the benzene ring is on the H-5 proton side with respect to the plane of H-3 protons.

Table II. Rotational Correlation Times of α-CD, Phenylalanine Containing Guests, and Their Inclusion Complexes

	α-CD overall (τ_o)	Guest overall (τ_o)	Phenyl ring internal (τ_i)
α-CD	370		
Phe		46	46
α-CD-Phe	410	60	190
Phe-Lys		130	90
α-CD-Phe-Lys	560	220	690

Correlation times are given in ps.

V. BRANCHED CD

Branched CDs are a group of CD to which one or more glucopyranosyl residues are covalently linked. They are more water-soluble and more resistant to enzymic degradation reaction than the normal CDs.[32,33] The double-quantum filtered COSY contour plot of 6-o-α-D-glucosyl α-CD (G_1-α-CD, see FIG. 13) is shown in FIG. 14 and the region a is expanded in FIG. 15. There are three sets of spin coupling networks arising from three magnetically different glucopyranosyl residues.[34] The connectivity starting from the anomeric proton resonance at 5.05 ppm leads to the relatively downfield shifted H-6 proton resonances around 3.57 ppm (see FIG. 15), indicating that this spin network arises from the B unit of FIG. 13. Therefore, the electronic structure of the B unit is slightly altered from that of the C unit due to the branching A unit. Such an asymmetric nature of G_1-α-CD appears to be enhanced upon complexation with the guest. The aromatic proton regions of the ^{1}H NMR spectra of G_1-α-CD in the presence and the absence of pNP are shown in FIG. 16. The degeneracy of the H-1 resonances from the C units is removed by complexation and this could be attributed to differences in the conformation around the glucosidic bond among the (1→4) linkages of G_1-α-CD caused by pNP insertion because the magnetic anisotropy effect arising from the lone-pair electrons of the glucosidic oxygen is known to be responsible for the deviation of the H-1 proton chemical shift in glucose derivatives. Therefore, an induced-fit type conformational change appears to be induced

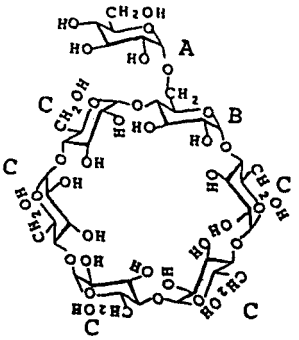


FIG. 13. Structure of 6-o-α-D-glucosyl α-CD(G_1-α-CD).

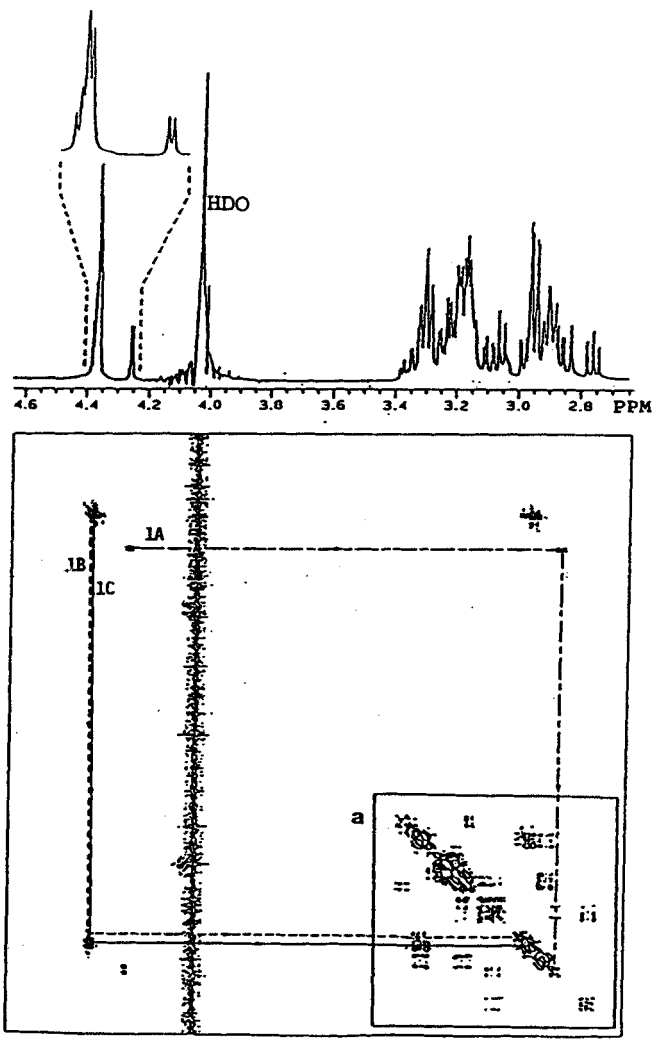


FIG. 14. Double-quantum filtered COSY spectrum of G_1-α-CD in D_2O at pD 7 and 30 °C. Region a is expanded in FIG. 15.

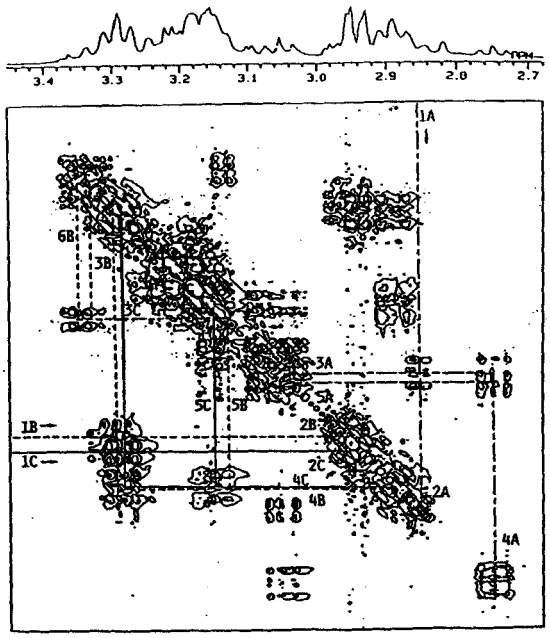


FIG. 15. Expanded spectrum of the region a in FIG. 14.

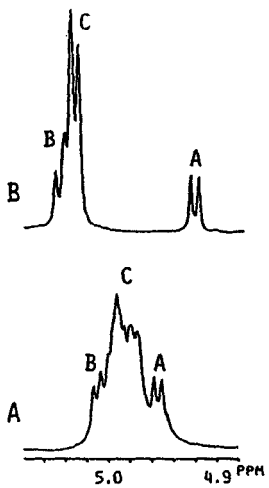


FIG. 16. Anomeric proton resonance regions of 500 MHz ^{1}H NMR spectra of G_1-α-CD in the presence(A) and the absence(B) of pNP. The degeneracy of H-1 proton resonances of the C units is removed upon complexation with the guest.

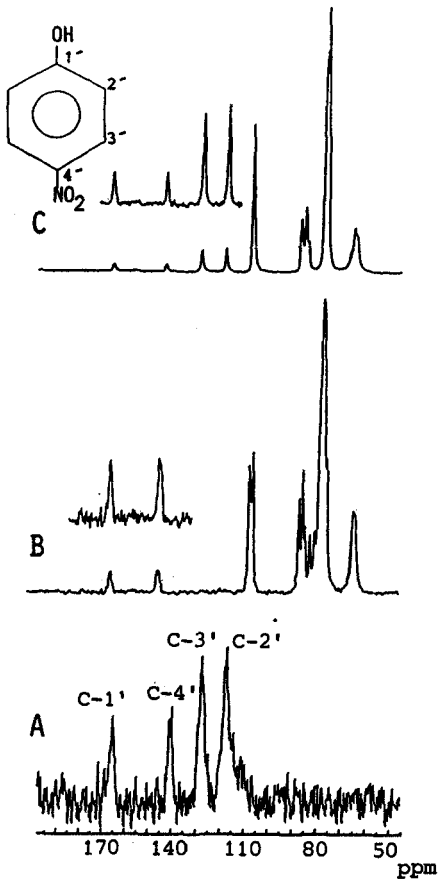


FIG. 17. CPMAS ^{13}C NMR spectra of pNP(A), α-CD-pNP(B), and β-CD-pNP(C) complexes. The C-2' and C-3' carbon resonances of pNP disappear completely in trace B, while they are clearly observed in trace C. The resonances at 60 ~ 100 ppm arise from CD.

on the cavity of G_1-α-CD upon complexation with pNP.[5] The binding studies indicate that the complexation ability of G_1-α-CD with pNP is almost the same as that of α-CD.[35]

VI. SOLID STATE NMR STUDIES OF CD INCLUSION COMPLEX

The characterization of CD inclusion complexes in the solid state is particularly important, as these complexes are utilized in drugs and foods in the form of solids. Cross-polarization magic-angle sample spinning (CPMAS) ^{13}C NMR is a powerful tool for this purpose. The resolution of

individual carbon chemical shifts not only makes CPMAS attractive as an analytical technique for solids but also provides a method for obtaining new information about solid-state structure and dynamics on the atomic level.[36-41] CPMAS ^{13}C NMR spectra of pNP, α-CD-pNP, and β-CD-pNP complexes are illustrated in FIG. 17. The striking result is the disappearance of the C-2' and C-3' carbon resonances of pNP in trace B. Those signals are clearly observed in trace C. The results indicate that the pNP molecule incorporated into the cavity of CD undergoes appreciable molecular motion. According to the theory of Rothwell and Waugh[42], line width of resonance reaches a maximun when the inverse of the correlation time(τ_c) for molecular motion is equal to the radio frequency of the proton decoupling field. Therefore, the disappearence of both C-2' and C-3' resonances due to the extensive broadening dictates the τ_c value of ~3×10^{-5} sec(at the magnetic field strength of 6.3 T) for the molecular tumbling of pNP in the cavity of α-CD.[39] On the other hand, the results from the dipolar-dephasing experiment of β-CD-pNP complex indicated τ_c ~7×10^{-7} sec for the guest.[41] The nature of the molecular dynamics of the guest molecule in its complexed state with CD depend on the host and detailed relaxation time studies will provide more quantitative information on the molecular motion of the CD-guest complex in the solid state.

VII. CONCLUSION

NMR is a powerful technique for studying molecular structure and dynamics of CD inclusion complexes not only in solution but also in the solid state. A combination of various 2D NMR experiments is almost essential for unambiguous signal assignment which forms a basis of NMR study. The complexation between CD and guest molecules leads to changes on various NMR parameters arising from both molecules. These results are interpretable, on the atomic level, in terms of CD-guest structural alteration induced upon complexation. The solution structure of the CD inclusion complexes with aromatic guests can be quantitatively determined solely by NMR. CPMAS NMR provides useful information about the structure and the dynamics of molecules in the solid state. Detailed relaxation studies should make possible quantitative analysis of the molecular dynamic coupling between CD and guest in the solid state.

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