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^{1}H-Nuclear Magnetic Resonance Study of 6-O-α-D-Glucopyranosyl α-Cyclodextrin - para-Nitrophenol Inclusion Complex

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6-O-α-D-Glucopyranosyl α-cyclodextrin - para-nitrophenol host-guest inclusion complex has been characterized by high resolution ^{1}H-NMR. Analysis on the NMR spectral parameters of the anomeric proton resonances of the host molecule has revealed that the asymmetric nature of 6-O-α-D-Glucopyranosyl α-cyclodextrin molecule is further enhanced by the complexation with para-nitrophenol, indicating an induced-fit type conformational change induced on the host upon the complexation.

6-O-α-D-Glucopyranosyl α-cyclodextrin (G_1-α-CD) is a branched α-cyclodextrin (α-CD) that is produced as a component via limited action of Bacillus macerance glucanotransferase on waxy corn starch and can be isolated from the other sugar derivatives produced and purified using procedures of Kobayashi et al.[1-2] As shown in Fig. 1, G_1-α-CD has one branching glucopyranose residue attaching to one of α-CD constituents through α(1-6) glucosidic linkage and due to this branch G_1-α-CD is more water-soluble and more resistant to enzymic degradation than the parent α-CD.[3] It is well-documented that CDs trap various substances as guests in their cavities to form inclusion complexes in solution and because of this property CD has received a considerable attention from a variety of field.[4-5] Although it is expected that G_1-α-CD forms inclusion complexes with guest molecules in solution in a manner similar to α-CD, neither the interaction between G_1-α-CD and guest molecule nor the structure of its inclusion complexes has been characterized in detail.

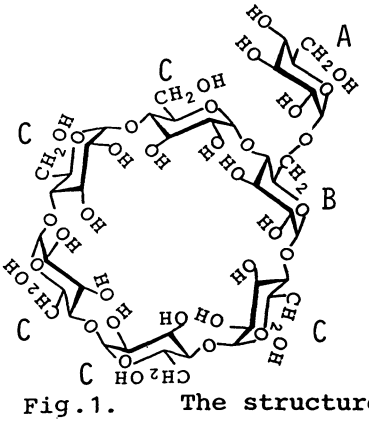

Fig.1. The structure of G_1-α-CD. The glucopyranose residues are divided into three different groups, A-C , on its NMR spectrum.

We therefore report herein on the results of 500MHz ^{1}H-NMR study of the interaction between G_1-α-CD and para-nitrophenol (pNP) molecule in aqueous solution which not only reveal that pNP is indeed inserted into the cavity of G_1-α-CD, but also indicate that a

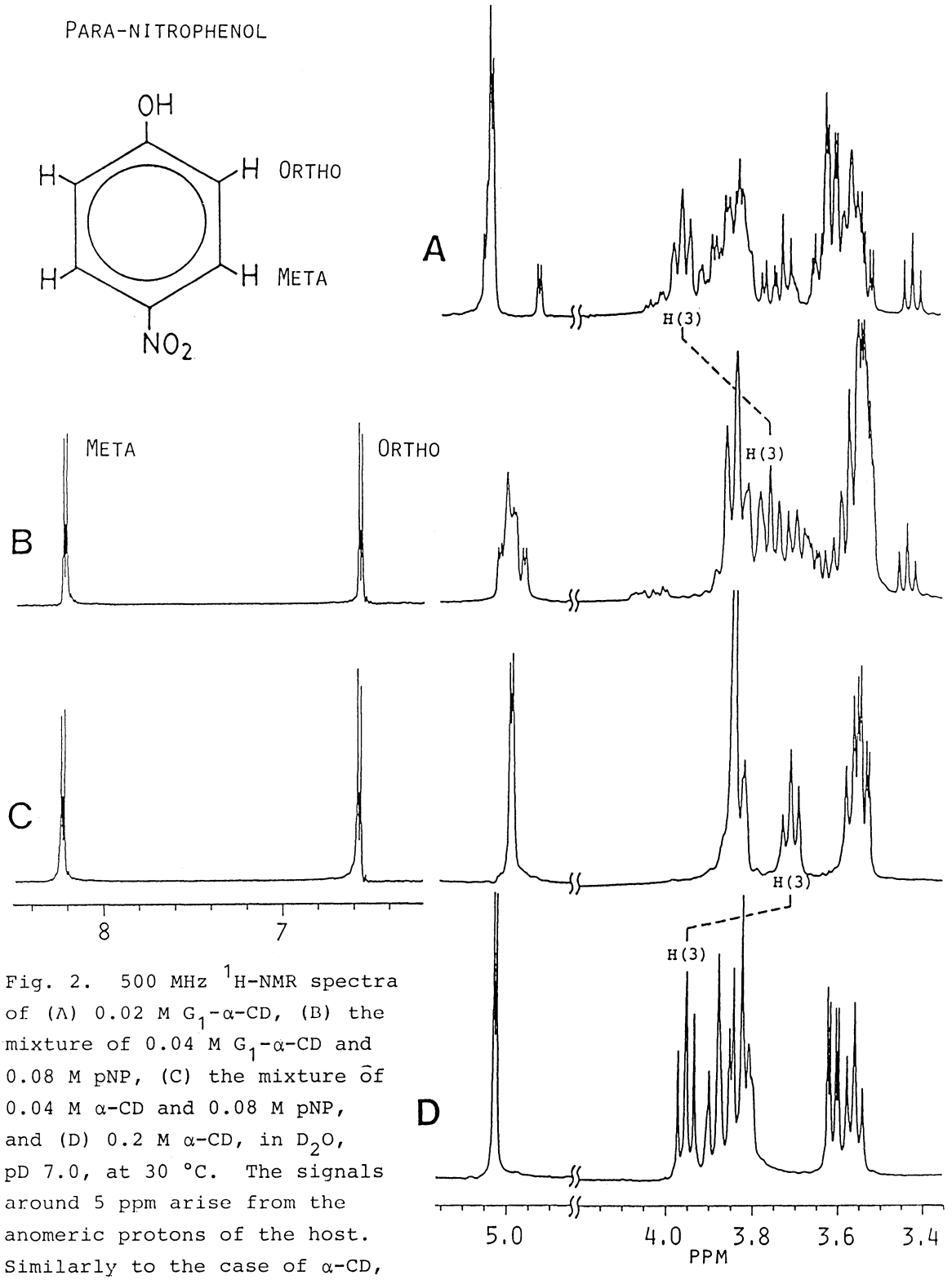

Fig. 2. 500 MHz ^{1}H-NMR spectra of (A) 0.02 M G_1-α-CD, (B) the mixture of 0.04 M G_1-α-CD and 0.08 M pNP, (C) the mixture of 0.04 M α-CD and 0.08 M pNP, and (D) 0.2 M α-CD, in D_2O, pD 7.0, at 30 °C. The signals around 5 ppm arise from the anomeric protons of the host. Similarly to the case of α-CD, the H(3) resonance of G_1-α-CD shifts toward upfield by the addition of pNP, indicating that pNP is inserted into the cavity of G_1-α-CD.

conformational change is induced on the macrocyclic ring structure of G_1-α-CD upon the complexation with pNP.

The 500 MHz ^{1}H-NMR spectra of G_1-α-CD and the mixture of G_1-α-CD and pNP in D_2O, pD 7.0, at 30 ℃ are illustrated in traces A and B of Fig.2, respectively, and are compared with those of α-CD and the mixture of α-CD and pNP in traces C and D, respectively. Due to the presence of the C_6 symmetry axis on α-CD molecule in solution, only single set of NMR signal pattern corresponding to the spin net-work of a glucopyranose constituent is observed[6](see trace D). Such symmetric property of α-CD is conserved even in the complex state with pNP owing to the fast averaging of the spatial orientation of pNP with respect to α-CD,[6-7] although the chemical shifts of α-CD resonances are influenced by the addition of pNP. A large upfield shift of H(3) resonance in trace C provides one of most direct evidence of complexation between α-CD and pNP (trace C).[8] On the other hand, in G_1-α-CD the symmetry-breaking constituent, unit A, perturbs the C_6 symmetry of α-CD, resulting in shifting the resonances of unit B relative to those of unit C's.[9] When two molar excess of pNP was added to G_1-α-CD, H(3) resonances of units B and C's shift toward upfield considerably due to the ring current effect of aromatic moiety of pNP, confirming that pNP is inserted into the cavity of G_1-α-CD. Only one set of the resonances arising from pNP is observed with G_1-α-CD (result not shown), indicating that the exchange process of pNP between free and complex states with G_1-α-CD is faster than NMR time scale. Effect of pNP on the spectral patterns of the anomeric proton resonances of G_1-α-CD is investigated in Fig.3 and the NMR spectral parameters obtained on those resonances are summarized in Table 1. The degeneracy of H(1) resonances of unit C's is removed by the complexation with pNP. Since the H(1) protons are oriented outside of the cavity, their chemical shift change may not be explained based on the aromatic ring current effect of pNP. In addition, the effects arising from the presence of both pNP and pNP^- at the pH value used here would be ruled out because the time scale of the reaction, $pNP \rightleftarrows pNP^- + H^+$, is so fast that only time-averaged signals from the host molecules interacting with pNP and pNP^- are observed in the NMR spectra. Therefore the dispersion of H(1) resonances of unit C's would be interpreted in terms of small differences in the conformation around the glucosidic bond among the α(1-4) linkages of G_1-α-CD in the complex state with pNP because the magnetic anisotropy effect arising from the lone-pair electrons of the glucosidic oxygen is known to account for the chemical shift of

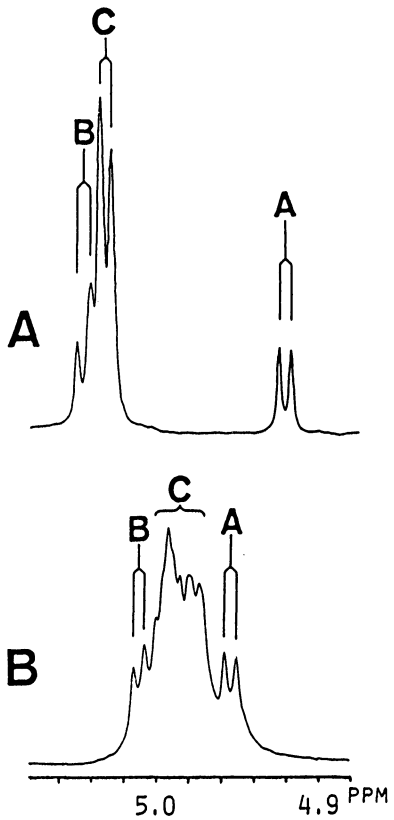

Fig. 3. Anomeric proton resonance regions of 500 MHz ^{1}H-NMR spectra of G_1-α-CD in the absence(A) and in the presence(B) of pNP.

the anomeric proton resonance in glucose derivatives.[10] In fact, the analysis of the coupling constant, J_{12}, revealed that an alternation of more than 10° in the dihedral angle (θ) defined in Table 1 is introduced on unit B upon the complexation. Such a conformational distortion energy may not localize at the particular site of the macrocyclic ring and so propagates along the chemical bonds. Due to the lack of C_6 symmetry on G_1-α-CD molecular structure, such conformational changes are not necessarily induced to the same extent among the α(1-4) glucosidic linkages of the macrocyclic ring.

Determination of more quantitative host-guest geometry in the system of G_1-α-CD and pNP inclusion complex in solution requires the measurements of dissociation constant (Kd)[11] and intermolecular nuclear Overhauser effect (NOE).[12] Such studies are planned.

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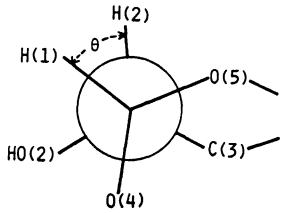
Table 1. ^1H Chemical shift (δ) and Coupling constant (J_{12}) of the anomeric proton resonances of G_1-α-CD in the absence and the presence of pNP

	Without pNP				With pNP			
Glucose Unit	A	B	C	α-CD	A	B	C	α-CD
δ /p.p.m.	4.92	5.04	5.03	5.03	4.95	5.01	4.98-5.00	4.99
J_{12} /Hz	3.66	4.77	3.29	3.42	3.48	3.48	3.11-3.67	3.36
θ [a]	46	36	49	48	47	47	51-46	49

a)Dihedral angle (in degree), θ, is defined as depicted in right and is calculated using the following Karplus type equation.[13]

$$^3J_{12} = (6.6 - 1.0\cos\theta + 5.6\cos 2\theta) * 0.36$$



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