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N.m.r. study on the formation and geometry of inclusion complexes of 6-*O*-(α-maltosyl)cyclomalto-hexaose and -heptaose with *p*-nitrophenol in aqueous solution

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ABSTRACT

The formation and molecular geometry of inclusion complexes of some branched cyclomaltaoses with *p*-nitrophenol in aqueous solution have been investigated by using high-resolution ^{1}H-n.m.r. spectroscopy. 6-*O*-(α-Maltosyl) cyclomalto-hexaose and -heptaose were found to form 1:1 inclusion complexes with *p*-nitrophenol, and the dissociation constants for their complexes are quite similar to those for corresponding unbranched cyclomaltaose–*p*-nitrophenol complexes, indicating that formation of these inclusion complexes is not hampered by the maltosyl branch. From measurement of nuclear Overhauser enhancements, it was concluded that the maltosyl branch is not situated over the entrance of the cavity.

INTRODUCTION

6-*O*-(α-Maltosyl)cyclomalto-hexaose (G_2-α-CD; Fig. 1) and -heptaose (G_2-β-CD) are branched cyclomaltaoses that consist, respectively, of six and seven (1→4)-α-D-glucopyranose residues linked to form macrocycles, together with one branching maltose residue attached to one of the residues of the macrocycle through a (1→6)-α-D-glucopyranosidic linkage. These compounds are of great interest as they have greater water solubility than the corresponding unbranched CDs, namely α- and β-CD[1–3]. It is well established that α- and β-CDs can trap various compounds as guests in their cavities to form inclusion complexes in solution[4–6]. On the basis of previous ^{1}H-n.m.r. studies[7], it was shown that 6-*O*-(α-D-glucopyranosyl)cyclomaltohexaose (G_1-α-CD) forms a 1:1 inclusion complex with *p*-nitrophenol (*p*NP) in aqueous solution, and the association constant for the complexation is quite similar to that for α-CD–*p*NP complexation, suggesting that G_1-α-CD and α-CD have similar abilities for complexation. Furthermore, it was found from measurements of the ^{1}H–^{1}H intramolecular nuclear Overhauser effects (n.O.e.) in the rotating frame that the glucopyranosyl branch of G_1-α-CD

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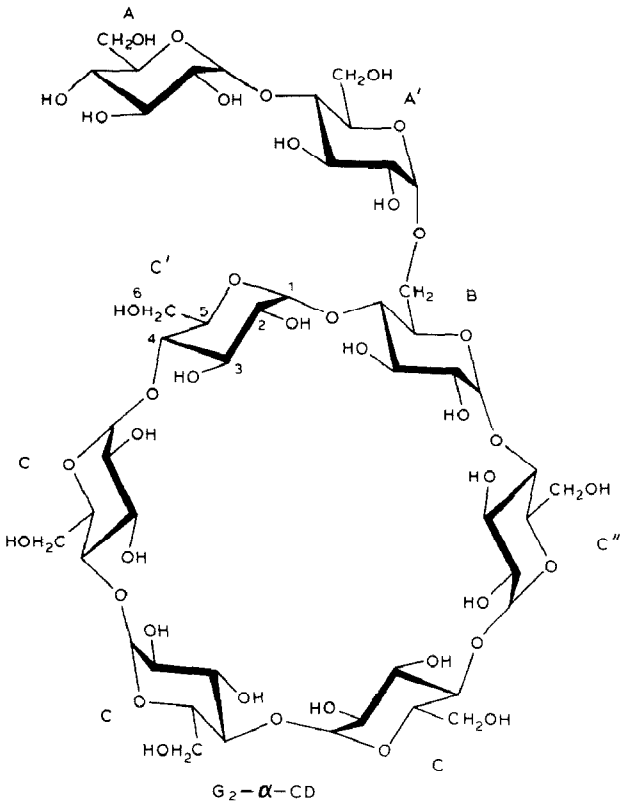

Fig. 1. The structure of G_2-α-CD. The D-glucopyranosyl residues are divided into six groups, A, A', B, C, C', and C'' based on its ^{1}H-n.m.r. spectrum.

is not situated over the entrance of the cavity[8], and consequently, the glucopyranosyl branch does not affect or alter the ability of α-CD to form an inclusion complex with *p*NP. The D-glucopyranosyl branch may be too short to alter the complexation ability of α-CD. Thus it is of interest to study the influence of longer sugar branches on the binding properties of CDs.

We report herein the results of a ^{1}H-n.m.r. study of the formation and the geometry of inclusion complexes of G_2-α-CD and G_2-β-CD with *p*NP in aqueous solution. This study was designed to investigate the effects of longer carbohydrate branches on the ability of α- and β-CDs to form inclusion complexes.

EXPERIMENTAL

Materials. — Branched CDs were prepared by the procedures of Kobayashi *et al.*[1,2]. The α- and β-CDs were a generous gift from Nihon Shokuhin Kako Co., Ltd, and were used after drying at 100° under high vacuum. *p*NP was a commercial, reagent-grade material and was purified by high-vacuum sublimation. Deuterium oxide, with an isotopic purity of 99.7%, used as solvent was purchased from Merck & Co.

Methods. — ^{1}H-N.m.r. spectra were recorded on a JEOL GX-500 spectrometer operated at 500 MHz in the quadrature mode. The two-dimensional (2D) ^{1}H-n.m.r. spectra were measured using the standard procedures described in the JEOL operations manuals, details of which can be found in previous papers[7,8]. A JEOL GSX-270 NMR spectrometer operated at 270 MHz for ^{1}H was also used for the determination of the dissociation constants for CD complexation. ^{1}H-N.m.r. chemical shifts are given in p.p.m. downfield from external Me_4Si.

RESULTS AND DISCUSSION

Dissociation constants for CD complexation. — The stoichiometry and the dissociation constant K_d for CD–*p*NP complexation were determined from changes in the ^{1}H.n.m.r. chemical shifts of *p*NP induced by complexation with CD. In these experiments, the concentration of *p*NP was fixed at 4.0×10^{-3}M while those of G_2-α- and -β-CD were changed from 2.0×10^{-3}M to 5.0×10^{-2}M. Since a single set of *p*NP resonances, which shifted downfield with increasing CD concentration, were observed for both G_2-CDs, the complexation reaction was reversible. The K_d value was derived by a least-squares fit of the modified Hildebrand–Benesi equation [9,10] (which was derived for 1:1 complexation) to the plot of ^{1}H chemical shifts *vs.* the [CD]/[*p*NP] molar ratio[7,10,11]. The values of K_d are given in Table I. The data for α-[7] and β-CD[11] and for G_1-α-CD[7] are also listed for comparison. For every α-CD–*p*NP system, the K_d value observed in alkaline solution was always smaller than that in neutral solution, indicating that the *p*NP anion was more prone to form a stable complex with CD. The K_d values for the G_2-α-CD–*p*NP complex at pD 7 and 10 were slightly larger than those of α-CD– and G_1-α-CD–*p*NP complexes at the corresponding pD values. The same trend was also found between β-CD– and G_2-β-CD–*p*NP complex systems. The K_d values suggest that both G_2-branched CDs and the corresponding unbranched CDs have almost the same ability to form complexes with *p*NP.

TABLE I

Dissociation constants (K_d) of some inclusion complexes of CDs with *p*NP determined by ^{1}H-n.m.r. spectroscopy

	pD 7	*pD 10*
	K_d *(10^{-3}M)*	K_d *(10^{-4}M)*
α-CD[a]	6.00 ±0.04	6.10
G_1-α-CD[a]	4.65 ±0.03	5.07 ±0.11
G_2-α-CD	7.94 ±0.49	8.66 ±0.29
	K_d *(10^{-3}M)*	K_d *(10^{-3}M)*
β-CD[b]	5.4	1.5
G_2-β-CD		3.64 ±0.13

[a] Data taken from ref. 7. [b] Data taken from ref. 11.

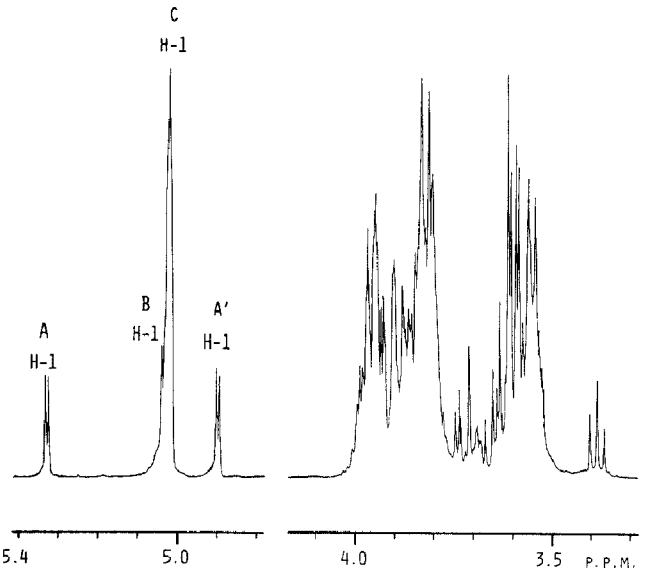

Fig. 2. 500 MHz ^{1}H-n.m.r. spectrum of G_2-α-CD in D_2O at pD 7 and 30°. The assignments are shown for the signals that arise from the anomeric protons.

Assignments of ^{1}H resonances of CDs. — The changes in the ^{1}H chemical shifts of CD induced by complexation with *p*NP provide information on the host–guest geometry in the CD–*p*NP complex[11,12]. However, full assignments for the ^{1}H-n.m.r. spectra of CDs in both the free and the complexed state are required. The branched CDs gave severely overlapped ^{1}H-n.m.r. spectra, which are more complex than those of unbranched CDs, even when observed at 500 MHz. As an example, Fig. 2 shows the 500 MHz, one-dimensional (1D) ^{1}H-n.m.r. spectrum of G_2-α-CD. These complexities are due to the structural and magnetic nonequivalence of the D-glucopyranosyl residues that form the branched CDs. In the case of both α- and β-CDs, a single set of n.m.r. resonances was observed as if there was only one type of D-glucopyranosyl residue present, even in the guest-included state[11,12]. This was because: (*i*) in either CD the six or seven D-glucopyranosyl residues are equivalent due to the presence of an apparent symmetry axis: and (*ii*) there is rapid intramolecular motion in solution. In contrast, in the case of branched CDs, the D-glucopyranosyl residues which form the macrocyclic CD ring are nonequivalent because of the symmetry-breaking moiety, *i.e.*, the branch. According to the ^{1}H-n.m.r. spectral assignments (described below) the 500 MHz ^{1}H-n.m.r. resonances of maltosyl-branched CDs were divided into at least six independent sets which were assigned to the D-glucopyranosyl residues: A and A′ in the branch; B at the branching site on the macrocycle; C′ and C″ next to residue B; and others denoted by C, as shown, for example, in Fig. 1.

The combined use of some 1D- and 2D-n.m.r. techniques makes it possible to assign almost all the resonances that appear in the ^{1}H-n.m.r. spectra of branched CDs in the free and complexed states. Two-dimensional n.O.e. measurements under spin-

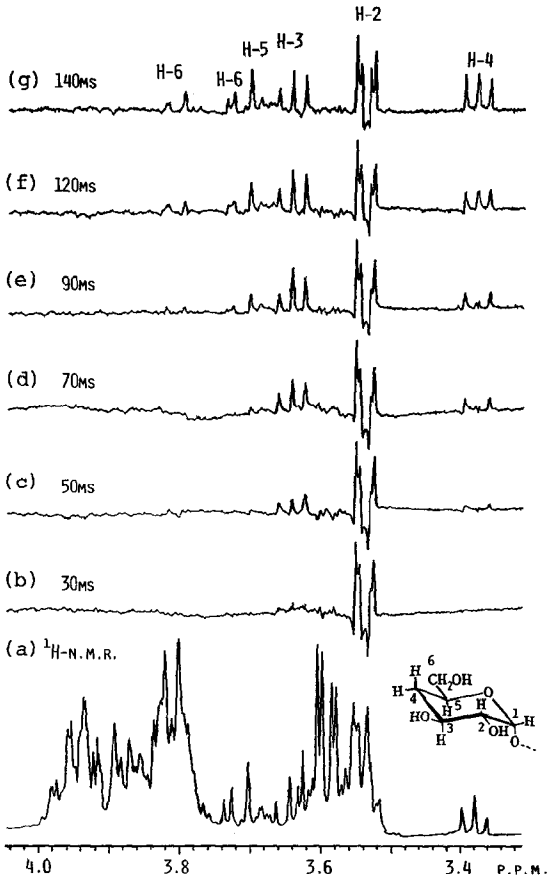

Fig. 3. A part of the 500 MHz ^{1}H-n.m.r. spectrum (a) and corresponding HOHAHA spectra (b–g) of G_2-α-CD in D_2O at pD 7 and 40°. In the HOHAHA spectra, the anomeric proton of residue A was selectively irradiated with different spin-lock times as shown on the left-hand side of each spectrum.

locked conditions (ROESY[13–15]) and HOHAHA[16] are especially useful[17]. By using the HOHAHA technique, it is possible to extract each set of resonances for protons belonging to the same D-glucopyranosyl residue. In the 1D-HOHAHA experiment, by changing the mixing time[16,18], the degree of magnetization transfer from a selectively irradiated proton to other protons can be controlled. An example is shown in Fig. 3, where H-1 resonating at 5.358 p.p.m. (see Fig. 2) was selectively excited. At the shortest mixing time, only the resonance of the proton nearest to H-1 (*i.e.*, H-2) should be observed. As the mixing time increases the resonances of the more distant protons appear (*i.e.*, H-3, H-4, H-5, and finally H-6). In this way, a set of resonances for protons of the whole D-glucopyranosyl residue (the so-called spin-network) can be assigned. These are given in Fig. 3. In this example, the resonances that appeared at 3.424 and at 5.358 p.p.m. were readily identified as those of H-4 and H-1 of the A-group D-glucopyranosyl residue, respectively. This is situated at the free end of the branch and is chemically very different from the other residues.

The 2D-ROESY (NOESY) spectrum provides information on sequence relations between the spin-networks. For example, the distance between H-1 of one D-glucopyranosyl residue and H-4 of its neighbouring residue across the common α-(1→4) glucosidic linkage is short enough to cause a detectable level of through-space n.O.e. enhancement. This allows the sequence-specific relation, between one residue and the neighbouring residue in the CD macrocycle and the maltosyl branch, to be assigned. On the basis of HOHAHA and n.O.e. information, ^{1}H resonances were sequence-specifically assigned. The chemical shift data are listed in Tables II–V for G_2-α-CD and G_2-β-CD in both the free and the complexed states. For comparison, the data for G_1-α-CD and G_1-α-CD–*p*NP system are also shown in Tables VI and VII.

Host–guest geometry. — In the case of G_1- and G_2-α-CD, upon complexation with *p*NP, the H-3 resonances of the D-glucopyranosyl residues forming the CD macrocycle, *i.e.*, the residues B, C, C′, and C″, show remarkably large upfield shifts that amount to about 0.3 p.p.m., whereas the complexation-induced chemical shift changes of other

TABLE II

^{1}H-N.m.r. chemical shift data[a] for G_2-α-CD observed in 0.02M solution in D_2O at 40° and pD 7

D-*Glucopyranose residue*	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6a*	*H-6b*
A	5.358	3.587	3.689	3.424	3.720[b]	3.855	3.760
A′	4.937	3.590	4.004	3.647	3.850	3.880	3.820
B	5.059	3.663	3.986	3.609	4.030	3.870	4.000
C′	5.069	3.637	3.983	3.580	3.860	3.900	3.860
C″	5.055	3.637	3.988	3.599	3.860	3.940	3.940
C	5.055	3.637	3.982	3.595	3.857	3.930	3.970
			3.977	3.584			
			3.979				

[a] Chemical shift in p.p.m. downfield from external Me_4Si. [b] Approximate chemical shift.

TABLE III

^{1}H-N.m.r. chemical shift data[a] for G_2-α-CD complexed with *p*NP observed in 0.02M G_2-α-CD–0.08M *p*NP solution in D_2O at 40° and pD 7.

D-*Glucopyranose residue*	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6a*	*H-6b*
A	5.374	3.609	3.708	3.443	3.762	3.780[b]	3.867
A′	4.983	3.609	4.032	3.671	3.589		
B	5.036	3.643	3.701	3.600	4.005	3.882	4.005
C′	5.058	3.620	3.715	3.605	3.849	3.893[c]	
C″	5.051	3.620	3.706	3.623	3.840	3.947	3.946
C_1	5.042	3.617	3.693	3.598	3.824	3.89	
C_2	5.040	3.617	3.719	3.601	3.847	3.89	
C_3	5.035	3.617	3.691	3.598	3.810	3.88	

[a] Chemical shift in p.p.m. downfield from external Me_4Si. [b] Approximate chemical shift. [c] Value indicates average chemical shift of H-6a and H-6b.

TABLE IV

^{1}H-N.m.r. chemical shift data[a] for G_2-β-CD observed in 0.02M solution in D_2O at 40° and pD 7

D-*Glucopyranose residue*	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6a*	*H-6b*
A	5.369[b]	3.606	3.708	3.445	3.746	3.875	3.781
A′	4.962	3.615	4.027	3.661	3.900[b]	3.883	3.836
B	5.092	3.701	3.991	3.630	4.060	3.880	4.020
C′	5.100	3.676	3.982	3.585	3.880	3.900[c]	
C″	5.100	3.679	3.988	3.608	3.880	3.950	3.950
C	5.092	3.676	3.982	3.603	3.880	3.900[c]	
				3.601			

[a] Chemical shift in p.p.m. downfield from external Me_4Si. [b] Approximate chemical shift. [c] Value indicates average chemical shift of H-6a and H-6b.

TABLE V

^{1}H-N.m.r. chemical shift data[a] for G_2-β-CD complexed with *p*NP observed in 0.02M G_2-β-CD–0.08M *p*NP solution in D_2O at 40° and pD 7

D-*Glucopyranose residue*	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6a*	*H-6b*
A	5.369	3.603	3.704	3.400	3.740[b]	3.871	3.777
A′	4.962	3.608	4.025	3.658	3.870	3.836	3.890
B	5.056	3.673	3.848	3.612	3.910	3.879	4.012
C′	5.073	3.647	3.847	3.571	3.710	3.870[c]	
C″	5.065	3.653	3.855	3.590	3.740	3.923	3.923
C	5.056	3.647	3.847	3.590	3.708	3.870[c]	
				3.584			
				3.587			

[a] Chemical shift in p.p.m. downfield external Me_4Si. [b] Approximate chemical shift. [c] Value indicates average chemical shift of H-6a and H-6b.

TABLE VI

^{1}H-N.m.r. chemical shift data[a] for G_1-α-CD observed in 0.04M solution in D_2O at 40° and pD 7

D-*Glucopyranose residue*	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6a*	*H-6b*
A	4.951	3.555	3.753	3.447	3.730	3.781	3.861
B	5.061	3.673	3.955	3.646	4.018	3.840	4.052
C′	5.073	3.643	3.990	3.591	3.870[b]	3.915	3.850
C	5.061	3.643	3.987	3.588	3.855	3.920	3.860
					~ 3.870		
C	5.061	3.643	3.990	3.580	3.870	3.944	3.870

[a] Chemical shift in p.p.m. downfield from external Me_4Si. [b] Approximate chemical shift.

TABLE VII

^{1}H-N.m.r. chemical shift data[a] for G_1-α-CD complexed with *p*NP observed in 0.04M G_1-α-CD–0.08M *p*NP solution in D_2O at 40° and pD 7

D-*Glucopyranose residue*	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6a*	*H-6b*
A	4.990	3.567	3.772	3.470	3.750[b]	3.850[b]	3.810[b]
B	5.033	3.617	3.796	3.639	4.093	3.834	4.044
C′	5.046	3.576	3.736	3.572	3.862	3.870[c]	
C_1	5.031	3.579	3.739	3.590	3.862	~3.88	
C_2	5.027	3.581	3.794	3.588	3.907	3.890[c]	
C_3	5.016	3.567	3.714	3.588	3.837	3.870[c]	
C_4	5.014	3.567	3.707	3.581	3.814	~3.88	

[a] Chemical shift in p.p.m. downfield from external Me_4Si. [b] Approximate chemical shift. [c] Value indicates average chemical shift of H-6a and H-6b.

protons are less than 0.07 p.p.m. Since the H-3 and H-5 protons of macrocyclic residues are located inside the cavity, their resonances are most likely to be influenced by the ring-current effect of the aromatic guest compound that is trapped inside the cavity. The magnitudes of *p*NP-induced chemical shift changes of branched α-CDs are comparable to those observed for α-CD, indicating that G_1- as well as G_2-α-CD form inclusion complexes with *p*NP having the same geometry as observed in the α-CD–*p*NP complex[11], namely, the phenyl moiety of *p*NP is included into the CD cavity from the end of the CD having secondary hydroxyl groups, with the nitro group of *p*NP at the front. The

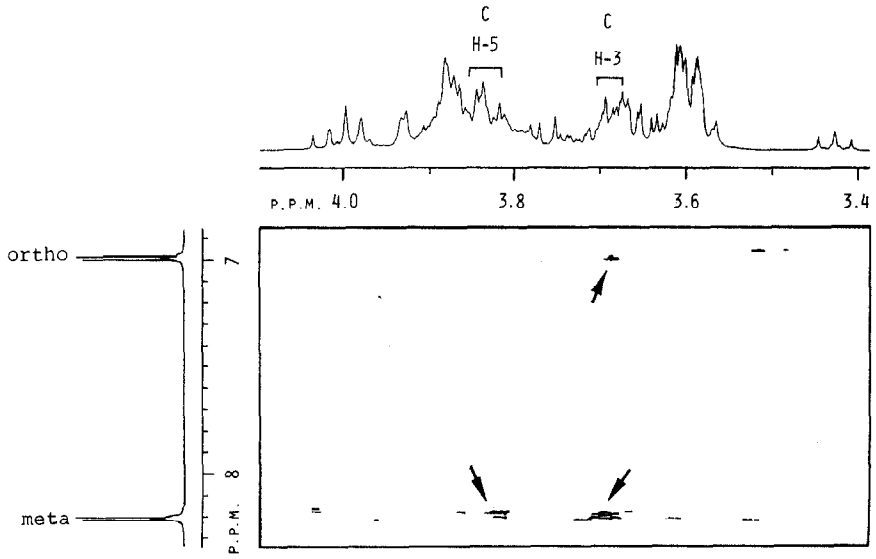

Fig. 4. A part of 500 MHz ROESY spectrum of the G_2-α-CD–*p*NP complex observed in D_2O at pD 7 and 40° with a spin-lock time of 250 ms. The arrows indicate the cross-peaks connecting the resonances of the H-3 and H-5 protons of G_2-α-CD to those of the *meta* and *ortho* protons of *p*NP.

depth of the *p*NP penetration into the α- and branched-α-CD cavity is rather small, with the center of the *p*NP phenyl ring on or near the plane formed from the six H-3 protons.

In the case of G_2-β-CD, the resonances of the H-5 and the H-3 protons of the macrocyclic D-glucopyranosyl residues also show relatively large *p*NP-induced chemical shift changes as compared to the other protons, indicating that the *p*NP is deeper in the cavity. The *p*NP phenyl ring may be situated approximately half-way between the planes of the H-3 and H-5 protons. This host–guest geometry is almost the same as that found for the β-CD–*p*NP complex[11,12].

The host–guest geometries deduced from the complexation-induced ^{1}H-m.n.r. chemical shift changes are supported by observation of the host–guest intermolecular ^{1}H homonuclear n.O.e. by using the 2D mode under spin-locked conditions, *i.e.*, the ROESY technique[8]. For example, Fig. 4 shows part of the ROESY spectrum of a G_2-α-CD–*p*NP mixture. This indicates clearly the cross-peaks connecting the H-3 resonances of both the B and C units to both the *meta* and *ortho* resonances of *p*NP and those connecting the H-5 peaks of the same units to only the *meta* resonance of *p*NP.

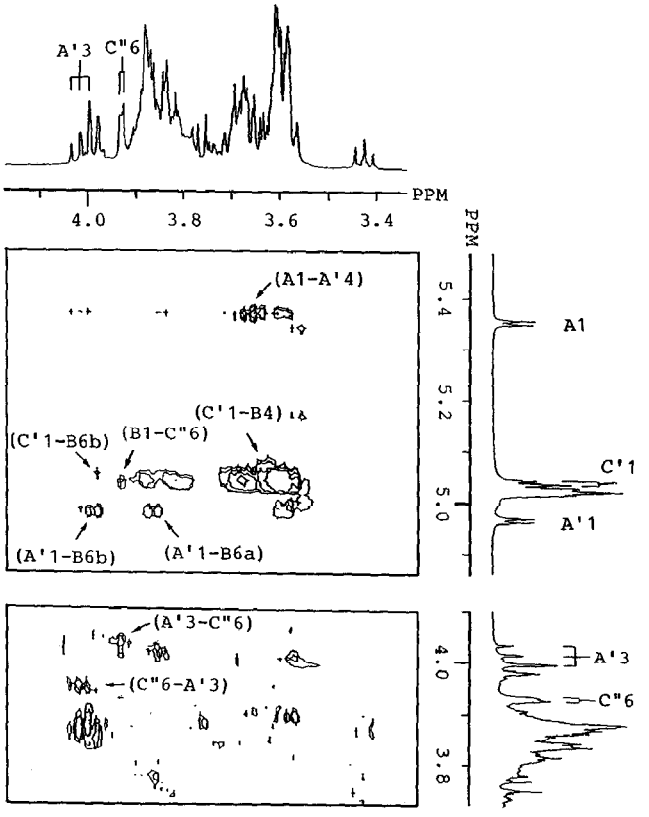

Fig. 5. A part of the 500 MHz ROESY spectrum of G_2-α-CD–*p*NP complex observed in D_2O at pD 7 and 40° with a spin-lock time of 250 ms. For simplification, some abbreviations have been introduced. For example, the resonance of H-1 of the D-glucopyranosyl unit A is depicted as A1, and the n.O.e. cross-peak between H-1 of A' unit and H-6a of the B unit is A'1–B6a, *etc.*

This cross-peak pattern is quite similar to those observed in the ROESY spectra of α-CD–*p*NP and G_1-α-CD–*p*NP complexes[8]. Therefore, the host–guest geometry in the G_2-α-CD–*p*NP complex appears to be similar to those observed in both the α-CD- and G_1-α-CD–*p*NP complexes, *i.e.*, in these complexes *p*NP is preferentially inserted into the CD cavity with the nitro group first, with the *meta* protons located close to both the H-3 and H-5 protons of the D-glucopyranosyl residues that form the macrocyclic CD ring. Therefore, they exhibit intermolecular n.O.e. cross-peaks with both CD protons.

Conformation of branched CDs. — The determination of the orientation of the branch with respect to the CD macrocycle is important in order to understand the influence of a maltosyl branch on the ability of the CD to form inclusion complexes. Information concerning the conformation of the branched CD molecule can be obtained from the ROESY spectrum. Fig. 5 shows another part of the ROESY spectrum of the G_2-α-CD–*p*NP mixture. In addition to the cross-peaks connecting the resonances of H-1 and H-4 located at the ends of the same D-glucosidic linkage (used for the sequence-specific assignments of the ^{1}H-n.m.r. spectrum), some intramolecular n.O.e. cross-peaks connecting the resonances which arise from the protons of both the branch and the macrocycle are also identified. The presence of two strong cross-peaks H-1(A′ unit)–H-6a (B unit) (simply shown as A′1–B6a and so on for simplicity in Fig. 5) and H-1(A′ unit)–H-6b(B unit) with nearly equal intensities clearly suggests that the preferential conformation about the C-5–C-6 linkage of the B unit is the *gauche–trans*(*gt*) rotamer. The preference of the *gt* conformation about this linkage is also suggested by the appearance of the H-3(A′ unit)–H-6(C″ unit). The through-space proton–proton relationships producing the n.O.e. cross-peaks are schematically shown in Fig. 6. According to the molecular model, the H-3 proton of the A′ unit can come close to two

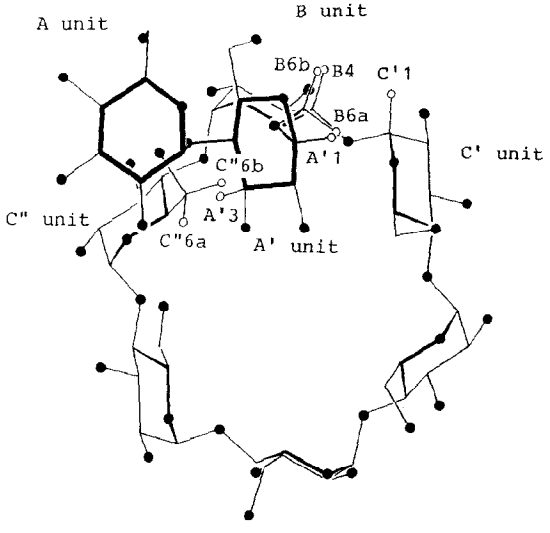

Fig. 6. Illustration indicating the spatial relationship between the maltosyl branch and part of the macrocycle of G_2-α-CD. Abbreviations used in Fig. 5 are also used here.

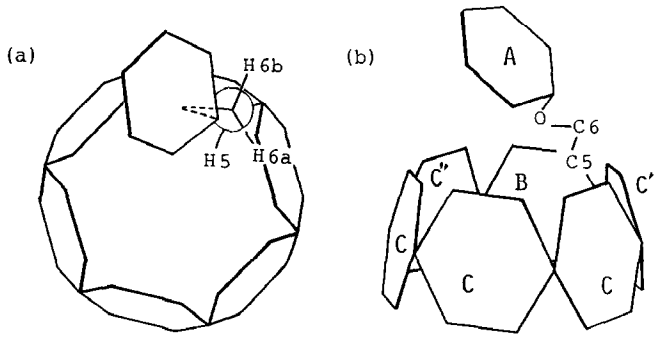

Fig. 7. Illustrations of the molecular geometry of G_1-α-CD expected from n.O.e. data[8]: (a) top and (b) side views. Each hexagon represents a D-glucopyranosyl residue. The C-5–C-6 linkage of the B unit is depicted as the *gt* rotamer.

H-6 protons of the C″ unit when the C-5–C-6 linkage of the B unit takes the *gt* conformation. A similar conformational preference of the C-5–C-6 linkage of the B unit is also suggested for G_2-α-CD in the free state, and G_2-β-CD in the free and complexed states, as quite similar H-1(A′ unit)–H-6(B unit) cross-peaks were also clearly demonstrated in their ROESY spectra.

The conformation about this linkage predominantly determines the orientation of the maltosyl branch with respect to the CD macrocyclic ring. In a previous paper[8], a ROESY experiment suggested for the G_1-α-CD–*p*NP system that the C-5–C-6 linkage of the B unit also prefers the *gt* conformation. This conformation is very consistent with that established by single-crystal X-ray analysis of G_1-α-CD · $8H_2O$ (ref. 19) where the C-5–C-6 linkage of the B unit takes the *gt* conformation and the branch D-glucopyranosyl residue A is located above the B unit and is not situated over the entrance of the CD cavity as shown schematically in Fig. 7. The ROESY results clearly indicate that the first D-glucopyranosyl unit of the maltosyl branch of G_2-α-CD, as well as that of G_2-β-CD, orients preferentially with respect to the B unit in a way similar to the A unit of G_1-α-CD. In this orientation, the free-end A D-glucopyranosyl unit of the maltosyl branch of G_2-α-CD, as well as that of G_2-β-CD, is situated far away from the entrance to the CD cavity. Any cross-peaks, connecting the resonances of protons of the A unit to those of the CD macrocycle, were not found in the ROESY spectra of G_2-α-CD and G_2-β-CD in the free and complexed state. Thus, the maltosyl branch tends to orient, not toward the CD cavity, but toward the aqueous surroundings. This preferential orientation well explains the experimentally observed roles of the hydrophilic maltosyl branch, that is, it improves the solubility in water of the CD molecule without significant modification of the ability of the CD to form inclusion complexes with a guest *p*NP molecule.

In conclusion, it was confirmed that the ability to form inclusion complexes of maltosyl-branched CDs is quite similar to that of unbranched CDs.

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