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^{1}H NMR spectra of branched-chain cyclomaltohexaoses (α-cyclodextrins)

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Abstract—The ^{1}H NMR spectra of seven branched α-cyclodextrins (α-CDs) were observed and analyzed in detail. They were compared with spectra of α-CD and amylose. Although these branched α-CDs consist only of α-D-glucose with the same α-(1→4) *O*-glucosyl binding, aside from one exception, differences in chemical shifts of corresponding signals were significantly large. Especially, differences in the chemical shift in anomeric protons were considerably large. Subtle differences in glucosyl binding directly influences chemical shifts of these protons because anomeric protons are located adjacent to the glucosyl binding sites.

Keywords: Branched cyclodextrin; α-Cyclodextrin; ^{1}H NMR; Chemical shift

1. Introduction

Saccharide chains in membrane proteins and lipids are considered to be responsible for molecular interactions with other proteins in hydrogen bonding or weaker bonding such as CH–aryl interactions. ^{1}H NMR spectroscopy is used widely to clarify these molecular structures and molecular interactions in solution because all hydrogens can be detected individually in the spectra. Changes in hydrogen signals in an interacting molecule can be detected in a single ^{1}H NMR spectrum. To analyze the structures of saccharide molecules and their molecular interactions with other molecules, for example, protein or peptide molecules, signal assignments are necessary in the first stages of the study. ^{1}H signal assignments are absolutely essential for analyzing NOE correlational peaks, which are very important for the structural analysis of saccharide molecules and the interaction with other molecules. However, it is very difficult to completely assign proton signals because saccharide molecules show severely overlapped signals in a limited range in the ^{1}H NMR spectra. Accordingly, only isolated signals for the methyl protons in acetyl groups in saccharide molecules were used for analysis of interaction with proteins.[1] There have been rare cases in which ^{1}H NMR signals of saccharide molecules were definitely used in analyses of molecular interactions.[2,3] Generally, accumulation of information about chemical shifts of ^{1}H NMR signals of saccharide molecules makes it possible to determine the structure of unknown saccharide molecules by determining the number of pyranosyl residues, the substitution pattern of the pyranosyl residues, and linkage positions to the next pyranose residues.

This study is intended to assign proton signals of saccharide molecules composed intensively of α-D-glucose. A series of branched α-cyclodextrins (α-CDs) were chosen as examples. In these molecules, a branch consists of α-D-glucose with an α-(1→4) glucosyl linkage; it is substituted to a hydroxyl group at a C-6 carbon of a glucose residue in α-CD with α-(1→6) linkage. This molecule consists entirely of α-D-glucoses with the same α-(1→4) *O*-glucosyl binding aside from one exception of α-(1→6) binding. We specifically addressed whether or not the ^{1}H NMR spectra of the substituted α-CD by the glucose derivatives are similar to ^{1}H NMR spectrum

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of α-CD. ^{13}C NMR spectra have been used for characterization of positional isomers of doubly and triply branched CDs with substitution by glucose,[4–7] maltose,[8,9] and longer glucosyl chains.[10] Overlapped signals were observed with core CD signals in the ^{13}C NMR spectra of those substituted CDs, and assignments of branch ^{13}C signals had been restrictedly examined. Though many branched CDs have been synthesized, there have been few reports on their ^{1}H NMR spectra. Although some studies to date have focused on ^{1}H NMR spectra using a 1D HOHAHA experiment to assign signals of branched α-CD[11] and inclusion phenomena of G1-α-CD with NMR spectroscopy,[12–14] no detailed analysis about the core CD signals has been reported.

This study performs detailed analysis of the complicated overlapped signals of the core CD ring and the branches in several CDs substituted by glucose chains.

2. Experimental

Seven branched α-CDs were used as samples: 6-*O*-α-glucopyranosyl-α-CD (G1-α-CD), 6-*O*-α-maltopyranosyl-α-CD (G2-α-CD), 6-*O*-α-maltotriosyl-α-CD (G3-α-CD), 6-*O*-α-maltotetraosyl-α-CD (G4-α-CD), 6-*O*-α-maltopentaosyl-α-CD (G5-α-CD), 6-*O*-α-maltohexaosyl-α-CD (G6-α-CD), and 6-*O*-α-maltoheptaosyl-α-CD (G7-α-CD). Nikken Chemicals Co., Ltd. (Omiya, Japan) and Ensuiko Sugar Refining Co., Ltd. (Yokohama, Japan) supplied branched α-CDs.[15,16] Commercially available α-CD and amylose were used without further purification for comparison of ^{1}H NMR spectra.

An ECA800 spectrometer (800.14 MHz for ^{1}H nucleus; JEOL Ltd.) recorded NMR spectra at 298 K in D_2O. Sample concentrations were about 10 mM, and the HOD signal at 4.785 ppm was used as the reference. Analysis used TOCSY, NOESY, and HMQC spectra. Glucose residues in the branched α-CDs were defined as shown in Figure 1 prior to assignment of signals.

3. Results and discussion

3.1. Overview of ^{1}H NMR spectra

^{1}H NMR spectra of seven branched α-CDs are shown in Figure 2b–h; they are compared with those of α-CD (Fig. 2a) and amylose (Fig. 2i). Introducing the branch saccharide to the α-CD, signals of the core CD were divided to split signals near the original position. Branch signals seemed to shift to a different range from the original α-CD. These changes were observed particularly with anomeric protons, as shown in Figure 2.

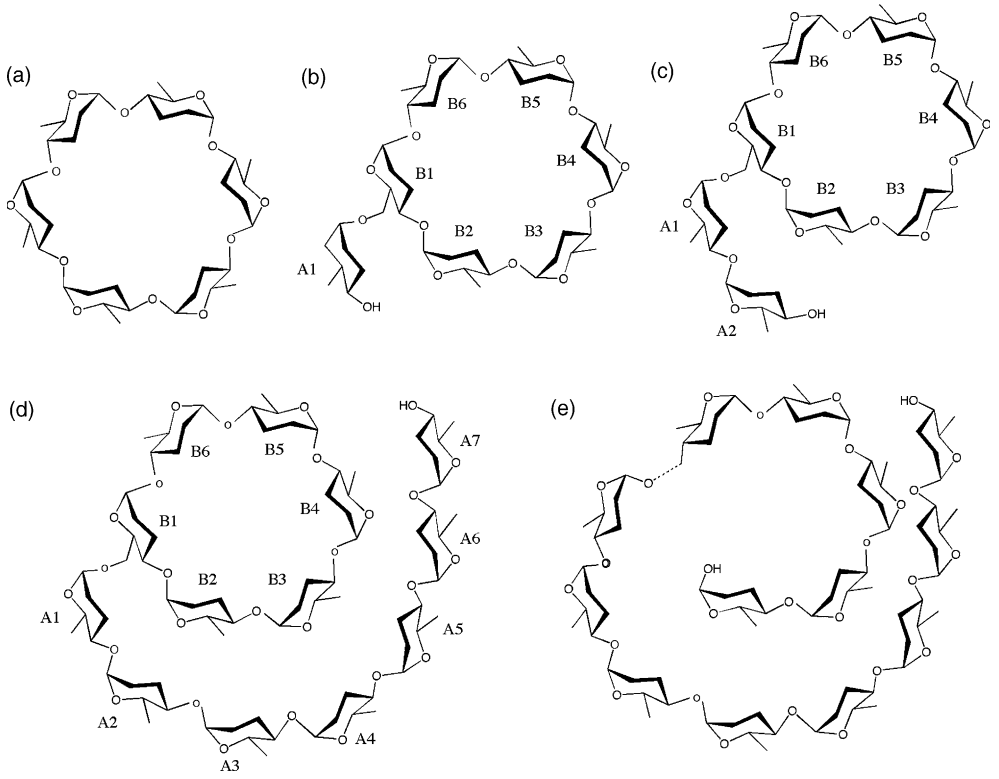

Figure 1. Branched α-CDs. (a) α-CD, (b) G1-α-CD, (c) G2-α-CD, (d) G7-α-CD, and (e) amylose. Similar definition of residues was applied for G3-, G4-, G5-, and G6-α-CD.

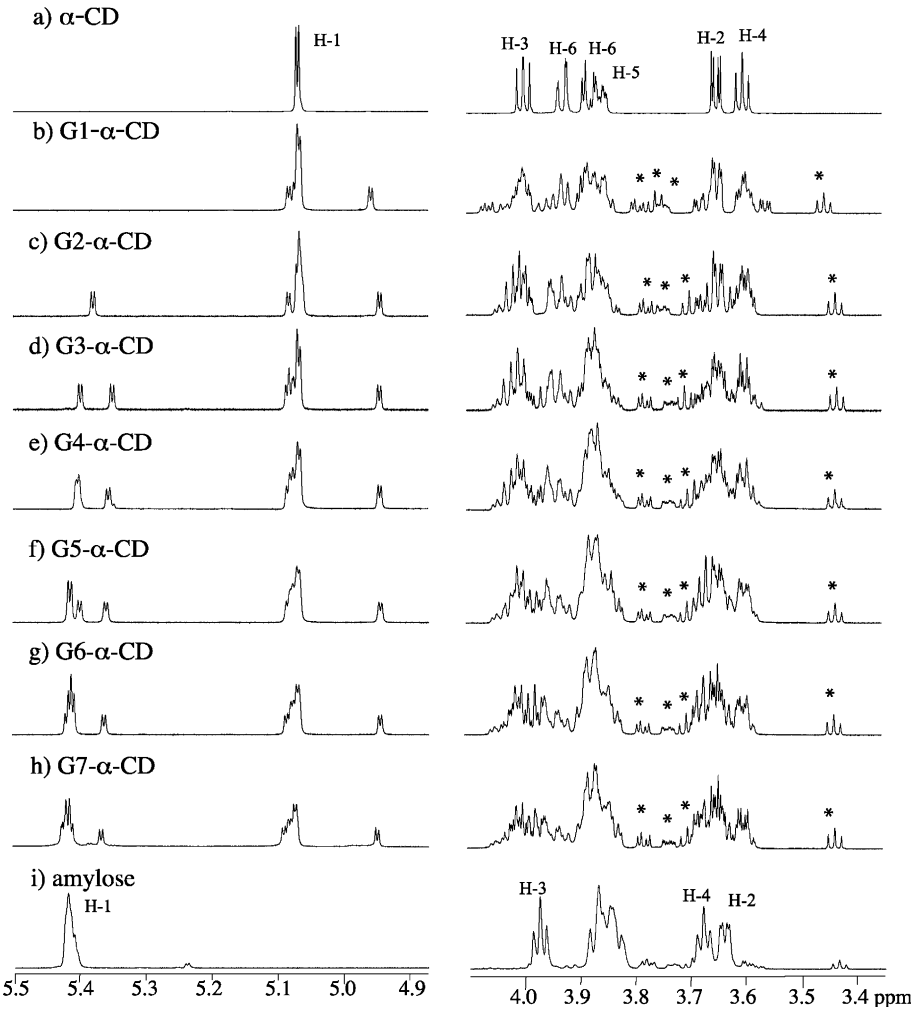

Figure 2. [1]H NMR spectra of branched α-CDs, α-CD, and amylose. (a) α-CD, (b) G1-α-CD, (c) G2-α-CD, (d) G3-α-CD, (e) G4-α-CD, (f) G5-α-CD, (g) G6-α-CD, (h) G7-α-CD, and (i) amylose. Marked signals (*) are H-6, H-5, H-3, and H-4 protons in the terminal residue in the branch accounting from low field, respectively.

Anomeric protons of the branched α-CD were observed in the range from 5.5 to 4.9 ppm. In G1-, G2-, and G3-α-CD, anomeric protons in branched residues were detected as isolated signals (5.4–4.9 ppm). In G4-, G5-, G6-, and G7-α-CD, several anomeric protons of branch moieties overlapped at lower field around 5.4 ppm. The signal at lower field increased as the number of glucose chains increased; consequently increasing signals coincided finally with the signal of the anomeric proton of amylose. Detailed analysis of anomeric protons by 2D spectra was made as the following. The H-4 signal of amylose was detected at lower field than the H-4 signal of α-CD, as shown in Figure 2. By analogy for the anomeric proton of the branch moiety, H-4 signals of the branch part, apart from the core CD in branched α-CDs, were expected to shift to low field with the elongation of the branch. On the other hand, the H-2, H-3, H-5, and H-6 signals of amylose remained in almost the same range as the corresponding signals of α-CD. These H-2, H-3, H-5, and H-6 signals of the branch appeared to show small changes by elongation of the branch. There were several small signals detected that were isolated from the large signals of the core CD in the region of 4.1–3.4 ppm. Among these signals, those marked (*) were commonly observed in all seven branched α-CDs. The signals were assigned characteristically to signals of the terminal glucose residue in the branch, as described below.

3.2. Anomeric protons

As for anomeric protons of the branch, isolated signals were detected with G1-, G2-, and G3-α-CD. For longer branches than G3-α-CD, overlap of the signals was observed in the lowest field. Assignments of these anomeric protons in the branch part were performed by correlation to ^{13}C signals.[11] Assignments of anomeric carbons in the branch were performed by analysis of the

relaxation time (T_1) using the conventional inversion recovery method. As reported previously,[11] longer relaxation time (T_1) was observed for the anomeric carbon in the terminal residue of the branch, reflecting larger mobility of the corresponding glucose ring. On the other hand, shorter T_1's were observed for anomeric carbons in the pyranose next to the core CD ring, reflecting less mobility between the core CD and remaining glucoses in the branch. Taking account of the results about relaxation times of the anomeric carbons,[11] anomeric protons in the branch were assigned in the corresponding HMQC spectrum. As one example, the HMQC spectrum of G5-α-CD is shown in Figure 3. In addition to the 1A1 proton in the high field resulting from the α-(1→6) binding and a large overlapped anomeric signal of the core CD, four anomeric protons were observed in the small range from 5.3 to 5.5 ppm. The order of four anomeric protons in chemical shifts was estimated from the high field, H-1A2, H-1A4, H-1A3, and H-1A5. The order did not simply reflect the distance from the core CD ring. Signals of anomeric protons in the branch appear by turns in the low field for longer branches than G3-α-CD. For example, a broadened signal of G4-α-CD at 5.404 ppm appears. It includes two signals: H-1A3 and H-1A4 (Fig. 2e). In the HMQC spectrum of G4-α-CD (not shown), two correlated peaks were detected clearly in the region around in 5.404 ppm; the H-1A3 signal is assigned in a slightly lower field than H-1A4 signal in consideration of relaxation times of the corresponding carbons. Although relaxation times of anomeric carbons of the branches in G6-α-CD and G7-α-CD were not completely individually detected, chemical shifts of the anomeric proton signals in the branch moiety in G6-α-CD and G7-α-CD were estimated by analogy to assignment results of G4-α-CD and G5-α-CD.

3.3. Terminal residue of the branch

The terminal residue of the branch has a free hydroxyl group on C-4; all other glucosyl residues in the branched α-CDs have no free hydroxyl groups on C-4. All hydroxyl groups that are combined to C-4, except the terminal one, are used to make the *O*-glucosyl binding. As shown in Figure 2b–h, the H-4 protons of the terminal residue were observed as a characteristic triplet signal in the highest field in every branched α-CD. Signals of the terminal residue can be detected easily in the TOCSY spectra. For example, Figure 4 shows the TOCSY spectrum of G5-α-CD. As shown in the upper side of Figure 4, H-2A5, H-3A5, H-5A5, and two H-6A5 signals were detected as individually isolated correlated

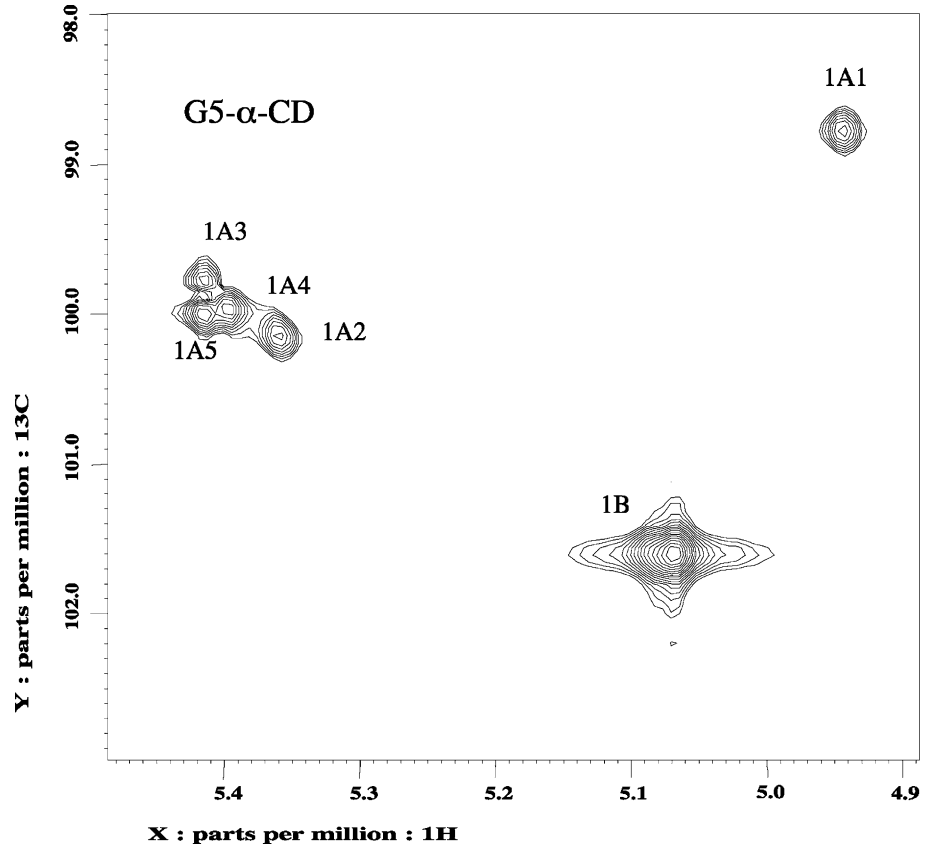

Figure 3. HMQC spectrum of G5-α-CD.

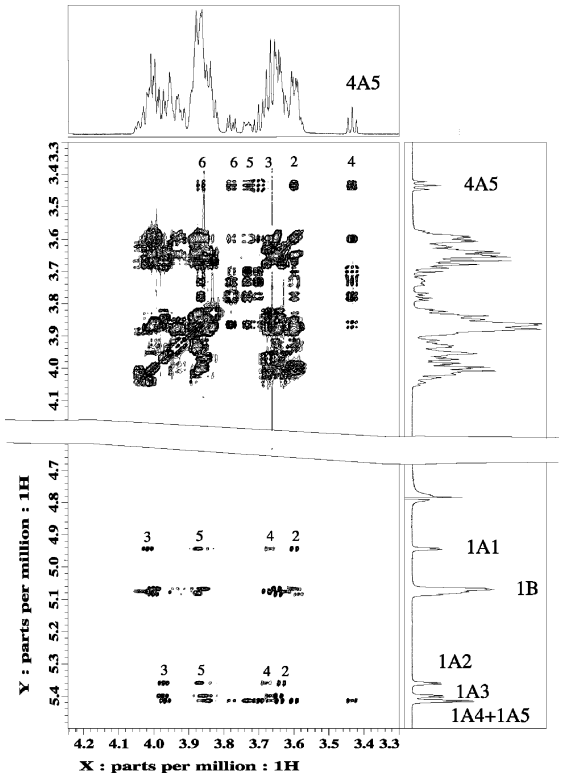

Figure 4. TOCSY spectrum of G5-α-CD. Mixing time was 100 ms.

peaks. Assignment of these signals was confirmed by the splitting pattern and intensity change in the TOCSY spectra by changing the mixing time. Among those signals, H-3A5, H-5A5, and one H-6A5 appeared as characteristically isolated weak signals, shown by the asterisk in Figure 2f. Similar signals of the terminal residue appeared in all ^{1}H NMR spectra of branched α-CDs, as shown by asterisks in Figure 2b–h.

3.4. Chemical shift of branch signals

Table 1 shows chemical shifts of proton signals of the branch part obtained from analyzing 2D TOCSY spectra. In the table, chemical shifts shown in bold typeface were obtained from 1D spectra with a spectral resolution of 0.0002 ppm. Chemical shifts written with normal typeface were obtained from 2D TOCSY spectra with a spectral accuracy of 0.004 ppm. Differences in corresponding signals in the branch part can be recognized from the table. As described above, the largest changes were observed in the anomeric protons. Figure 5 shows the ^{1}H NMR spectrum of the anomeric protons of G7-α-CD. The largest difference of anomeric protons was 0.477 ppm between H-1A1 and H-1A4. This is larger than those in the H-2–H-6 signals in α-CD. This large difference may arise from the difference of two kinds of glucosyl binding. The highest field signal was assigned to be H-1A1 and far away from the other anomeric protons in the branch of the G7-α-CD. The H-1A1 proton belongs to only one glucosyl residue with α-(1→6) binding in G7-α-CD molecule. Other anomeric protons belong to glucosyl residues with α-(1→4) glucosyl binding. Variation in chemical shift of anomeric protons of the branch glucose with α-(1→4) binding was, at most, 0.057 ppm.

It is interesting that anomeric protons of the core CD were observed in that relatively high field despite α-(1→4) binding, whereas anomeric protons with the same α-(1→4) binding in the branch part appeared in a range around 5.4 ppm. Comparing α-CD to larger CDs, for example, β-CD, γ-CD, δ-CD, and ε-CD, anomeric signals of these CDs shifted to low field as the number of pyranoses in the CD ring increased.[17] It is not known precisely what causes the variance in the chemical shifts of signals. However, comparison of α-CD to larger CDs shows that strain in *O*-glucosyl binding is one possible cause of variance in the chemical shifts of anomeric signals.

Table 1 shows that the second large variance is in the H-3 signals. It is interesting that the variance in H-3 was larger than that in H-4. We infer that the H-4 protons are affected more by *O*-glucosyl bindings than the H-3 protons, because H-4 protons exist next to the binding position. As shown in Table 1, the largest difference in H-3 was 0.320 ppm with G6-α-CD and with G7-α-CD. For example, two H-3 protons, H-3A1 and H-3A6, show difference in chemical shifts in G6-α-CD of 0.320 ppm. The largest difference in chemical shift of H-4 protons in the branch is 0.247 ppm; it is shown by two protons, H-4A2 and H-4A6 in G6-α-CD. The reason for such larger variance in chemical shifts of H-3 protons than those of H-4 protons remains unclear. Variance in chemical shifts of H-5 protons in the branch was relatively small; the largest between two protons, H-5A1 and H-5A6, is 0.144 ppm in G6-α-CD. The smallest variance was detected with H-2 protons in the branch. It was, at most, 0.045 ppm. These variances in chemical shifts of H-2, H-3, H-4, and H-5 protons are engendered largely in chemical shifts of the terminal residue, as shown in Table 1. Differences in chemical shifts of H-3, H-4, and H-5 protons in nonterminal residues are considerably small. Interestingly, chemical shifts of H-2 protons in the terminal residue and in the residue next to the core CD were almost equal. Further chemical shifts of H-3, H-4, and H-5 signals in nonterminal residues are similar to the corresponding signals of amylose. We cannot discuss differences in chemical shifts of the H-6 protons in this paper because chemical shifts of the H-6 protons in branched α-CDs were obtained only partly.

3.5. Signals of the core CD protons

As shown in Figure 5, the signal of the anomeric protons of the core CD in G7-α-CD is not a simple doublet as observed in α-CD. Two kinds of weak doublets were

Table 1. Chemical shifts of protons in the branch part[a]

	1	2	3	4	5	6a	6b
G1-α-CD							
δ (A1)	**4.957**	3.558	**3.758**	**3.452**	**3.743**	**3.790**	3.861
G2-α-CD							
δ (A1)	**4.943**	3.592	4.015	3.665	3.864	—	—
δ (A2)	**5.381**	3.595	**3.695**	**3.430**	**3.743**	**3.770**	3.861
G3-α-CD							
δ (A1)	**4.944**	3.601	4.021	3.663	3.878	3.855	—
δ (A2)	**5.352**	3.635	3.965	3.664	3.870	3.848	—
δ (A3)	**5.401**	3.596	**3.705**	**3.429**	**3.732**	**3.776**	3.865
G4-α-CD							
δ (A1)	**4.943**	3.599	4.018	3.665	3.873	3.835	3.889
δ (A2)	**5.358**	3.637	3.970	3.675	3.867	3.847	—
δ (A3)	**5.404**	3.642	3.967	3.668	3.851	3.826	3.873
δ (A4)	**5.403**	3.601	**3.702**	**3.432**	**3.731**	**3.775**	3.865
G5-α-CD							
δ (A1)	**4.943**	3.599	4.018	3.669	3.874	3.840	3.889
δ (A2)	**5.360**	3.636	3.973	3.678	3.871	3.849	—
δ (A3)	**5.415**	3.641	3.968	3.667	3.844	—	—
δ (A4)	**5.401**	3.643	3.975	3.668	3.856	3.829	3.879
δ (A5)	**5.415**	3.599	**3.701**	**3.433**	**3.732**	**3.777**	3.866
G6-α-CD							
δ (A1)	**4.943**	3.596	4.018	3.667	3.875	3.854	—
δ (A2)	**5.363**	3.633	3.972	3.680	3.868	3.850	—
δ (A3)	**5.415**	3.641	3.971	—	3.849	—	—
δ (A4)	**5.411**	3.641	3.973	—	3.852	—	—
δ (A5)	**5.420**	3.640	3.969	3.669	3.847	—	—
δ (A6)	**5.415**	3.598	**3.698**	**3.433**	**3.731**	**3.776**	3.866
G7-α-CD							
δ (A1)	**4.948**	3.601	4.022	3.672	3.878	3.859	—
δ (A2)	**5.368**	3.639	3.975	3.683	3.875	3.854	—
δ (A3)	**5.413**	3.645	3.974	3.673	3.851	—	—
δ (A4)	**5.425**	—	—	—	—	—	—
δ (A5)	**5.418**	—	—	—	—	—	—
δ (A6)	**5.418**	—	—	—	—	—	—
δ (A7)	**5.418**	3.600	**3.702**	**3.437**	**3.736**	**3.783**	3.869
Amylose							
δ	**5.421**	**3.641**	**3.975**	**3.679**	**3.854**	**3.832**	**3.876**

[a] Expressed in ppm downfield from Me_4Si.

observed at the low field side of the original signal of nonsubstituted CDs. The largest difference in anomeric protons of the core CD was 0.021 ppm. Similarly, by introducing a branch into α-CD, two doublet signals were observed at the low-field side in all seven branched α-CDs. Although appearance of anomeric signals of the core CD was not identical (see Fig. 2), signals of the core CD were divisible, temporarily, into three groups, a, b, and c, defining from low field. The lowest field signal, (a), was assigned to anomeric protons of the residue next to the branching residue. The anomeric proton signal of the branching residue was in the middle group, (b). Other anomeric signals were major signals in the original field (c). These assignments of three groups in the anomeric proton region are supported by the following four results and discussions.

Figure 6 shows three 2D spectra of G4-α-CD. The branched residue (B1) includes no hydroxyl group caused by the *O*-glucosyl binding at the C-6 carbon (see Fig. 7); the other residues (B2, B3, …, B6) have free hydroxyl groups at the C-6 carbons. Two H-6 protons of the branched residue (H-6B1) show signals in a relatively low field caused by *O*-glucosyl binding far from the other H-6 protons of the core CD. At the same time, shift of the C-6 carbon in the ^{13}C spectrum of the branched residue to low field was caused by the *O*-glucosyl binding (Fig. 6). Consequently, chemical shifts of two H-6 protons on the branched residue were easily assignable by the HMQC spectrum, as shown in Figure 6a. This was the first evidence for the assignments of three groups (a, b, and c). Two H-6 signals of the branched residue were included in the middle group

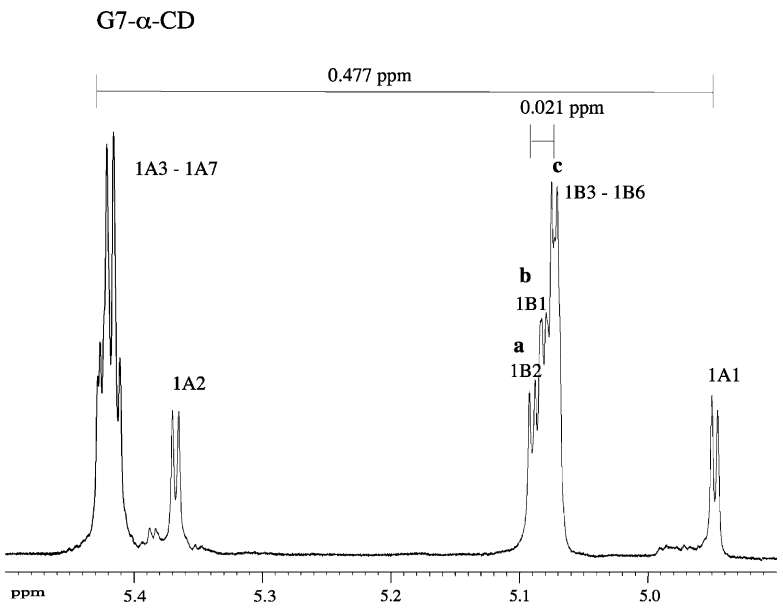

Figure 5. [1]H NMR spectrum of G7-α-CD in the region of anomeric protons.

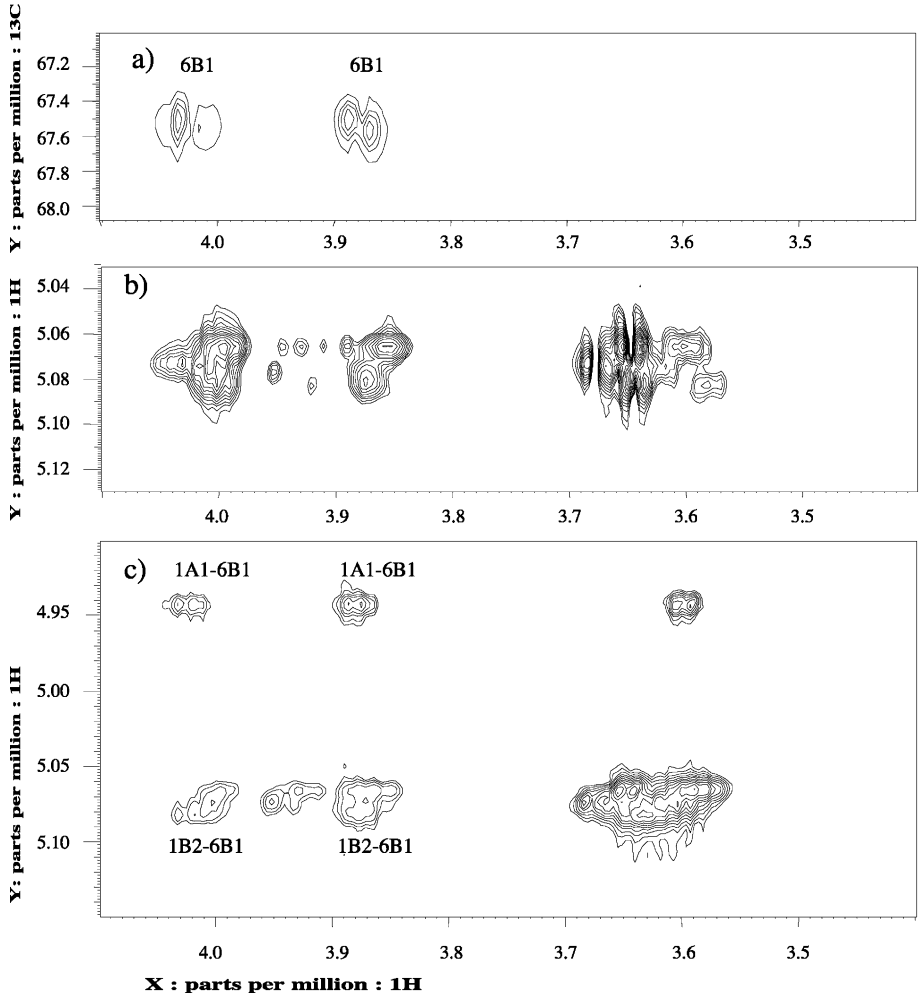

Figure 6. Three 2D spectra of G4-α-CD were used for assignments of anomeric protons of core CD. (a) HMQC, (b) TOCSY, and (c) NOESY.

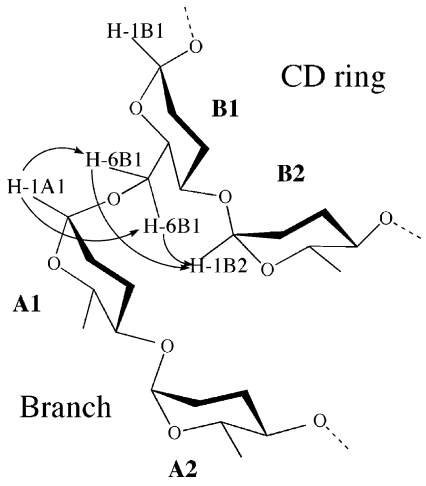

Figure 7. Branched residue (B1), the next residue (B2), and branch residues (A1 and A2).

(b) of the core CD in the TOCSY spectrum, as shown in Figure 6b (the second result). These facts demonstrate that the middle signal (b) of the anomeric protons of the core CD is exactly responsible for H-1B1. The two H-6B1 protons show NOE correlation with the anomeric proton of the first residue in the branch (H-1A1), as shown in Figure 6c, indicating the connection with the *O*-glucosyl binding (the third result, see Fig. 7). This supports the assignment of the H-1B1 proton. Generally, an NOE between the H-1 proton in a residue and the two H-6 protons in the next glucosyl residue is detected weakly in CDs. As shown in the lower part of Figure 6c, NOEs were detected between the anomeric proton of the core CD in the highest field (H-1B2) and two H-6B1 protons of the branched residue. This is the fourth result, suggesting that the signal (a) in the lowest field is assignable to the anomeric proton in the residue next to the branched one (B2). In the case of G1-α-CD and G2-α-CD, the weight ratio of three groups (a:b:c) in anomeric protons of the core CD was 1:1:4. In the case of G3-α-CD, G4-α-CD, G5-α-CD, G6-α-CD, and G7-α-CD, the weight ratio was 1:2:3. Although the middle signal (b) includes one more proton of the core CD in larger branched α-CD than G3-α-CD, assignments of the three groups described above are valid. It was diffi-

Table 2. Chemical shifts of protons in the core CD part[a]

	Weight	1	2	3	4	5	6	6
G1-α-CD								
δ (a)	1	**5.082**	3.646	3.998	3.594	3.873	—	—
δ (b)	1	**5.073**	**3.678**	4.026	—	—	3.840	4.064
δ (c)	4	**5.066**	3.646	3.999	3.594	3.854	—	—
G2-α-CD								
δ (a)	1	**5.082**	3.643	3.997	—	3.873	—	—
δ (b)	1	**5.069**	**3.674**	4.014	3.619	3.880	3.907	4.040
δ (c)	4	**5.068**	3.644	3.994	3.597	3.852	—	—
G3-α-CD								
δ (a)	1	**5.084**	3.640	4.006	3.577	3.873	—	—
δ (b)	2	**5.079**, **5.072**	3.656, 3.678	—	—	—	3.874	4.038
δ (c)	3	**5.067**	3.646	3.999	3.601	3.855	—	—
G4-α-CD								
δ (a)	1	**5.085**	3.648	3.991	3.600	3.873	—	—
δ (b)	2	**5.079**, **5.077**	3.678	4.031	3.621	—	3.892	4.038
δ (c)	3	**5.069**	3.644	4.002	3.580	3.854	—	—
G5-α-CD								
δ (a)	1	**5.084**	3.646	4.001	3.585	3.875	—	—
δ (b)	2	**5.071**	3.678	4.019	3.622	3.888	3.907	4.038
δ (c)	3	**5.064**	3.649	3.997	3.598	3.858	—	—
G6-α-CD								
δ (a)	1	**5.087**	3.647	4.002	3.587	3.875	—	—
δ (b)	2	**5.082**, **5.074**	3.679	4.010	3.620	—	3.915	4.042
δ (c)	3	**5.067**	3.648	3.996	3.598	3.854	—	—
G7-α-CD								
δ (a)	1	**5.090**	3.649	4.002	3.593	3.878	—	—
δ (b)	2	**5.077**	3.680	4.018	—	—	3.910	4.042
δ (c)	3	**5.069**	3.648	3.996	3.603	3.859	—	—
α-CD								
δ		**5.069**	**3.648**	**3.998**	**3.600**	**3.860**	**3.878**	**3.927**

[a] Expressed in ppm downfield from Me_4Si.

cult to determine one more residue contributing middle signal (b) in the anomeric region in larger branched α-CD than G3-α-CD, despite many efforts using a high-resolution spectrometer.

Chemical shifts of the core CD signals obtained from 2D TOCSY spectra are shown in Table 2: the chemical shifts of the anomeric protons were estimated from the 1D spectra. Chemical shifts written in bold typeface were obtained from 1D spectra in the same manner as those in Table 1. Although several signals could not be determined in the 2D TOCSY spectra, almost all protons in the branched residues seemed to appear at a slightly lower field than those in nonbranched residues with seven branched α-CDs. The exceptional case was that of the anomeric protons, as described above.

Difference in the anomeric protons of the core CD was 0.021 ppm at most as shown in Figure 5. Variation in chemical shifts of the H-2 protons in the core CD was 0.032–0.034 ppm, as estimated from Table 2. The largest differences in the chemical shifts of H-3 and H-4 were 0.040 and 0.041 ppm, respectively, for G4-α-CD. Moreover, the largest variance in H-5 protons of the core CD was 0.030 ppm with G5-α-CD. Thus, respective variances of H-1, H-2, H-3, H-4, and H-5 of the core CD were as small as the variance in H-2 of the branch moiety. The chemical shift of the H-6 protons of the branched residue was obtained exclusively. We infer that the difference in chemical shifts of the H-6 protons in the core CD would be the largest because the *O*-glucosyl binding at the C-6 carbon is the only difference in glucose residues in the core CD. As discussed above, existence of *O*-glucosyl binding at C-6 carbon causes the chemical shift of H-6 protons in the branching residue to the lower field. The magnitude of variance in chemical shift of the H-6 protons in the core CD is reasonably expected to be comparable with the variance in anomeric protons in branch moiety with α-(1→4) *O*-glucosyl binding.

4. Conclusions

The ^{1}H NMR spectra of seven branched α-cyclodextrins (α-CD) were analyzed in detail with a high-resolution NMR spectrometer and compared with spectra of α-CD and amylose. Deducing from the ^{1}H NMR spectra of these branched cyclodextrins, signals of the branch part were shown to resemble those of amylose with increasing residues in the branch. Variance in chemical shift of both branch-part protons and core CD protons were caused mainly by existence of α-(1→4) or α-(1→6) *O*-glucosyl binding at the corresponding carbons. Four signals (H-3, H-4, H-5, and H-6) of the terminal residue in the branch were easily determined as characteristically isolated resonances in the ^{1}H NMR spectra in all seven branched α-CDs. Variance in chemical shifts of all of the core CD protons, except for the H-6 protons, was smaller by one order than those of the branch moiety. The largest variation in chemical shifts could be observed with H-6 protons among the protons of the core CD with the magnitudes comparable with those in the anomeric protons in the branch moiety with α-(1→4) *O*-glucosyl binding.

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