Note

NMR Spectra of Partially Deacetylated Chitotrisaccharides

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For study of the specificity of chitinase, it is important to identify the structures of the enzymatic hydrolyzate from partially acetylated chitosan. The reaction products contained a mixture of partially deacetylated chito-oligosaccharides consisting of GlcNAc and GlcN.^{1,2)} The structures of the partially deacetylated chito-oligosaccharides have been identified by a combination of *N*-acetylation, enzyme digestion, and nitrous acid degradation.^{1–3)} Recently, NMR spectroscopy has been usefully applied to a simpler and more definitive determination of oligosaccharide structures. In this study, we analyzed the structure of two partially deacetylated chitotrisaccharides by comparing the NMR spectra of these compounds with those of chitotriose [(GlcNAc)₃] and chitosatriose [(GlcN)₃].

Partially deacetylated chito-oligosaccharides were obtained by chitinase digestion of 25–35% *N*-acetylated chitosan according to the method of Ohtakara *et al.*²⁾ In this study, two partially deacetylated chitotrisaccharides, GlcN–GlcNAc–GlcNAc (F-3) and GlcN–GlcN–GlcNAc (F-7) (see Fig. 2 in ref. 2), were employed for NMR measurements. Their ¹H- and proton-decoupled ¹³C-NMR spectra were obtained using a 5 mm C/H probe on a JNM GX-500 spectrometer (JEOL) at 27°C. Three mg of each sample was lyophilized three times from 99.96% D₂O and dissolved in 0.5 ml of 99.96% D₂O. Chemical shifts were referenced directly to sodium 2,2-dimethyl-2-silapentanesulfonate for proton shifts and to *p*-dioxane for carbon shifts (67.4 ppm downfield from TMS).

Figure 1 shows the carbonyl and methyl carbon regions of the ¹³C-NMR spectra of F-3 (D, E), F-7 (G, H) and (GlcNAc)₃ (A, B). The methyl proton region of the ¹H-NMR spectra of these compounds are also shown in

Fig. 1 (C, F, I). According to the signal assignments of Dahlquist and Raftery,⁴⁾ three singlet proton signals of (GlcNAc)₃ were assigned to the acetyl methyl protons of the nonreducing end residue (Peak 1, 2.051 ppm), the internal residue (Peak 2, 2.046 ppm), and the reducing end residue (Peak 3, 2.023 ppm), respectively. The signal of Peak 1, which appeared at the lowest field in the spectrum of (GlcNAc)₃ (Fig. 1C), is missing in the spectrum of F-3 (Fig. 1F). The carbonyl carbon signal of the nonreducing end residue of (GlcNAc)₃ (Peak 1 in Fig. 1A) is also missing in F-3 (Fig. 1D). The relative intensity of a signal derived from the methyl carbons of the nonreducing and internal residues of (GlcNAc)₃ (Peak 1, 2 in Fig. 1B) is reduced in the spectrum of F-3 (Fig. 1E). These results confirmed the structure of F-3 as GlcN–GlcNAc–GlcNAc.

In the case of F-7, one acetyl methyl signal was detected in the ¹H-spectrum (Fig. 1I), and two signals derived from the α - and β -anomers of the reducing end residue were detected in the methyl and carbonyl carbon regions of the ¹³C-spectrum (Figs. 1G and 1H). The results confirmed the structure of F-7 as GlcN–GlcN–GlcNAc.

All the carbon resonances of F-3, F-7, (GlcNAc)₃, and (GlcN)₃ were assigned by comparing these data with the data for (GlcNAc)₂, (GlcN)₂, and GlcN–GlcNAc.^{5–7)} The effects of deacetylation on the ¹³C-chemical shifts of pyranose ring carbons have been investigated by comparison between the chemical shifts of GlcN–GlcNAc and (GlcNAc)₂,⁵⁾ and were taken into consideration in these signal assignments. In addition, we took account of the intensity ratio of α -anomer to β -anomer of the reducing end carbons in the assignments. The results are listed in Table I. The chemical shifts of C-1, C-2, C-3, and C-4 were changed in a complicated manner by deacetylation, while the values of C-5, C-6, carbonyl carbon, and methyl carbon were not changed significantly. In particular, most of the signals of C-1, C-3, and C-4 of F-7 were shifted to the upfield in comparison with those of F-3. The upfield shifts were predominant in C-1 and C-4 carbons which participate in the β -1,4-glycosidic linkage. However, no such large upfield shifts of the C-1 and C-4 carbon signals were observed between (GlcNAc)₃ and F-3, and between F-7 and (GlcN)₃. Saito *et al.*⁶⁾ suggested that ¹³C-chemical shifts of carbons at the glycosidic linkage are very sensitive to conformational change. Tsukada and Inoue⁷⁾ also reported the empirical correlation between C-1 carbon chemical shift and the glycosidic torsion angle $\Delta\psi$. From this correlation, it is expected that the upfield shift of C-1 carbon signal by 4 ppm corresponds to alteration in the glycosidic torsion angle $\Delta\psi$ by about 30°. Thus, the conformation of the glycosidic linkages of (GlcNAc)₃ and F-3 is considerably different from that of F-7 and (GlcN)₃. In the latter two oligosaccharides, there should be an electrostatic repulsion between the ammonium groups of

Abbreviations: GlcN, 2-amino-2-deoxy-D-glucopyranose; GlcNAc, 2-acetamido-2-deoxy-D-glucopyranose; GlcN–GlcNAc, 2-amino-2-deoxy- β -D-glucopyranosyl-(1 $\rightarrow$ 4)-2-acetamido-2-deoxy-D-glucopyranose; (GlcN)_{*n*} or (GlcNAc)_{*n*}, β -1,4-linked *n*-mer of GlcN or GlcNAc; NMR, nuclear magnetic resonance.

Fig. 1. ^1H - and ^{13}C -NMR Spectra of $(\text{GlcNAc})_3$ (A, B, C), F-3 (D, E, F), and F-7 (G, H, I). C, F, and I show the methyl proton region of the ^1H -spectra. A, D, and G show the carbonyl carbon region, and B, E, and H the methyl carbon region of the ^{13}C -spectra. Individual resonances in the $(\text{GlcNAc})_3$ spectra (A, B, C) have been assigned as indicated in the figure.⁴⁻⁶⁾ Numerals 1, 2, and 3 indicate the resonances derived from the nonreducing end residue, the internal residue, and the reducing end residue, respectively.

two adjacent glucosamine residues due to their positive charges. However, the repulsion does not exist between the monosaccharide units of the former two oligosaccharides. This situation may cause the different conformations of glycosidic linkages of the oligosaccharides. Although the chemical shift changes in C-2 and C-3 by the deacetylation should partially reflect the difference in the electron withdrawing capacity between NH_3^+ and $\text{NH}(\text{C}=\text{O})\text{CH}_3$, the data for these carbons cannot fully be explained at present. Other conformational parameters should be obtained to explain all of the ^{13}C -chemical

shift changes induced by the deacetylation.

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Table I. ^{13}C -NMR DATA OF THE PARTIALLY DEACETYLATED CHITOTRIOSSES, CHITOTRIOSE, AND CHITOSATRIOSE

		(GlcNAc) ₃	GlcN-GlcNAc-GlcNAc (F-3)	GlcN-GlcN-GlcNAc (F-7)	(GlcN) ₃
C-1	α	91.25	91.27	91.41	89.78
	β	95.63	95.65	95.58	93.48
	int.*	102.09	102.15	98.05	98.39
	nonred.*	102.28	103.11	98.60	98.64
C-2	α	54.45	54.48	54.78	55.06
	β	56.93	56.95	57.45	57.53
	int.	55.85	56.14	56.57	56.74
	nonred.	56.41	57.43	56.66	56.74
C-3	α	70.06	70.09	69.56	68.77
	β	73.29	73.33	72.63	71.16
	int.	72.98	72.79	71.00	71.03
	nonred.	74.25	76.07	72.57	72.65
C-4	α	80.45	80.58	77.86	77.34
	β	79.95	80.15	77.57	77.53
	int.	80.00	78.76	77.35	77.41
	nonred.	70.53	70.40	70.42	70.48
C-5	α	70.83	70.82	70.68	70.79
	β	75.42	75.41	75.14	75.43
	int.	75.31	75.47	75.58	75.66
	nonred.	76.72	77.05	77.22	77.28
C-6	α	60.79	60.85	60.85	60.92
	β	60.95	60.94	61.30	61.10
	int.	60.79	60.94	61.23	61.03
	nonred.	61.36	61.43	61.11	61.20
C=O	α	175.28	175.31	175.35	—
	β	175.54	175.57	175.61	—
	int.	175.39	175.41	—	—
	nonred.	175.42	—	—	—
CH ₃	α	22.70	22.71	22.74	—
	β	22.99	23.00	23.01	—
	int.	22.93	22.96	—	—
	nonred.	22.93	—	—	—

* int., the internal residue; nonred., the nonreducing end residue.

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