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The Conformational Study of Chitin and Chitosan Oligomers in Solution

Hiroshi Sugiyama,[a,*] Kanehiko Hisamichi,[b] Kazuo Sakai,[c] Taichi Usui,[d] Jun-Ichi Ishiyama,[e] Hideaki Kudo,[f] Hiroki Ito[f] and Yasuhisa Senda[f]

[a]*Institute for Chemical Reaction Science, Tohoku University, Sendai 980-8577, Japan*
[b]*Institute of Development, Aging and Cancer, Tohoku Univeristy, Sendai 980-8575, Japan*
[c]*Yaizu Suisankagaku Industry Co., Ltd., Yaizu 425-8570, Japan*
[d]*Department of Applied Biochemistry, Shizuoka University, Shizuoka 422-8529, Japan*
[e]*Miyagi National College of Technology, Natori 981-1239, Japan*
[f]*Department of Chemistry, Yamagata University, Yamagata 990-8560, Japan*

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Abstract—The inter-residual dihedral angles ϕ and φ of chitin and chitosan oligomers were determined from experimental $^{3}J_{C\text{-}H}$ constants and ROESY cross peaks.

Recently chitin and chitosan, which are mainly produced from crustacean shells, have become very important, particularly for medicinal applications.[1] Their very high wound healing ability when used as a dressing material has drawn attention.[2] This ability is attributed to both their primary chemical structures and their conformation. Ogawa and his co-workers have reported that the chitosan conformation in the crystal state ranges from a 2-fold helix to 8/5 helical structure (left-handed helix), depending on its counter anion.[3–5] In addition, Gardner and Blackwell determined the crystal structure of β-chitin[6] and Mo and Jensen reported the crystal structures of the α and β anomers of chitin dimer, which have inter-residual bonds with very different rotational angles.[7,8] Therefore, we investigated the rotational angles of the inter-residual bonds of their oligomers in solution by the NMR method.

Typically, this is done by measuring the nuclear Overhauser effects of saccharides and then estimating the energy minima from theoretical calculations with the NOE constraint, which contains the distance information. However, the distance information from NOE is not exact.[9–11] Moreover, there is some ambiguity in the theoretical calculation, particularly when it includes solvent effects. Our recent experimental conformational study of maltose-type oligomers showed that the dihedral angles between the glucose residues obtained from spin–spin coupling constants $^{3}J_{C\text{-}H}$ could not be interpreted using existing theoretical calculations.[12] Hricovini et al. reported a similar discrepancy for xylobiose.[13] Therefore, we tried to determine the inter-residual $^{3}J_{C\text{-}H}$ constants of chitin and chitosan oligomers from the dimer to the hexamer in the solvents [$^{2}H_2$]O (D_2O) and [$^{2}H_6$]-dimethylsulfoxide (DMSO). These solvents are very commonly used as NMR solvents for saccharides, and produce different environments for the solutes. Unfortunately, the $^{3}J_{C\text{-}H}$ constants of chitin oligomers longer than the trimer could not be determined because of their very low solubility in both solvents.

The proton NMR spectra were assigned by the phase-sensitive DQFCOSY and HMQC methods. In D_2O, the proton NMR spectra of these oligomers have very similar chemical shifts and vicinal coupling constants (around 9 Hz) between the ring protons (Tables 1 and 2), so these oligomers should have the $^{4}C_1$ ring conformation.

In DMSO, the proton spectra showed very similar chemical shifts for the oligomers, but the same protons of different middle residues in the same oligomer had slightly different chemical shifts. Particularly, the hexamers in DMSO showed broad, poorly separated absorption lines between 3.25 and 3.75 ppm, just like the NMR spectra of solid state or quadrapolar elements. Therefore, we gave up trying to assign the exact proton chemical shifts of the chitin hexamer, although it was almost the

*Corresponding author.

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Table 1. Proton NMR assignments of chitin oligomers in D_2O, ppm from TSP ($J_{n,n+1}$ and $J_{6,6'}$ in Hz)[a]

		1	2	3	4	5	6	6′	CH_3
Dimer	α	5.20 (2.2)	3.87 (10.6)	3.90 (8.4)	3.64 (10.6)	3.89 (5.0, 2.2)	3.68 (−12.0)	3.80	2.05
	β	4.72 (8.6)	3.71 (10.4)	3.67 (8.4)	3.62 (10.4)	3.66 (5.0, 2.2)	3.66 (−12.0)	3.84	2.05
	n	4.61 (8.4)	3.77 (10.0)	3.58 (8.4)	3.48 (10.6)	3.50 (5.0, 2.2)	3.76 (−11.5)	3.93	2.08
Trimer	α	5.20 (2.2)	3.86 (10.6)	3.88 (8.4)	3.63 (10.0)	3.89 (5.0, 2.2)	3.68 (−12.0)	3.80	2.05
	β	4.72 (8.4)	3.69 (10.6)	3.66 (8.4)	3.62 (10.6)	3.52 (5.0, 2.2)	3.67 (−12.0)	3.84	2.05
	m	4.60 (8.4)	3.79 (10.6)	3.74 (8.4)	3.65 (10.0)	3.56 (5.0, 2.2)	3.66 (−12.0)	3.85	2.07
	n	4.60 (8.0)	3.75 (10.6)	3.58 (8.4)	3.47 (10.0)	3.48 (5.0, 2.2)	3.73 (−12.0)	3.92	2.07
Tetramer	α	5.20 (2.6)	3.86 (10.4)	3.88 (8.2)	3.63 (8.2)	3.89 (5.0, 2.2)	3.72 (−12.0)	3.80	2.05
	β	4.71 (8.4)	3.69 (10.4)	3.66 (8.4)	3.62 (10.2)	3.53 (5.0, 2.2)	3.67 (−12.0)	3.84	2.05
	m	4.60 (8.8)	3.77 (10.6)	3.73 (8.4)	3.65 (10.0)	3.56 (5.0, 2.2)	3.66 (−12.0)	3.85	2.07
	n	4.59 (8.8)	3.75 (10.4)	3.58 (8.4)	3.47 (10.0)	3.48 (5.0, 2.2)	3.73 (−12.0)	3.93	2.07
Pentamer	α	5.21 (3.3)	3.86 (10.2)	3.88 (8.4)	3.63 (10.0)	3.88 (5.0, 2.2)	3.72 (−12.0)	3.79	2.05
	β	4.71 (8.4)	3.69 (10.2)	3.66 (8.4)	3.62 (10.0)	3.52 (5.0, 2.2)	3.67 (−12.0)	3.84	2.05
	m	4.59 (8.4)	3.77 (10.6)	3.72 (8.2)	3.65 (10.0)	3.54 (5.0, 2.5)	3.66 (−12.5)	3.86	2.06
	n	4.59 (8.4)	3.75 (10.6)	3.56 (8.2)	3.47 (10.0)	3.48 (5.0, 2.2)	3.75 (−12.5)	3.93	2.07
Hexamer	α	5.20 (2.4)	3.86 (10.6)	3.88 (8.4)	3.63 (10.0)	3.88 (5.0, 2.2)	3.72 (−12.5)	3.78	2.05
	β	4.70 (8.4)	3.69 (10.2)	3.66 (8.2)	3.62 (10.0)	3.53 (5.0, 2.2)	3.67 (−12.5)	3.83	2.05
	m	4.59 (8.4)	3.75 (10.6)	3.72 (8.4)	3.65 (10.2)	3.55 (5.0, 2.2)	3.67 (−12.5)	3.85	2.06
	n	4.59 (8.4)	3.75 (10.6)	3.56 (8.2)	3.47 (10.0)	3.49 (5.0, 2.2)	3.75 (−12.5)	3.93	2.07

[a]α or β, reducing end α or β anomer residue; m, middle residue; n, non-reducing end residue.

Table 2. Proton NMR assignments of chitosan HCl salt oligomers in D_2O, ppm from TSP ($J_{n,n+1}$ and $J_{6,6'}$ in Hz)[a]

		1	2	3	4	5	6	6′
Dimer	α	5.44 (3.7)	3.34 (10.6)	4.03 (9.0)	3.87 (10.0)	4.03 (5.0, 2.2)	3.77 (12.5)	3.83
	β	4.97 (9.0)	3.05 (8.0)	3.86 (8.2)	3.71 (9.0)	3.88 (5.0, 2.2)	3.74 (12.5)	3.94
	n	4.83 (8.0)	3.13 (10.6)	3.69 (8.0)	3.49 (10.0)	3.54 (5.0, 2.2)	3.77 (12.5)	3.84
Trimer	α	5.44 (3.7)	3.34 (10.6)	4.03 (8.8)	3.88 (10.0)	4.04 (5.2, 2.2)	3.77 (12.5)	3.84
	β	4.96 (8.4)	3.03 (10.6)	3.86 (8.4)	3.71 (10.0)	3.88 (5.0, 2.0)	3.75	3.94
	m	4.87 (8.4)	3.16 (10.4)	3.88 (8.4)	3.74 (10.0)	3.94 (5.0, 2.0)	3.76 (12.5)	3.95
	n	4.84 (8.4)	3.12 (10.6)	3.69 (8.4)	3.49 (9.9)	3.55 (5.2, 2.0)	3.76 (12.5)	3.95
Tetramer	α	5.45 (3.7)	3.35 (10.6)	4.04 (8.8)	3.88 (10.0)	4.04 (5.0, 2.0)	3.77 (12.5)	3.84
	β	4.95 (8.4)	3.05 (10.6)	3.88 (8.4)	3.71 (10.0)	3.88 (5.0, 2.0)	3.75	3.94
	m	4.88 (8.4)	3.16 (10.6)	3.89 (8.4)	3.74 (10.0)	3.93 (5.5, 2.0)	3.77 (12.5)	3.94
	n	4.85 (8.4)	3.14 (10.6)	3.70 (8.4)	3.50 (10.0)	3.55 (5.0, 2.0)	3.77 (12.5)	3.94
Pentamer	α	5.44 (3.7)	3.34 (10.6)	4.04 (8.8)	3.87 (10.2)	4.04 (5.0, 2.0)	3.78 (12.5)	3.84
	β	4.96 (8.4)	3.05 (10.6)	3.88 (8.3)	3.70 (10.3)	3.88 (5.0, 2.0)	3.74	3.96
	m	4.87 (8.4)	3.16 (10.3)	3.90 (8.4)	3.73 (10.0)	3.94 (5.0, 2.0)	3.77 (12.5)	3.94
	n	4.85 (8.4)	3.14 (10.6)	3.70 (8.4)	3.49 (9.9)	3.55 (5.0, 2.0)	3.77 (12.5)	3.94
Hexamer	α	5.45 (3.7)	3.35 (10.6)	4.04 (9.0)	3.88 (10.2)	4.04 (5.0, 2.0)	3.77 (12.5)	3.84
	β	4.96 (8.4)	3.05 (10.6)	3.88	3.70	3.89	3.75	3.95
	m	4.88 (8.4)	3.16 (10.3)	3.90 (8.4)	3.73 (10.2)	3.94 (5.5, 2.0)	3.77 (12.5)	3.94
	n	4.85 (8.4)	3.12 (10.6)	3.70 (8.4)	3.50 (10.0)	3.55 (5.5, 2.0)	3.77 (12.5)	3.94

[a]α or β, reducing end α or β anomer residue; m, middle residue; n, non-reducing end residue.

same as that of the other oligomers. For the same reason, the vicinal coupling constants of the middle residues of the higher oligomers could not be estimated. However, the ring conformation of each residue should be the $^4C_1$ form, because their 2-amino or 2-acetylamino groups, 3-hydroxyl groups, inter-residual oxygen atoms, and hydroxylmethyl groups are directed towards the equator in the $^4C_1$ form (Tables 3 and 4).

There are 16 possible inter-residual bond rotation angles in a disaccharide, when we use the combined data of the Karplus type equation and the $^3J_{C\text{-}H}$ constant for the estimation. However, if we add the nuclear Overhauser effect data, the possibilities decrease markedly.[15] Therefore, we measured the phase-sensitive ROESY spectra of these oligomers in addition to their inter-residual $^3J_{C\text{-}H}$ (Fig. 1). The ROESY cross peaks between the anomeric proton and other protons and the $^3J_{C\text{-}H}$ constants of H1′-C1′-O-C4 (ϕ) and C1′-O-C4-H4 (φ), which were obtained using the Karplus type equation that Mulloy et al. derived experimentally,[14] are shown in Tables 5 and 6. In DMSO, the constant for the chitosan tetramer had the biggest coupling constants, while in D_2O both the tetramer and pentamer had the smallest coupling constants. The oligomers had more open inter-residual dihedral angles in D_2O than in DMSO, just like maltose oligomers.[12] Our data showed that chitin oligomers had the same tendency.

Table 3. Proton NMR assignments of chitin oligomers in DMSO, ppm from TSP ($J_{n,n+1}$ and $J_{6,6'}$ in Hz)[a]

		1	2	3	4	5	6	6′	CH_3
Dimer	α	5.01 (2.9)	3.68 (8.6)	3.73 (10.6)	3.40	3.66	3.57 (−13.0)	3.63	1.93
	β	4.53 (8.1)	3.52 (9.1)	3.37	3.38	3.51	3.51 (−12.1)	3.80	1.97
	n	4.44 (8.4)	3.61 (8.5)	3.38 (9.5)	3.20 (9.3)	3.33 (9.2, 2.1)	3.51 (−12.1)	3.80	1.97
Trimer	α	4.94 (2.5)	3.67	3.67	3.36	3.66	3.52 (−13.5)	3.59	1.88
	β	4.49 (8.1)	3.46 (10.2)	3.21 (7.9)	3.36	3.51			1.86
	m	4.41 (7.8)	3.59 (9.3)	3.50 (8.1)	3.31	3.32	3.45 (−12.1)	3.69	1.89
	n	4.40 (7.8)	3.51 (7.8)	3.32 (7.2)	3.09 (9.3)	3.25 (8.4, 1.2)	3.43 (−12.9)	3.77	1.89
Tetramer	α	4.99 (2.6)	3.69	3.71	3.41	3.68	3.56	3.62	1.88
	β	4.51 (8.2)	3.51 (10.7)	3.27 (8.2)	3.41		3.50	3.62	
	m	4.50 (8.4)	3.63 (9.3)	3.52 (8.2)	3.37	3.38	3.47	3.71	1.89
	n	4.42 (8.4)	3.57 (9.5)	3.36	3.17 (9.3)	3.31 (8.4, 2.6)	9.48 (−10.3)	3.79	1.89
Pentamer	α	4.95 (2.9)	3.66 (9.6)	3.70 (9.8)	3.35 (8.8)	3.66 (8.4, 2.6)	3.49 (−11.0)	3.58	1.88
	β	4.50 (9.8)							
	m	4.43 (7.3)	3.56	3.51	3.29	3.30	3.44	3.70	1.89
	n	4.39 (8.5)	3.48 (10.0)	3.33 (8.9)	3.07	3.21	3.43 (−11.0)	3.77	1.89
Hexamer	α	4.97							
	β	4.50							
	m	4.45							
	n	4.40							

[a]α or β, reducing end α or β anomer residue; m, middle residue; n, non-reducing end residue.

Table 4. Proton NMR assignments of chitosan HCl salt oligomers in DMSO, ppm from TSP ($J_{n,n+1}$ and $J_{6,6'}$)[a]

		1	2	3	4	5	6	6′
Dimer	α	5.28 (3.3)	2.95 (10.3)	3.83 (9.5)	3.66 (9.0)	3.83 (9.8, 2.7)	3.59 (−12.4)	3.76
	β	4.78 (8.1)	2.67 (10.0)	3.75 (10.8)	3.66 (9.0)	3.43		
	n	4.86 (8.4)	2.82 (9.3)	3.55 (9.0)	3.22 (9.2)	3.33 (8.3, 2.1)	3.48 (−12.3)	3.77
Trimer	α	5.29 (2.9)	2.97 (10.1)	3.82 (9.9)	3.68 (9.5)	3.78	3.63	3.73
	β	4.79 (8.1)	2.68 (9.3)	3.79	3.68	3.38		
	m	4.96 (8.4)	2.89 (9.3)	3.84 (9.9)	3.67 (9.6)	3.48	3.68	3.77
	n	4.80 (9.5)	2.80 (8.8)	3.55 (10.4)	3.19 (9.0)	3.28	3.54	3.77
Tetramer	α	5.28 (3.3)	2.98 (10.3)	3.83 (9.9)	3.68 (9.4)	3.74	3.64	3.73
	β	4.79 (8.1)	2.69 (8.9)	3.79 (9.8)	3.68	3.39		
	m	4.96 (8.4)	2.91 (9.4)	3.84 (9.9)	369	3.50	3.56	3.77
	n	4.89 (8.5)	2.81 (9.3)	3.56 (9.9)	3.20 (9.3)	3.29	3.46	3.77
Pentamer	α	5.27 (3.3)	2.98 (9.9)	3.82 (9.9)	3.69	3.84	3.56	3.74
	β	4.77 (8.4)	2.68 (9.3)	3.77 (9.7)		3.42		
	m	4.93	2.89 (9.4)	3.83 (10.4)	3.72	3.53	3.57	3.76
	n	4.87 (8.4)	2.80 (9.2)	3.54 (10.4)	3.19 (9.0)	3.29 (8.2,)	3.49 (−10.3)	3.77
Hexamer	α	5.27 (3.44)	3.01	3.83	3.68	3.85	3.60	3.72
	β	4.77 (7.28)	2.72	3.69				
	m	4.83 (9.70)	2.91	3.78	3.72	3.67	3.56	3.73
	n	4.77 (9.3)	2.83 (9.3)	3.51 (8.8)	3.22 (8.8)	3.34 (9.3, 2.4)	3.49	3.78

[a]α or β, reducing end α or β anomer residue; m, middle residue; n, non-reducing end residue.

The ROESY cross peaks between the anomeric and other protons of the chitosan dimer and trimer in D_2O did not appear at a mixing time shorter than 90 ms. At a mixing time of 90 ms, we obtained the peaks shown in the table. In the other cases, we obtained the cross peaks in the table at a mixing time of 30 ms. Presently, we are unable to explain this difference.

In addition to the ϕ and φ angles obtained in these experiments, the diatomic distances between the H-1 and H-4 of contiguous residue were estimated using the crystallographic data for the anhydrous chitosan HCl salt[3] for chitosan oligomers and for β-chitin[6] for chitin oligomers. As shown in Table 7, the diatomic distances between H-1 and H-3 in the same residue of an anhydrous chitosan HCl salt[3] and the *N,N′*-diacetyl methyl α-[7] and β-chitobiosides[8] are longer than those between H-1 and H-5, because the bond angle of ring C–O–C is usually smaller than that of C–C–C. However, for β-chitin the intra-residual distance between H-1 and H-3 is shorter than that between H-1 and H-5.

For chitosan oligomers in D_2O, there were ROESY cross peaks between H-1 and H-4 of the contiguous residue, so the dihedral angles ϕ and φ should be less than 90°. However, there are four possible combinations of dihedral angles ϕ and φ: + +, − −, + −, and − +. The distance between H-1 and H-4 of the contiguous residues is

Table 5. Phase-sensitive ROESY cross peaks between anomeric and other protons, $^3J_{C\text{-}H}$ constants, and dihedral angles of chitosan oligomers[a]

	DMSO			D_2O	
Dimer	[(H-αβ4)(H-n1)] $J_{C4\text{-}H1'}$ = 3.6 Hz ϕ = 47.3°	[(H-n3,n5)(H-n1)] $J_{C1'\text{-}H4}$ = 4.8 Hz φ = 35.7°		[(H-α4)(H-n1)] $J_{C4\text{-}H1'}$ = 1.2 Hz ϕ = 74.2°	[(H-n3,n5)(H-n1)] $J_{C1'\text{-}H4}$ < 1 Hz φ = 90°
Trimer	[(H-αβ4)(H-m1)] $J_{C4\text{-}H1'}$ = 3.3 Hz ϕ = 50.2°	[(H-m4,n5)(H-n1)] $J_{C1'\text{-}H4}$ = 4.9 Hz φ = 34.6°		[(H-α4)(H-m1)] $J_{C4\text{-}H1'}$ = 1.1 Hz ϕ = 76.2°	[(H-m4)(H-n1)] $J_{C1'\text{-}H4}$ < 1 Hz φ = 90°
Tetramer	[(H-αβ4)(H-m1)] [(H-m3)(H-m1)] $J_{C4\text{-}H1'}$ = 4.2 Hz ϕ = 41.6°	[(H-m4)(H-m1)] [(H-n5)(H-n1)] $J_{C1'\text{-}H4}$ = 4.9 Hz φ = 34.6°	[(H-m4)(H-n1)]	[(H-m4)(H-m1)] $J_{C4\text{-}H1'}$ < 1 Hz ϕ = 90°	[(H-m3)(H-m1)] $J_{C1'\text{-}H4}$ < 1 Hz φ = 90°
Pentamer	[(H-αβ4)(H-m1)] [(H-m3)(H-m1)] $J_{C4\text{-}H1'}$ = 3.6 Hz ϕ = 47.3°	[(H-m4)(H-m1)] [(H-n5)(H-n1)] $J_{C1'\text{-}H4}$ = 4.8 Hz φ = 35.7°	[(H-m4)(H-n1)]	[(H-m4)(H-m1)] $J_{C4\text{-}H1'}$ < 1 Hz ϕ = 90°	[(H-m3)(H-m1)] $J_{C1'\text{-}H4}$ < 1 Hz φ = 90°
Hexamer	[(H-αβ4)(H-m1)] $J_{C4\text{-}H1'}$ = 3.6 Hz ϕ = 47.3°	[(H-m4)(H-m1)] $J_{C1'\text{-}H4}$ = 4.0 Hz φ = 43.5°	[(H-m3)(H-m1)]	[(H-m4)(H-m1)] $J_{C4\text{-}H1'}$ = 3.6 Hz ϕ = 47.3°	[(H-m3)(H-m1)] $J_{C1'\text{-}H4}$ < 1 Hz φ = 90°

[a]α or β, reducing end α or β anomer residue; m, middle residue; n, non-reducing end residue.

Table 6. Phase-sensitive ROESY cross peaks between anomeric and other protons, $^3J_{C\text{-}H}$ constants, and dihedral angles of chitin oligomers[a]

	DMSO		D_2O	
Dimer	[(H-αβ4)(H-n1)] $J_{C4\text{-}H1'}$ = 4.6 Hz ϕ = 37.7°	[(H-n3,n5)(H-n1)] $J_{C1'\text{-}H4}$ = 3.9 Hz φ = 44.5°	[(H-α4)(H-n1)] $J_{C4\text{-}H1'}$ = 3.4 Hz ϕ = 49.3°	[(H-n3,n5)(H-n1)] $J_{C'\text{-}H4}$ = 4.5 Hz φ = 38.7°
Trimer	[(H-αβ4)(H-m1)] $J_{C4\text{-}H1'}$ = 4.5 Hz ϕ = 38.7°	[(H-m4)(H-n1)] $J_{C1'\text{-}H4}$ = 4.7 Hz φ = 36.7°	[(H-αβ4)(H-m1)] $J_{C4\text{-}H1'}$ = 3.6 Hz ϕ = 47.3°	[(H-m4)(H-n1)][(H-m5)(H-m1)] $J_{C'\text{-}H4}$ = 4.6 Hz φ = 37.7°
Tetramer	[(H-m4)(H-m1)] [(H-m4)(H-n1)][H-m3)(H-m1)] [(H-n5)(H-n1)]		[(H-m4)(H-m1)] [(H-m4)(H-n1)][H-m5)(H-m1)]	
Pentamer	[(H-m4)(H-m1)] [(H-m4)(H-n1)][H-m3)(H-m1)] [(H-m5)(H-m1)] [(H-n5)(H-n1)]		[(H-m4)(H-m1)] [(H-m4)(H-n1)] [(H-m5)(H-m1)] [(H-n5)(H-n1)]	
Hexamer	[(H-m4)(H-m1)] [(H-m4)(H-n1)]		[(H-m4)(H-m1)] [(H-m4)(H-n1)]	

[a]α or β, reducing end α or β anomer residue; m, middle residue; n, non-reducing end residue.

longer than 0.35 nm for pairs with the same sign and from 0.276 to 0.305 nm for the pairs with different signs. Comparing the diatomic distances between H-1 and H-3 or H-5 in the same residue with the crystallographic data, the +− pair should be selected. Since there were ROESY cross peaks not only between H-1 and H-4 in contiguous residues, but also between H-1 and H-3 or H-5 in the same residue, the distance between H-1 and H-4 closest to the distance between H-1 and H-3 or H-5 should be selected. For the +− pair, the dimer, trimer, and hexamer were part of a left-handed helical coil. This conformation is the same as that of chitosan in crystals with various anions, and the tetramer and pentamer had conformations similar to the anhydrous chitosan HCl salt crystal, but with larger ϕ and φ angles.

For chitosan oligomers in DMSO, the pairs of ϕ and φ with the same sign gave distances from 0.255 to 0.272 nm between H-1 and H-4 of the contiguous residue, while pairs with different signs gave distances from 0.223 to 0.231 nm. The distances of pairs with the same sign were closer to the distances between H-1 and H-3 or H-5, and the left-handed helical coil should have the pair −−. The pairs with different signs gave distances from 0.223 to 0.231 nm between H-1 and H-4 of the contiguous residue. These distances were too short compared with the distance between H-1 and H-3 or H-5 in the same residue in the anhydrous chitosan HCl crystal.

The crystallographic data for chitin oligomers suggest very different ϕ and φ angles. As shown in Table 7, the distances between H-1 and H-3 or H-5 of the reducing end residue and those of the non-reducing end residue of *N,N′*-diacetyl methyl β-chitobioside are different. However, the differences between H-1 and H-3 or H-5 of the non-reducing end of the β-chitobioside and that of the α-chitobioside are more similar. In the crystallographic data, the difference in the distances between H-1 and H-3 and between H-1 and H-5 is the smallest in β-chitin. Since the dimer in both solvents had ROESY cross peaks between H-1 and H-3 and between H-1 and H-5 in the non-reducing end residue, we used the crystallographic data for β-chitin. If the pairs of ϕ and φ with opposite signs were used, the distances between H-1 and H-4 in the contiguous residue of the dimer and

Table 7. Crystallographic data of distances between anomeric and other protons in the same residue (nm unit)

	N,N′-Diacetyl[7] methyl-α-chitobioside	*N,N′*-Diacetyl[8] methyl-β-chitobioside	β-Chitin[6]	Anhydrous chitosan HCl salt[3]
H1-H3	0.251 (Non-reducing end)	0.277 (Reducing end) 0.262 (Non-reducing end)	0.243	0.270
H1-H5	0.231 (Non-reducing end)	0.218 (Reducing end) 0.238 (Non-reducing end)	0.251	0.242
	$\phi = 36.3°$ $\varphi = 11.7°$	$\phi = 30.2°$ $\varphi = -44.9°$	$\phi = -23.5°$ $\varphi = 36.8°$	$\phi = 26.4°$ $\varphi = -29.7°$

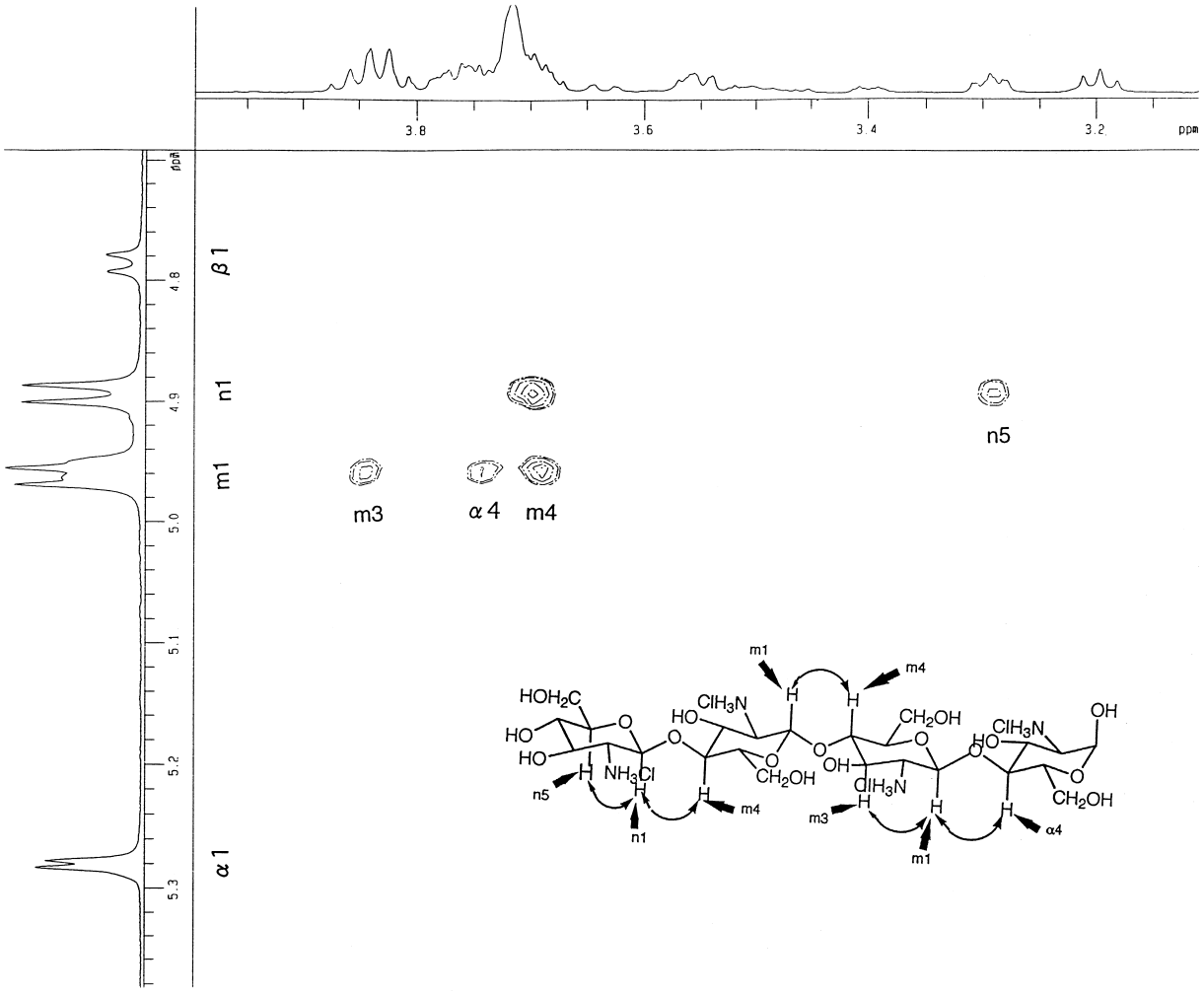

Figure 1. The ROESY spectrum of chitotetraose HCl salt in DMSO.

trimer in both solvents were shorter than 0.214 nm. On the other hand, pairs with the same sign had distances from 0.239 to 0.256 nm, which are much closer to the crystallographic distances between H-1 and H-3 or H-5 of β-chitin. For the crystallographic data, the sign pairs of ϕ and φ produce very different results. *N,N′*-Diacetyl methyl chitobiosides are probably more affected by the crystal field forces than β-chitin, which might have greater oligomeric or polymeric effects. Therefore, the $-+$ pair was selected. In D_2O, the chitin dimer and trimer had a left-handed coil conformation with this pair. In DMSO, however, the dimer was a right-handed coil, while the trimer was almost flat, like the crystal of the anhydrous chitosan HCl salt.

Experimental

Materials

Chitin oligomers were obtained by the method of Rupley[16] and chitosan oligomers were obtained by the method of Barker et al.[17]

Methods

Most NMR spectra were obtained using a JEOL Lambda 600 spectrometer and some were obtained on a JEOL Alpha 400 spectrometer at 600 MHz and 400 MHz for protons and 150 MHz and 100 MHz for carbon, respectively. The solvents used were 99.8% [$^2H_2$]O (D_2O) and

99% [$^2H_6$]-dimethylsulfoxide (DMSO). The chemical shift reference was sodium 3-trimethylsilyl-[$^2H_4$]-propionate (TSP) in both solvents. The DMSO solution contained 10 vol% D_2O for removing hydroxyl proton splitting on the sugar ring protons. The large HDO peak was suppressed by using the homo-gated decoupling method.

The spectra of chitosan HCl salt oligomers in both solvents were measured at 277 K because of their instability. Those of chitin oligomers were measured at an ambient temperature of around 295 K.

Phase-sensitive ROESY spectra were measured at least 600 Hz higher than the highest ring proton absorption for the observed irradiation,[15] and the mixing times were 30, 50, 70, 90, 120, and 150 ms with a 3.5 s waiting time between scans.[18]

The inter-residual $^3J_{C\text{-}H}$ constants were measured using the 2-D version method of Wilker and Leibfritz.[19] The Karplus type equation of Mulloy et al.[14] was compared with the crystallographic data and the experimental inter-residual $^3J_{C\text{-}H}$ constants for cyclo-hexa-, -hepta-, and -octa-amyloses.[20] The differences between the inter-residual $^3J_{C\text{-}H}$ constants in both solvents and between the inter-residual $^3J_{C\text{-}H}$ constants and the crystallographic data were within 0.1 Hz.

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