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HIGH RESOLUTION ^{13}C NMR SPECTRA OF CHITIN OLIGOMERS IN AQUEOUS SOLUTION

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^{13}C NMR spectra of chitin oligomers in aqueous solution were analyzed for a reference of assignment of solid state ^{13}C NMR spectra of chitin and N-acyl chitosans, and also of ^{13}C chemical shift data in aqueous solution.

In the preceding paper, [1] we have shown that measurements of solid state ^{13}C chemical shifts of chitin and N-acyl chitosans are very useful in predicting conformation of the latters in solid state with reference to that of the former. For this approach, it is essential to have a knowledge of displacement of the ^{13}C chemical shifts exhibiting conformation-dependent changes of this particular polymer, although our previous data[2] suggested that ^{13}C chemical shifts of carbons at the glucosidic linkage are very sensitive to conformational change. Here we attempted to record ^{13}C NMR spectra of chitin oligomers in aqueous solution, instead of chitin polymer insoluble in ordinary solvents, to obtain unambiguous assignment of ^{13}C chemical shifts and a reference of those in aqueous solution.

N,N-Diacetyl chitobiose and chitin oligomers were prepared by partial hydrolysis of chitin by hydrochloric acid, followed by gel-filtration by a Bio-Gel P-2 column and selective N-acetylation.[3] ^{13}C NMR spectra were recorded by a Bruker CXP-300 spectrometer at 75.46 MHz.

Figure 1 shows ^{13}C NMR spectra of 2-acetamido-2-deoxy-D-glucopyranose (monomer), N, N-diacetyl chitobiose (dimer), and chitin oligomers (DP 2-10) in aqueous solution. The previous assignment of the C-3 and C-5 shifts[4] in the α-anomer of the monomer should be reversed (Table 1), as revealed by the method of deuterium-induced isotope shifts using double coaxial tube.[5] We assigned ^{13}C peaks of N, N-diacetyl chitobiose and chitin oligomers by considering effect of displacement of ^{13}C shifts due to formation of glucosidic linkages[6] and also the intensity ratio of

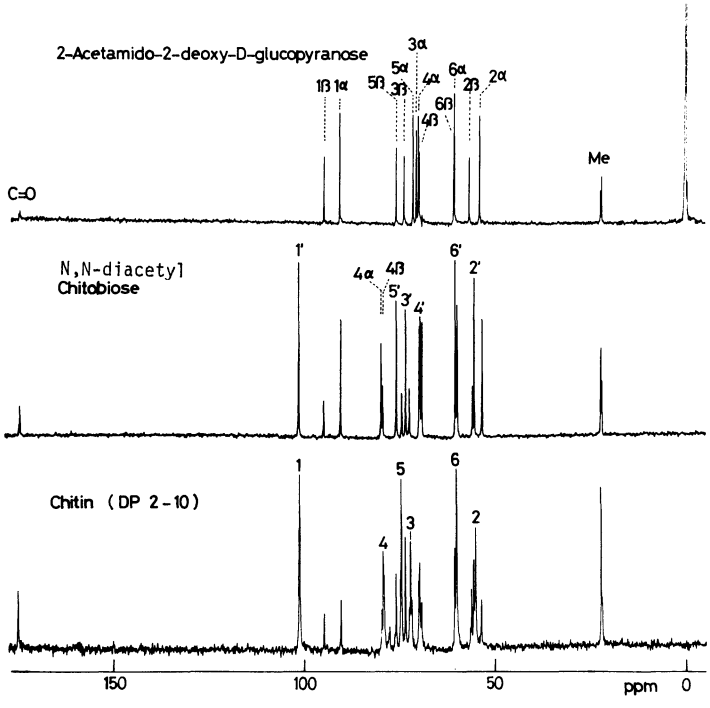

Figure 1. 75.46 MHz ^{13}C NMR spectra of 2-acetamido-2-deoxy-D-glucopyranose (top), N,N-diacetyl chitobiose (middle), and chitin oligomers (bottom). pH 7; 20 mg/ml; ~2000 transients.

α-anomer to β-anomer with reference to those of the monomer. The prime refers to the residue close to the reducing end. It is not attempted to assign all the carbon signals of unfractionated chitin oligomers. Instead, peaks related to chitin polymer (not primed) were chosen as those giving the maximum peak heights among the cluster of peaks of respective carbons. For this approach, peaks arising from the residues of the reducing ends can be easily subtracted from those of N, N-diacetyl chitobiose.

As expected, only ^{13}C chemical shifts of the C-1 and C-4 signals at the glucosidic linkages are uniquely displaced upfield, compared with those of solid-state chitin as recorded by cross-polarization/magic angle spinning ^{13}C NMR method.[1] The presence of such unique differences of the ^{13}C chemical shifts was explained by that chitin oligomers in aqueous solution take different conformation from that of solid chitin.[1] It is also expected that comparison of the ^{13}C chemical shifts of chitin oligomers with those of N-acyl chitosan gel reveals conformation of the gel sample for the portion other than cross links.[2] Further works along with this line will be published shortly.

Table 1. Assignment of ^{13}C chemical shifts of chitin oligomers (pH 7, ppm from ext. TMS)

	2-acetamido-2-deoxy-D-gluco-pyranose[7]	N,N-diacetyl chitobiose	Chitin oligomers (DP 2-10)
C-1			101.4
C-1α	91.0	90.6	90.6
C-1β	95.1	95.0	95.0
C-1'		101.6	101.6
C-2			55.2
C-2α	54.2	53.7	53.8
C-2β	56.7	56.3	56.3
C-2'		55.8	55.7
C-3			72.3
C-3α	70.9	69.4	69.4
C-3β	74.0	72.6	72.6
C-3'		73.6	73.6
C-4			79.4
C-4α	70.3	80.1	79.9
C-4β	70.1	79.7	79.2
C-4'		69.9	69.9
C-5			74.7
C-5α	71.7	70.1	70.1
C-5β	76.1	74.7	74.8
C-5'		76.0	76.1
C-6			60.2
C-6α	60.8	60.2	60.2
C-6β	60.9	60.3	60.2
C-6'		60.8	60.7
C=O	174.7[a] 174.8[b]	174.7	174.7
CH_3	22.0[a] 22.3[b]	22.0 22.3	22.3

[a] α-anomer, [b] β-anomer.

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7) There appears difference of chemical shifts observed by super-conducting system with those observed in iron magnet system by amount of 0.4-0.6 ppm as referenced to external TMS.

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