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CONFORMATION OF CHITIN AND N-ACYL CHITOSANS IN SOLID STATE AS REVEALED BY ^{13}C CROSS POLARIZATION/MAGIC ANGLE SPINNING (CP/MAS) NMR SPECTROSCOPY

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^{13}C chemical shifts of solid chitin and N-acyl chitosans were measured by cross polarization/magic angle spinning (CP/MAS) NMR spectroscopy. It was found that a regenerated chitin, N-acetyl chitosan, adopts conformation almost identical to that of naturally occurring chitin in solid state. Conformation of N-decanoyl and N-stearoyl chitosans is also similar to that of chitin except for the moiety of amide groups.

Chitin is a cellulose-like polysaccharide, insoluble in ordinary solvents, consisting of β-(1→4)-linked 2-acetamido-2-deoxy-D-glucose residues found as in fungal and bacterial cell walls, in insect cuticles and in the shell of crustaceans.[1] In contrast, a regenerated chitin, N-acetyl chitosan, exhibits a distinct property to form transparent, rigid gel in aqueous media.[2-6] A number of analogs of gel-forming regenerated chitins were prepared by N-acylation of chitosan derived from N-deacetylation of chitin with alkali. It appears to be very important to clarify molecular architecture of N-acyl chitosans in solid and gel states, in order to gain a clue in understanding of such an obvious distinction of physical properties between naturally occurring and regenerated chitins.

Generally, polysaccharide gels are composed of the three-dimensional networks formed by the cross-links. Such cross-links are formed by association of polymer chains with ordered conformation.[7,8] As an excellent tool in studying conformation of polysaccharides, we previously demonstrated that ^{13}C chemical shifts of carbons at the glucosidic linkages are substantially changed depending on the conformation of polysaccharides in solution, gel and solid states.[9-15] For this approach, however, it is essential to have a knowledge of ^{13}C chemical shifts of fixed conformer as observed in solid state, as a reference of the conformation-dependent ^{13}C chemical shifts.[13-15]

Here, we present a high resolution ^{13}C NMR study of solid chitin and N-acyl chitosans using cross polarization/magic angle spinning (CP/MAS) NMR spectroscopy, to reveal conformational behavior of naturally occurring and regenerated chitins.

Solid N-acyl chitosan samples were used after lyophilization of chitosan gels prepared by the method described previously.[2-6] Naturally occurring chitins were prepared from crab shells of Chionecetes opilio,O.Fabrcius and also purchased from Sigma Chemical Company (also from crab shells). ^{13}C CP/MAS NMR spectra were recorded by a Bruker CXP-300 spectrometer at 75.46 MHz with a CP/MAS accessary. Contact time was chosen as 1 ms and repetition time was 2 s. Samples(ca. 300 mg) were contained in an Andrew-Beams type rotor machined from perdeuterated poly(methyl methacrylate) and

spun as fast as 3-4 kHz.

Figure 1 shows ^{13}C CP/MAS NMR spectra of chitin and N-acyl chitosans in solid state. Obviously, all carbon signals of chitin (Figures 1A and 1B) are well resolved, resulting in a good quality of high resolution ^{13}C NMR spectra comparable to those of aqueous solution. Assignment of peaks given in Figure 1 is straightforward, in comparison with that of water-soluble lower molecular-weight oligomers of chitin (see accompanying paper[16]).

Interestingly, ^{13}C chemical shift positions of lyophilized N-acetyl chitosan are almost identical to those of naturally occurring chitin within the experimental error (± 0.5 ppm)(see Table 1), although the C-3 and C-5 signals are not resolved in the former. This finding implies that conformational property of a regenerated chitin, N-acetyl chitosan, is very similar to that of naturally occurring chitin. It should be mentioned that ^{13}C chemical shift is more sensitive to conformation of the individual polymer chain than the extent of molecular packing to the crystalline region.[14,15] The effect of the latter can be seen indirectly as the line-broadening or small splitting of peaks, depending on the extent of the dispersion of chemical shifts arising from polymer chains of slightly different conformations, if crystallinity is very low. Thus, as far as crystallinity is concerned, naturally occurring chitin samples are much better than the lyophilized N-acetyl chitosan as judged from the linewidths.

The peak-intensities of sugar carbons of N-decanoyl and N-stearoyl chitosans were diminished compared with those of chitin and N-acetyl chitosan, because relative amount of the sugar moiety is simply decreased in the presence of bulky fatty acyl groups. In addition, it appears that a plausible poor crystalline packing results in the increased linewidths or smaller splitting of peaks.[18] As pointed out previously,[15] ^{13}C chemical shifts at the glucosidic linkages (C-1 and C-4, in this case) are very sensitive to the conformational angles around the glucosidic linkages (ϕ and ψ). In this connection,

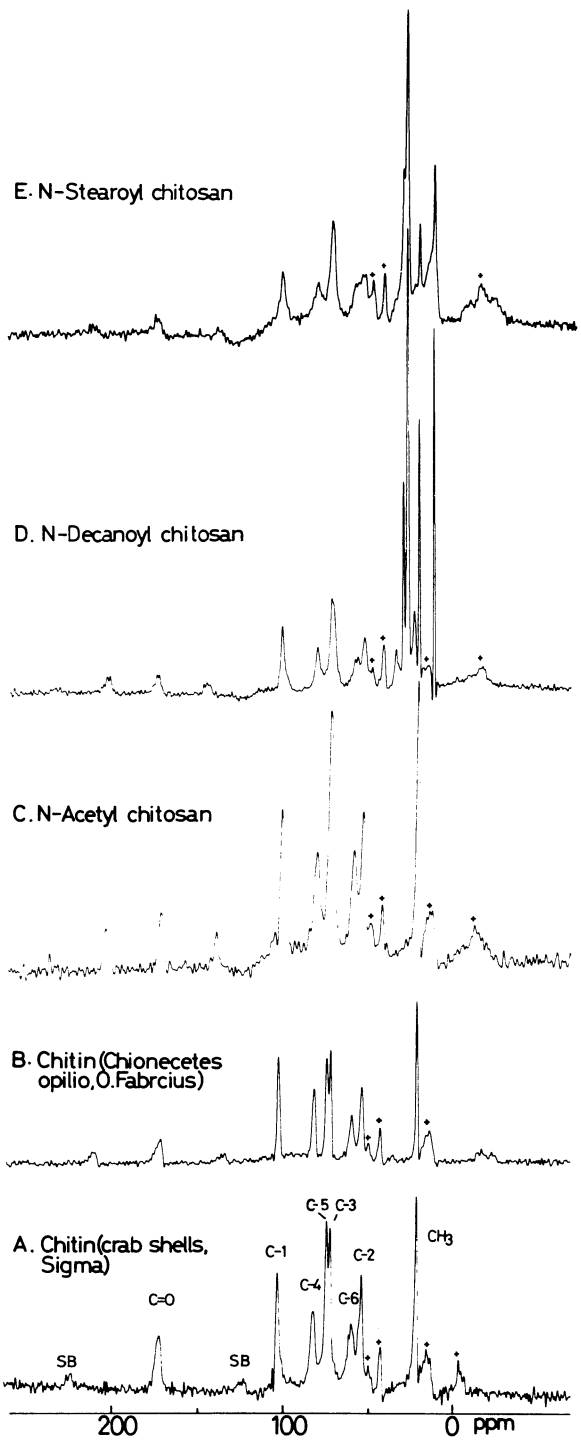

Figure 1. 75.46 MHz ^{13}C CP/MAS NMR spectra of solid chitin and N-acyl chitosans. Peaks marked by + are from the rotor and probe. Number of transients are: 850(A), 915(B), 660(C), 1213(D) and 5000(E). Sharp peaks at 0-40 ppm in D and E are from the fatty acyl moiety.

Table 1. Comparison of ^{13}C chemical shifts of chitin and N-acyl chitosans in solid state with those of aqueous solution (ppm from TMS, ± 0.5 ppm for CP/MAS)

	Solid state					Aqueous solution
	chitin (crab shell, Sigma)	chitin (crab shell, Chionecetes opilio)	N-acetyl chitosan	N-decanoyl chitosan	N-stearoyl chitosan	chitin oligomers [b] (DP 2-10)
C-1	103.7	103.9	103.3	103.4	103.8	101.4
C-2	55.2	54.6	55.2	55.2	56.8 55.2	55.2
C-3	73.2	73.2	74.5[a]	73.7[a]	74.8[a]	72.3
C-4	83.5	83.0	83.1	83.2	83.2	79.4
C-5	75.6	75.4	74.5[a]	74.9[a]	74.8[a]	74.7
C-6	60.6	60.7	60.5	60.7 59.3	60.8 59.3	60.2
CH_3	22.6	22.4	22.7	-	-	22.3
C=O	173.7	172.9	173.3	176.6	176.6	174.7

[a] C-3 and C-5 signals are overlapped. [b] Data taken from Ref. 16.

it appears that the conformational angles of N-decanoyl and N-stearoyl chitosans are not strongly deviated from those of chitin and N-acetyl chitosan, as viewed from the similarity of the ^{13}C chemical shifts of the C-1 and C-4 carbons (see Table 1). However, it is clearly seen that the carbonyl ^{13}C shifts of N-decanoyl and N-stearoyl chitosans are substantially displaced downfield (ca. 3 ppm) compared with those of naturally occurring and regenerated chitins. For α-chitin, the most common crystal structure of chitin, X-ray diffraction studies by Carlstrom and others[19-21] revealed that an intermolecular NH--CO hydrogen bond is formed between the amide groups which lies almost perpendicular to the fiber axis. Thus, it is plausible that such a hydrogen bond system might be distorted by the presence of bulky acyl groups in N-decanoyl and N-stearoyl chitosans. However, the observed downfield displacements mentioned above are inconsistent with the general trend observed in solution that the carbonyl ^{13}C shift is displaced downfield by formation of hydrogen bond.[22] Further works are necessary to clarify this problem.

Further, it is interesting to compare the ^{13}C chemical shifts of solid chitin with those of chitin oligomers recorded in aqueous solution. The ^{13}C chemical shifts of solid and solution states are in good agreement with slight experimental deviation with < 1 ppm, except for the C-1 and C-4 peaks (Table 1). The differences of the ^{13}C chemical shifts of the C-1 and C-4 carbons between samples of solid and aqueous solution are 2.3 and 4.1 ppm, respectively, and comparable to those obtained in other polysaccharides such as (1→3)-β-D- and (1→4)-α-D-glucans.[14,15] This finding is consistent with a view that the C-1 and C-4 carbons at the glucosidic linkages in chitin exhibit conformation-dependent change of ^{13}C shifts, as pointed out already. In particular, it is obvious that chitin oligomers in aqueous solution take conformational behavior like random-coil due to the rapid conformational isomerism around the glucosidic linkages within NMR time scale ($< 10^{-3}$s), as estimated on the basis of the well known formula of chemical exchange.[15,23]

Finally, it is most probable, on the basis of the ^{13}C CP/MAS NMR data mentioned above, that cross-links of N-acyl chitosan gels are primarily formed by the association of polymer chains adopting conformation similar to that of naturally occurring chitin. The extent of such association, however, is not necessary the same as that of naturally occurring chitin, as mani-

fested from the difference in the linewidths between two kinds of samples. On the contrary, only small amount of cross-links is sufficient to retain property of gels, as inferred from our previous work of gels of synthetic polymers.[24] Thus, one of the striking difference in physical property between naturally occurring and regenerated chitin samples might be explained by these packing properties.

In conclusion, it is shown that measurement of ^{13}C NMR spectra of chitin and N-acyl chitosans in solid state is very useful in clarifying conformation of polymer chains in solid and the portion relevant to the cross-links in gel state.

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17) There appear the spinning side-bands (marked by SB) with equal spacing around the carbonyl signals. This is obviously caused by the insufficient magic angle spinning rate compared with the increased width of the anisotropy of ^{13}C shifts under high frequency condition. This interference, however, is not serious for our present purpose, because such pairs are easily discriminated and are not overlapped with any important signals.
18) It appears that the C-6 signals of N-decanoyl and N-stearoyl chitosans, and the C-2 signals of the latter, are split into doublets. A plausible explanation for the former splittings may be that there exist at least two types of orientation in the hydroxymethyl groups (see Ref.15).
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