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Distribution of the Acetamide Group in Partially Deacetylated Chitins

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ABSTRACT

The distribution of the acetamide group in partially deacetylated chitins (DAC) was investigated by nitrous acid deamination. Deamination products of various DAC (DAC-66, DAC-77, DAC-84 and DAC-91) were mainly trisaccharide, disaccharide, and monosaccharide. These results would suggest random distribution of the acetamide group in the DAC molecule prepared by heterogeneous deacetylation.

INTRODUCTION

Chitin, a natural and (1,4)-linked polysaccharide composed of 2-acetamide-2-deoxy-β-D-glucopyranose residues, is widely distributed in nature as the cell wall of bacteria and the skeletal material of crustaceans, insects, etc. Partially deacetylated chitins (DAC) were prepared by alkaline deacetylation of chitin (Tokura *et al.*, 1986) or *N*-acetylation of chitosan (Hirano & Yamaguchi, 1976). Sannan *et al.* (1976) prepared water-soluble DAC (45–55% of deacetylation) by homogeneous deacetylation of chitin. On the other hand, DAC prepared by heterogeneous deacetylation were insoluble. The difference of water-solubility is presumed to be due to the distribution of the acetamide group in the DAC molecule (Kurita *et al.*, 1977). However, there has been little study with respect to the distribution of the acetamide group in the DAC molecule.

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In this study, the distribution of the acetamide group in DAC prepared by heterogeneous deacetylation was investigated by nitrous acid deamination and gel permeation chromatographic (GPC) method.

EXPERIMENTAL

Various DAC (Wako Pure Chemical Industries, Ltd) were prepared from chitin powder by the heterogeneous deacetylation in 40 wt% aqueous NaOH at 100°C for various time intervals. The degree of deacetylation of DAC was evaluated by the elemental analysis and IR method (Sannan *et al.*, 1978). *N*-Acetyl-D-glucosamine oligomer $(GlcNAc)_n$ standards ($n = 2-6$) were purchased from Seikagaku Kogyo Co., Ltd. 2,5-Anhydro-D-mannitol was prepared from D-glucosamine by the method of Horton & Philips (1973).

To a 10% acetic acid solution (50 ml) of DAC (0·5 g) was added 5% aqueous $NaNO_2$ (15 ml), and the mixture was stirred for 3 h at 2–4°C followed by standing for 40 h at room temperature. Insoluble materials were removed by centrifugation (15000 rev/min) and the pH of the supernatant was adjusted to 5·5 with Amberlite IRA-400 (OH^- form, 50 ml). To the supernatant was added 6% aqueous $NaBH_4$ (5 ml), and the solution was stirred for 1 day at room temperature followed by desalting with Amberlite IR-120B (H^+ form, 100 ml) and Amberlite IRA-400 (OH^- form, 100 ml). The desalted solutions were concentrated to 10 ml and stored at 2–4°C after filtration. The deamination products of DAC were recovered quantitatively from the desalted solutions and insoluble materials.

The molecular weight of the deamination products was determined by means of GPC with *N*-acetyl-D-glucosamine oligomer standards and 2,5-anhydro-D-mannitol on a Shimadzu LC-6A apparatus equipped with a Shimadzu RID-6A RI detector (column: Hitachi GL-W520 (10·7 mm × 300 mm); eluent: distilled water; flow rate: 1 ml/min; column temperature: 50°C).

RESULTS AND DISCUSSION

Nitrous acid deamination is a well-known reaction for the structural analysis of mucopolysaccharides (Foster *et al.*, 1963; Wolfrom *et al.*, 1969; Isemura & Schmidt, 1971; Horton & Philips, 1973; Hirano *et al.*, 1985). In this paper, nitrous acid deamination was performed to study the distribution of the acetamide group in the DAC molecule in 10%

aqueous acetic acid. A typical gel permeation chromatogram of deamination products is shown in Fig. 1. The product ratio (%) of deamination products is given by the equation: the peak area/total peak area in the chromatogram. The results are summarized in Table 1. The

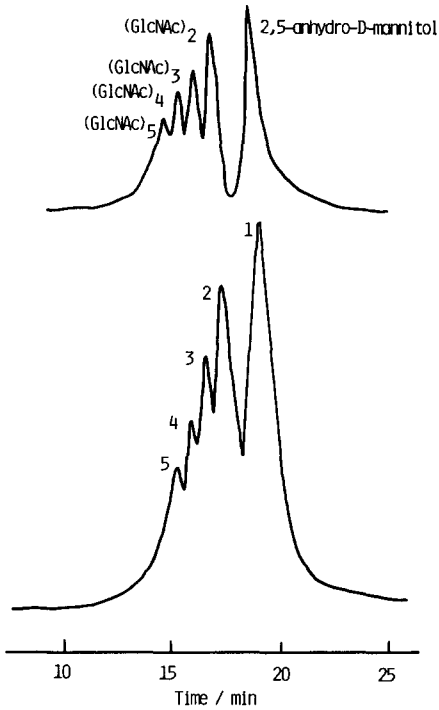


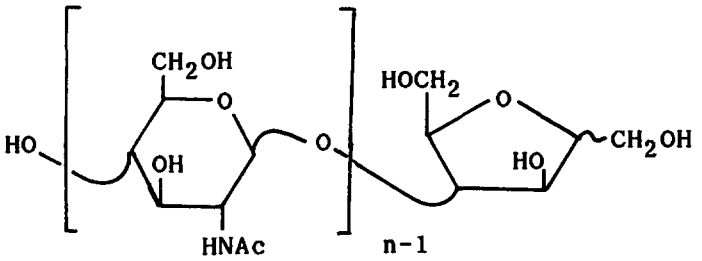
Fig. 1. Gel permeation chromatogram of deamination products of DAC-66.

TABLE 1
Deamination of Various DAC

DAC[a]	*Product*[b] *(%)*					
	Polymer[c]	5	4	3	2	1
DAC-66	4·7	9·3	6·7	15·5	26·8	37·0
DAC-77	3·4	0·6	4·4	10·8	25·2	55·6
DAC-84	3·0	—	3·5	8·4	19·2	65·9
DAC-91	2·9	—	—	6·6	17·6	72·9

[a]DAC-66 means the 66% deacetylated chitin.
[b]Calculated from peak area of GPC.
[c]Water-insoluble material (wt%).

main product obtained by the deamination of the 91% deacetylated chitin (DAC-91) was monosaccharide (1). The composition (%) of di- (2), tri- (3), tetra- (4), and penta-saccharide (5) increased with a decrease in the degree of deacetylation. After deamination of DAC, there were water-insoluble materials (polymer) of 3–5 wt%, of which IR spectra were similar to those of DAC used in this deamination reaction. Since deamination products of 66% or above deacetylated chitin were oligomers less than five residues, these DAC would have random distribution of the acetamide group.



1 : n=1
2 : n=2
3 : n=3
4 : n=4
5 : n=5

In order to make this statement, furthermore, a statistical analysis was performed according to the method of Bernoullian statistics (Table 2). For oligomers 1–5, the calculated values (Table 2) are in excellent agreement with the experimental values (Table 1). These results would support a random distribution of the acetamide group in the DAC molecule. We would have to say that the method described above is preferable as one of the procedures to clarify how the acetamide group is distributed in the DAC molecule.

Table 3 shows the composition of a GlcNAc segment in DAC which was calculated from the product ratio (%) in Table 1 and the degree of *N*-acetylation which was calculated from the proportion (wt%) of GlcNAc residue obtained by summation of the GlcNAc segment composition. These values agreed approximately with those evaluated by the elemental analysis and IR method. Hence we would like to propose this procedure to calculate the degree of *N*-acetylation.

It was reported that the deacetylation under the heterogeneous condition proceeded preferentially in the amorphous region to give block-type copolymers of *N*-acetyl-D-glucosamine and D-glucosamine units (Kurita *et al.*, 1977). Our results, however, suggest that the acetamide group distributes at random in 66% or above DAC prepared by heterogeneous deacetylation. These methods and results would assist in resolving the mechanism on the immunoadjuvant activities through mouse peritoneal macrophage activation (Nishimura *et al.*, 1985) and lysozyme suscepti-

TABLE 2
The Weight Fraction (W_n) of GlcNAc Oligomers[a]

DAC	M_0	W_n (%)				
		5	4	3	2	1
DAC-66	175·3	3·2	7·5	16·4	31·0	40·8
DAC-77	170·7	0·9	3·3	10·5	29·3	57·0
DAC-84	167·7	0·3	1·3	6·1	24·7	69·0
DAC-91	164·9	—	0·3	2·3	16·6	82·4

[a]The weight fraction (W_n) of each oligomer was calculated from the following equation:

$$W_n = n \times F_D^2 \times F_A^{n-1} \times M_n \times (n \times M_0)^{-1}$$

where n = oligomer (1–5), F_D = degree of deacetylation, F_A = degree of acetylation, M_n = molecular weight of the nth oligomer (1, 164; 2, 367; 3, 570; 4, 773; 5, 976) and M_0 = average molecular weight of monomer in polymer.

TABLE 3
Composition of GlcNAc Segments in DAC[a]

DAC	*Composition (wt%)*				*Degree of N-acetylation*[b] *(%)*
	$(GlcNAc)_4$	*$(GlcNAc)_3$*	*$(GlcNAc)_2$*	*GlcNAc*	
DAC-66	7·7	5·2	11·0	14·8	33 (34)
DAC-77	0·5	3·5	7·7	14·0	22 (23)
DAC-84	—	2·7	5·9	10·6	16 (14)
DAC-91	—	—	4·6	9·8	12 (9)

[a]Calculated from the equation as follows:

$$(GlcNAc)_{n-1}(wt\%) = W_n(\%) \times \{203(n-1)+1\}/\{203(n-1)+164\}$$

[b]Based on the proportion (wt%) of GlcNAc residue obtained by summation of GlcNAc segment compositions. Parentheses mean the degree of *N*-acetylation evaluated by the elemental analysis and IR method.

bility (Sashiwa *et al.*, 1990) of various DAC prepared by heterogeneous deacetylation.

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