N-Acylchitosan Gel Preparation of Partially and Heterogeneously N-Acylated Chitosans

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A series of partially and heterogeneously *N*-acylated chitosans was prepared and isolated in 50~100% yields. The structure of *N*-acyl groups influenced the gelation. The minimum requirement for the gelation was defined as *ca.* 0.4 *N*-lauroyl (C_{12}), *ca.* 0.6 *N*-fatty acyl (C_8 – C_{10}) or *ca.* 0.7 *N*-benzoyl groups per hexosaminide residue. However, the gelation did not occur with *N*-high fatty acyl (C_{14} – C_{18}) groups.

N-Acylchitosan gel^{1,2)} was originally prepared in our laboratory from chitosan [(1→4)-2-amino-2-deoxy- β -D-glucan]. Partially *N*-acylated chitosans are interesting polymers for the study of the formation of polyionic complexes with specific acidic compounds and with the surface of some specific living cells.³⁾

We now wish to report a series of partially and heterogeneously *N*-acylated chitosans prepared for the purpose of studying the gelation mechanism.

RESULTS AND DISCUSSION

As shown in Table I, a series of partially *N*-acylated chitosans was prepared with 0.75 molar equivalent or less of carboxylic anhydrides per hexosaminide residue⁴⁾ and isolated in 69~100% yields as hydroacetate. The presence of *N*-acylamino groups in these products was reflected by IR absorptions at 1650 and 1550 cm^{-1} (C=O and NH in *N*-acyl) and at *ca.* 2900 cm^{-1} (CH in fatty acyl).

Table II summarizes CD and ORD data of the partially *N*-benzoylated derivatives of chitosan and *N*-desulfated heparin. Each of these derivatives showed a similar Cotton effect owing to the *N*-benzoyl chromophore, but the Cotton effect of *N*-benzoylchitosans was negative and that of *N*-benzoylheparin was positive (Fig. 1). The d.s. values for the *N*-benzoyl group per hexosaminide residue are almost parallel, with regard to 2-benzamido-2-deoxy-D-glucopyranoside residue, to the

Fig. 1. CD and ORD Spectra of *N*-Benzoylheparin (the d.s.=0.82) and *N*-Benzoylchitosan (the d.s.=0.56).

-----, CD curve; —, ORD curve; ○, *N*-benzoylheparin; ●, *N*-benzoylchitosan. Analysis was performed in 0.01 *N* HCl (*c*=0.1%) at 17°C, using a cell of 1-mm path length.

absolute values of $[\alpha]_{242}^{17}$ for *N*-benzoylheparin and of $[\alpha]_{250}^{17}$ for partially *N*-benzoylated chitosans, and to those of $[\theta]_{230}^{17}$ for *N*-benzoylheparin and of $[\theta]_{235}^{17}$ for partially *N*-acylated chitosans. This result accords with an α -configuration of the 2-benzamido-2-deoxy-D-glucopyranoside residue in heparin and with a β -configuration of that in chitosan.

As indicated by GLC, NMR and elemental analysis data (Table I), 60~100% of carboxylic anhydride added was used for *N*-acylation

TABLE I. SOME PARTIALLY AND HETEROGENEOUSLY N-ACYLATED CHITOSANS

N-Acyl group	The d. s. values for N-acyl group ^{a)}		Yield (%)	Gelation ^{b)}	Solubility in 10% acetic acid ^{c)}	$[\alpha]_D^{17}$ degree (<i>c</i> =10% acetic acid)	Nitrogen (%) ^{d)}	
	GLC	NMR					Calcd.	Found
Propionyl	0.19 (0.25)	0.18	99	—	s	—16.3 (1.1)	6.36	6.61
Propionylbutyryl	0.19/0.81		90	+	i		6.13	6.00
Propionylmyristoyl	0.14/0.86		77	ppt.	i		4.00	4.36
Propionyl	0.32 (0.50)	0.35	98	—	s	—10.8 (1.0)	6.37	6.90
Propionylbutyryl	0.33/0.67		90	+	i		6.18	6.51
Propionylmyristoyl	0.30/0.70		80	ppt.	i		4.31	4.61
Propionyl	(0.75)		91	±	i			n. d.
Butyryl	0.27 (0.25)	0.25	77	—	s	—6.7 (1.1)	6.26	6.70
Butyrylhexanoyl	0.16/0.84		85	+	i		5.50	5.80
Butyrylpalmitoyl	0.27/0.73		66	ppt.	i		3.96	4.10
Butyryl	(0.50)	0.36	100	—	s		6.23	6.61
Butyryl	(0.75)		99	±	i			n. d.
Hexanoyl	0.15 (0.25)	0.13	100	—	s	n. d.	6.17	6.50
Hexanoyloctanoyl	0.15/0.85		54	+	i		4.95	5.31
Hexanoylstearyl	(0.25/1.75)		98	ppt.	i			n. d.
Hexanoyl	(0.50)	0.40	64	—	s		5.85	5.98
Hexanoyl	(0.75)		94	±	i			n. d.
Octanoyl	0.16 (0.25)	0.17	91	—	s	—19.1 (1.1)	6.03	6.31
Octanoyldecanoyl	0.14/0.86		93	+	i		4.50	4.81
Octanoylbenzoyl	0.16/0.84		93	+	i		5.17	5.03
Octanoyl	0.30 (0.50)		89	—	s	—8.8 (1.1)	5.81	6.36
Octanoylhexanoyl	0.30/0.70		88	+	i		5.23	5.54
Octanoylstearyl	(0.50/1.50)		100	ppt.	i			n. d.
Octanoyl	(0.75)		69	±	i			n. d.
Decanoyl	0.18 (0.25)		75	—	s	—11.2 (0.98)	5.88	5.96
Decanoylpropionyl	0.18/0.82		92	+	i		4.12	4.01
Decanoyllauroyl	0.13/0.87		91	+	i		5.97	6.06
Decanoyl	0.36 (0.50)		82	—	s	—4.7 (0.43)	5.49	5.77
Decanoyloctanoyl	0.24/0.36		82	—	i			n. d.
Decanoylpalmitoyl	0.36/0.64		78	ppt.	i		3.79	3.96

Decanoyl	(0.75)	74	±	i	—	—	5.62	n. d.
Lauroyl	0.23 (0.25)	85	—	s	—	7.4 (0.40)	5.93	5.93
Lauroylbutyryl	0.16/0.84	88	+	i	—	—	5.62	5.68
Lauroylmyristoyl	0.23/0.77	81	+	i	—	—	3.84	3.91
Lauroyl	(0.50)	85	±	i	—	—	—	n. d.
Lauroyl	(0.75)	86	—	i	—	—	—	n. d.
Myristoyl	0.25 (0.25)	69	—	s	—	—	6.30	6.51
Myristoylhexanoyl	(0.25/1.75)	75	+	i	—	—	—	n. d.
Myristoylpalmitoyl	0.25/0.75	78	ppt.	i	—	—	3.96	3.91
Myristoyl	(0.50)	84	ppt.	i	—	—	—	n. d.
Myristoyl	(0.75)	71	ppt.	i	—	—	—	n. d.
Palmitoyl	(0.25)	87	ppt.	i	—	—	—	n. d.
Palmitoyl	(0.50)	52	ppt.	i	—	—	—	n. d.
Palmitoyl	(0.75)	65	ppt.	i	—	—	—	n. d.
Stearoyl	(0.25)	66	ppt.	i	—	—	—	n. d.
Stearoyl	(0.50)	70	ppt.	i	—	—	—	n. d.
Stearoyl	(0.75)	62	ppt.	i	—	—	—	n. d.
Benzoyl ^{a)}	0.19*	n. d. ^{f)}	—	s	—	n. d.	7.29	7.29
Benzoyl	0.24 (0.25)	83	—	s	—	n. d.	6.93	7.01
Benzoylpropionyl	0.24/0.76	89	+	i	—	—	—	n. d.
Benzoylmyristoyl	0.24/0.76	50	ppt.	i	—	—	—	n. d.
Benzoyl ^{b)}	(0.40) 0.37*	n. d.	—	i	—	—	6.73	6.73
Benzoyl	0.44 (0.50)	85	—	i	—	—	5.82	6.21
Benzoylpropionyl	0.44/0.56	99	+	i	—	—	5.88	5.80
Benzoyllauroyl	0.37/0.63	95	+	i	—	—	4.45	4.35
Benzoyl ^{b)}	(0.60) 0.56*	n. d.	—	s	—	—	6.26	6.31
Benzoyl	(0.75)	80	±	i	—	—	—	n. d.

^{a)} The d.s. values, which were not determined, indicate the amounts of carboxylic anhydrides added as shown in the brackets. * indicates the d.s. values determined by the elemental analysis. ^{b)} + gel formed; — gel not formed; ppt. precipitate formed. ^{c)} i, insoluble. s, soluble. ^{d)} The nitrogen values of partially N-acylated chitosans are calculated for $[(C_6H_{10}NO_2)(C_2H_5O_2)]_n$, in which y is an observed d.s. value for the corresponding acyl group and $x + y$ equals 1.00. The nitrogen values of heterogeneously N-acylated chitosans are calculated for $[(C_6H_{10}NO_2)(C_7H_9O_2)]_n$ (fatty acyl) or $[(C_6H_{10}NO_2)(C_8H_7O_2)]_n$ (aromatic acyl) in which x and y are the observed d.s. values for the corresponding acyl group. ^{e)} Calcd. for $[(C_6H_{10}NO_2)(C_7H_9O_2)]_n$ (fatty acyl) or $[(C_6H_{10}NO_2)(C_8H_7O_2)]_n$ (aromatic acyl) group. ^{f)} n.d., not determined. ^{g)} Calcd. for $[(C_6H_{10}NO_2)(C_7H_9O_2)]_n$ (fatty acyl) or $[(C_6H_{10}NO_2)(C_8H_7O_2)]_n$ (aromatic acyl) group. ^{h)} Calcd. for $[(C_6H_{10}NO_2)(C_7H_9O_2)]_n$ (fatty acyl) or $[(C_6H_{10}NO_2)(C_8H_7O_2)]_n$ (aromatic acyl) group. ⁱ⁾ Calcd. for $[(C_6H_{10}NO_2)(C_7H_9O_2)]_n$ (fatty acyl) or $[(C_6H_{10}NO_2)(C_8H_7O_2)]_n$ (aromatic acyl) group. ^{j)} Calcd. for $[(C_6H_{10}NO_2)(C_7H_9O_2)]_n$ (fatty acyl) or $[(C_6H_{10}NO_2)(C_8H_7O_2)]_n$ (aromatic acyl) group.

TABLE II. CD AND ORD DATA OF THE PARTIALLY *N*-BENZOYLATED DERIVATIVES OF CHITOSAN AND *N*-DESULFATED HEPARIN^(a)

Compound	ORD						CD		UV		
	Peak (nm)	$[\alpha]_{\text{peak}}^{17}$ (degree)	Trough (nm)	$[\alpha]_{\text{trough}}^{17}$ (degree)	Amplitude (degree)	Inflection point(nm)	Sign	Peak (nm)	$[\theta]_{\text{peak}}^{17}$ (degree)	Sign	$\lambda_{\text{max or min}}$ (nm)
<i>N</i> -Benzoylchitosan d.s.=0.19 ^{b)} d.s.=0.37 d.s.=0.56	223	-380	250	-410	30	236.5	-	235	-380	-	236.5
	223	0	250	-640	640	236.5	-	235	-820	-	236.5
	223	+200	250	-820	1020	236.5	-	235	-1180	-	236.5
<i>N</i> -Benzoylheparin d.s.=0.82	242	+740	220	-200	940	231	+	230	+1100	+	231

^{a)} Analyzed in 0.01 *N* hydrochloric acid ($c=0.1\%$) at 17°C.^{b)} The d.s. value/hexosaminide residue.

under the present conditions. The minimum requirement for the gelation was defined as *ca.* 0.4 *N*-lauroyl (C_{12}), *ca.* 0.6 *N*-fatty acyl (C_8 - C_{10}) or *ca.* 0.7 *N*-benzoyl groups per hexosaminide residue. The gelation did not occur with *N*-high fatty acyl (C_{14} - C_{18}) groups. The inhibition of the gelation may be due to steric hindrance by the long fatty acyl chains. The distribution of *N*-acyl groups in these partially *N*-acylated chitosans is unknown.

The nitrogen contents were calculated as hydroacetate for these *N*-acylchitosans on the basis of the observed d.s. values. The observed nitrogen contents were higher than the calculated ones as shown in Table I. This is due to the presence of some free amino groups in place of hydroacetate in the polymers.

In order to determine both the d.s. values of the *N*-acylchitosans by GLC analysis and the structural influence of *N*-acyl groups on the gelation, the *N*-acylchitosans soluble in 10% acetic acid were further *N*-acylated with carboxylic anhydrides differing from the original *N*-acyl group. The heterogeneously *N*-acylated chitosans thus prepared had the d.s. value = 1.00⁽⁴⁾ and were isolated in 50~100% yields (Table I). The structural influence of *N*-acyl groups on the gelation was essentially similar to that of the partially *N*-acylated chitosans described above.

EXPERIMENTAL

General. Gas-liquid chromatography analysis was performed at 60~230°C using a Shimadzu GC-5A apparatus with a hydrogen flame ionization detector, with nitrogen as carrier gas and with a glass column (2 m × 0.4 cm) packed with silicon OV-17 (5%) on celite 545, 80~100 mesh. The CD and ORD spectra (1 mm path length) were recorded using a JASCO J-20 recording spectropolarimeter at 17°C ($c=0.1\%$, 0.01 *N* hydrochloric acid).

The d. s. values for *N*-acyl group per hexosaminide residue were determined by 1) GLC analysis of the fatty acyl composition of heterogeneously *N*-acylated chitosans (d. s. = 1.00) which were derived from the partially *N*-acylated chitosans under examination, 2) the ratio of signal intensities of (*N*-acyl protons/methine and methylene protons of the sugar residue) in the NMR spectra (D₂O), and 3) elemental analysis.

References to the other analytical methods and

materials have been cited previously.²⁾

Preparation of the sample for the GLC analysis of N-acyl composition. Each of the heterogeneously N-acylated chitosans (20 mg) was hydrolyzed with 12 N H₂SO₄ (1 ml) in a sealed tube at 100°C for 3 hr. Free fatty acids were extracted with 3 ml of ether. After drying over anhydrous Na₂SO₄, 2 ml of methanolic solution containing 1% *p*-toluenesulfonic acid was added, and the mixture was refluxed for 1 hr to afford the methyl esters of fatty acids. Their composition was analyzed by GLC.

N-Benzoylheparin. N-Benzoylheparin containing 0.82 *N*-benzoyl and 0.18 *N*-acetyl groups per hexosaminide residue was prepared as previously reported,⁵⁾ $[\alpha]_D^{21} + 40.2^\circ$ ($c = 0.84$, water).

Partially N-acylated chitosans. Chitosan (100 mg) was dissolved in 2 ml of 10% acetic acid by standing at room temperature overnight, and the viscous solution was diluted with 10 ml of methanol.⁴⁾ To the solution was added 0.25, 0.50 or 0.75 molar equivalent of carboxylic anhydride per hexosaminide residue, and the mixture was stored at room temperature. The reaction mixture was suspended in *ca.* 100 ml of acetone and then in *ca.* 100 ml of a mixture of acetone-chloroform (1:1, v/v). The precipitate was collected by filtration and washed with ether, and then dried over P₂O₅ *in vacuo* at 100°C for 5 hr to afford the hydroacetate. To obtain the free amine, the hydroacetate was suspended in *ca.* 100 ml of ethanol con-

taining 0.5 M KOH, and the suspension was stirred at room temperature overnight. The precipitate was filtered and resuspended in ethanol. The product was filtered and washed successively with ethanol and ether, and the *N*-acylchitosan was isolated as free amine.

Heterogeneously N-acylated chitosans. Chitosan (100 mg) was dissolved in aqueous acetic acid-methanol as described above. To the solution was added 0.25 or 0.50 molar equivalent of a carboxylic anhydride per hexosaminide residue, and the mixture was stored at room temperature overnight. To the solution was added 1.50 or 1.75 molar equivalents of another carboxylic anhydride per hexosaminide residue.⁴⁾ The heterogeneously *N*-acylated chitosans thus prepared were suspended in 70~100 ml of acetone and the reaction products were isolated as described above.

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