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Regioselective chlorination of chitin with *N*-chlorosuccinimide-triphenylphosphine under homogeneous conditions in lithium chloride–*N*,*N*-dimethylacetamide

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Abstract

Purified chitin was chlorinated with an equimolar mixture of *N*-chlorosuccinimide and triphenylphosphine under homogeneous conditions in a 5% (w/v) solution of LiCl in *N*,*N*-dimethylacetamide at 70–85°C. ^{13}C NMR spectroscopy of the chlorinated products and gas chromatographic–mass spectrometric analysis of their hydrolyzates, both as trifluoroacetyl derivatives, showed that the chlorine substitution took place regioselectively at C-6. Chlorodeoxychitins with degrees of substitution up to 1.0 could be prepared easily by use of an excess of reagents. Polymer chain scission took place to some extent, especially when the chlorination was carried out at higher temperatures with higher concentrations of reagents.

Keywords: Regioselective chlorination of chitin; Chitin, regioselective chlorination of; Polysaccharide, homogeneous chlorination

1. Introduction

Chitin is one of the most abundant polymers in Nature [1] but its industrial utilization is undeveloped at present as compared to the structurally similar cellulose. In recent years, however, basic and applied studies on chitin have flourished [2–4]. Chitin is a rigid structural polysaccharide as is cellulose, but it can be digested in the body by the action of lysozyme whereas cellulose is non-degradable. Chitin and its derivatives show various bioactivities, and their uses in biomedical fields is an active research area. Chemical mod-

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ification of chitin is difficult in general, because chitin is a highly crystalline, intractable material with a strongly hydrogen-bonded network structure. Most chitin derivatives are prepared from chitosan, *N*-deacetylated chitin, or partly deacetylated, water-soluble chitin because only a few solvents for chitin are known that can be used as reaction media and the reactivity of chitin under heterogeneous conditions is generally low.

A binary solvent system, LiCl–*N*,*N*-dimethylacetamide (DMA) dissolves purified chitin [5]. Homogeneous acylation reactions in this solvent system have been briefly reported [6]. The LiCl–DMA system also dissolves cellulose, after preactivation treatment [7]. Chlorodeoxycelluloses of various ds values have been synthesized in this solvent system under homogeneous reaction conditions with *N*-chlorosuccinimide (NCS) and triphenylphosphine (TPP) [8] and with sulfuryl chloride [9]. This solvent system is suitable for chlorination because the chloride ion in the system will function as an additional reagent.

Here we report the homogeneous chlorination of chitin with NCS and TPP in LiCl–DMA. Chlorodeoxychitin may be expected to be as useful a precursor for the preparation of chitin derivatives as is chlorodeoxycellulose for cellulose derivatives [10], but the synthesis of chlorodeoxychitin has not been reported. The only halodeoxychitin reported is iododeoxychitin of ds 0.58, prepared from tosylated chitin [11].

2. Results and discussion

Synthesis and properties of chlorodeoxychitins.—The concentration of chitin in the chlorination mixture was fixed [0.5% (w/v), molar concentration of polymer repeating unit (PRU) of chitin, [PRU] = 2.57 mol/L) in all chlorination experiments. The concentration of LiCl in the reaction mixture was 5% (w/v). Equimolar amounts of NCS and TPP were used for chlorination and their concentrations are expressed as their molar ratios to PRU of chitin ([NCS·TPP]/[PRU]). Chlorination of chitin did not take place to an appreciable extent when the reagent molar ratio was equal to or <2, and it did not proceed appreciably even at higher reagent molar ratios when the reaction temperature was <70°C. Chitin solutions in LiCl–DMA gelled when the temperature was raised to around 95°C, as described in the literature [12]. Therefore, the chlorination was studied below the gelation temperature. Chlorination of chitin proceeded appreciably under appropriate conditions and pale-yellow to tan powdery products were obtained in moderate to high yields. The crude products were purified by treating with dilute alkali followed by dialysis. The color of the products faded upon this treatment. The ds was calculated from the chlorine content of the product.

Fig. 1 shows the time-dependence of chlorination at temperatures between 70 and 85°C at reagent molar ratios between 5 and 10. The ds value of the product leveled off in 2–4 h and the leveled-off ds value was dependent on the reaction variables. When the reagent molar ratio was low ([NCS·TPP]/[PRU] = 5), the leveled-off ds depended greatly on the reaction temperature and increased with increasing temperature: from 0.1 at 70°C to 0.5 at 85°C. When the reagent molar ratios were higher (8 or 10), the leveled-off ds value obtained was close to 1.0, independent of the reagent molar ratio and the reaction temperature. Chlorodeoxychitins of ds >1.0 could not be obtained under these conditions, in contrast to the case of chlorination of cellulose under similar reaction conditions, where chlorodeoxycellulose having ds as high as 1.9 was obtained [8].

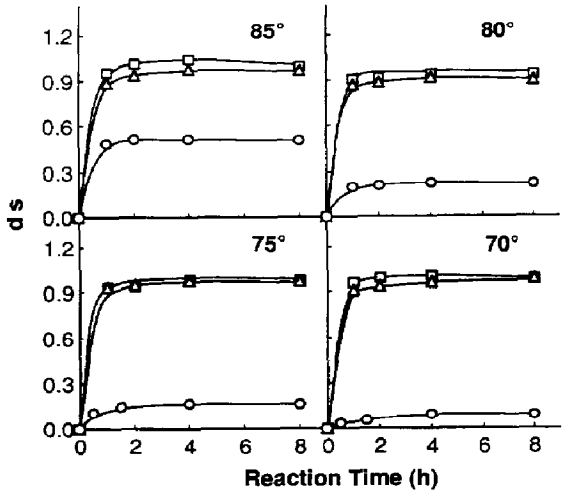

Fig. 1. Chlorination of chitin under various conditions: [NCS·TPP]/[PRU]; 5 (○), 8 (△), and 10 (□).

Chlorodeoxychitins were soluble in 5% (w/v) LiCl–DMA, as was the original chitin, and transparent films could be made by casting the solutions and effecting coagulation in ethanol. The solubilities of chlorodeoxychitins in other solvents at 40°C are shown in Table 1. All samples except chitin were soluble in formic acid under the experimental conditions used (chitin dissolves in formic acid by repeated freezing and defreezing treatments [13]). The solubility of chlorodeoxychitin tended to increase with increasing ds: chlorodeoxychitin of ds 0.94 dissolved in pyridine whereas that of ds 0.51 only swelled, and that of ds 0.06 did not swell.

Structure of chlorodeoxychitins.—The IR spectra of chlorodeoxychitins of different ds were compared with that of chitin. Chlorodeoxychitins of high ds showed $\nu_{C\text{-}Cl}$ absorptions at 753 and 723 cm^{-1}. Crude chlorodeoxychitins produced at high molar ratios of reagents often showed a weak peak at 1745 cm^{-1} assignable to ester carbonyl. This peak disappeared after the sample was treated with dilute alkali. The formation of ester carbonyl could be due

Table 1
Solubility of chlorodeoxychitins [a]

Ds	Organic solvent [b]							
	HCO_2H	Pyridine	NMP	Me_2SO	DMA	HMPT	THF	$CHCl_3$
0.94	+	+	±	±	±	±	±	−
0.79	+	±	±	±	−	−	−	−
0.51	+	±	±	−	−	−	−	−
0.21	+	±	−	−	−	−	−	−
0.06	+	−	−	−	−	−	−	−
0.0	−	−	−	−	−	−	−	−

[a] Samples were kept in 1000-fold solvent for 2 days at 40°C: (+) dissolved, (±) partially dissolved or swollen, (−) not dissolved.

[b] NMP, *N*-methyl-2-pyrrolidone; Me_2SO, dimethyl sulfoxide; DMA, *N*,*N*-dimethylacetamide; HMPT, hexamethylphosphoric triamide; THF, tetrahydrofuran.

[c] Purified chitin.

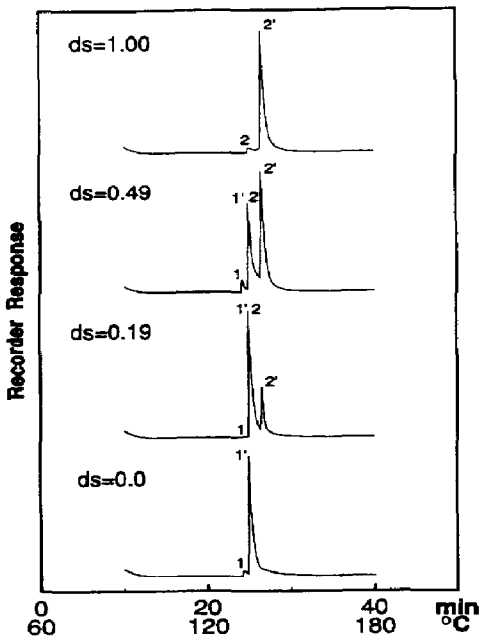

Fig. 2. GLC traces of hydrolyzates of chitin and chlorodeoxychitins as *N,O*-TFA derivatives: 1 and 1′, GlcN*p*; 2 and 2′, 6-Cl-GlcN*p*.

to the formation of iminium groups followed by hydrolysis during isolative treatment [14]. The $N \rightarrow O$ acetyl migration reaction of chitin [11] could also explain these results; however, as mentioned later, acetyl migration did not occur during chlorination.

Chitin and chlorodeoxychitins were dissolved in 35% HCl, heated for 6 h at 80°C, and the hydrolyzates were subjected to GLC and GLC–MS analyses after conversion into the volatile *N,O*-trifluoroacetyl (TFA) derivatives. The yields of monosaccharides after hydrolysis were > 95% as revealed by GLC analysis using 1,6-hexanediol as an internal standard. Fig. 2 shows GLC traces of chitin and chlorodeoxychitins of different ds. The chitin-derived product gave two anomeric peaks for the *N,O*-TFA derivative of glucosamine (GlcN) but no peaks for the *O*-TFA derivative of *N*-acetylglucosamine (GlcNAc). Both GlcN and 6-chloro-6-deoxyglucosamine (6-Cl-GlcN) gave two anomeric peaks for the pyranose form. In each case the peak area of the slower-eluting peak was much larger than that of the other. Although the slower-eluting peak of GlcN and the faster-eluting peak of 6-Cl-GlcN overlap each other, Fig. 2 clearly shows that the chlorination took place regioselectively at C-6. Chlorination of cellulose took place at C-6 initially, and then a second chlorine was introduced at C-3 but not at C-2. Chlorodeoxycelluloses of ds > 0.85 showed peaks of 3,6-dichloro-3,6-dideoxyallose [8], whereas chlorodeoxychitin of ds 1.0 showed only peaks of 6-Cl-GlcN.

The assignment of the peaks of GlcN in Fig. 2 was based on the comparison of the retention values with those of an authentic sample and GLC–MS analysis. The assignment of 6-Cl-GlcN was based solely on GLC–MS analysis. Table 2 summarizes the MS fragmentation patterns of *N,O*-TFA derivatives of GlcN and 6-Cl-GlcN. The nomenclature proposed by Kochetkov and Chizhov [15] for fragment-ions is used here. The structures of the most of the fragment-ions observed could be explained based on the fragmentation

Table 2
Mass fragmentation patterns of glucosamines as *N,O*-trifluoroacetyl derivatives

Fragment [a]	Peak 1 (GlcN*p*) [b]		Peak 2 (6-Cl-GlcN*p*) [c]	
	m/z [e]	r.a. [d]	*m/z* [e]	r.a. [d]
M^+	659	0.0	581(d)	0.0
A_1	546	0.5	468(d)	2.5
A_2	432	1.6	432(s)	0.1
			354(d)	1.3
A_3	318	20.1	318(s)	0.2
			240(d)	28.0
E_1	532	0.75	532(s)	2.0
E_2	418	0.3	418(s)	0.1
E_3	304	8.2	304(s)	2.8
C_2	404	13.6	404(s)	4.6
			327(d)	12.7
F_1	265	7.7	265(s)	45.6
CF_3CO	97	18.8	97(s)	14.9
C_5H_5O	81	7.9	81(s)	7.0
CF_3	69	100	69(s)	100

[a] Nomenclature for fragment ions, see text and Ref. [15].
[b] Peak 1 for chitin.
[c] Peak 2 for chlorodeoxychitin.
[d] r.a., Relative abundance (%).
[e] Mass numbers for 6-Cl-Glc are based on ^{35}Cl: d, 3:1 doublet at *m* and *m* + 2; s, singlet.

of the corresponding glucose derivatives [8]. The assignment of some of the fragment-ions, such as A_1 (doublet), for 6-Cl-GlcN were confirmed by the isotopic pattern due to chlorine substitution. The E series ions of GlcN and 6-Cl-GlcN indicated they were all in the pyranose form. The E_1 ions of 6-Cl-GlcN corresponded to the loss of a CH_2Cl moiety from the molecular ion, indicating the attachment of chlorine at C-6. Two ions of different *m/z* were observed for the fragment ions, A_2, A_3, and C_2 for 6-Cl-GlcN. These ions corresponded to the loss of either HCl or CF_3CO_2H.

For further identification of the structure of chlorodeoxychitins, ^{13}C NMR spectra were measured in trifluoroacetic acid-*d* as TFA derivatives, and together with those of chitin and chitosan. The spectrum of chitin was similar to those of chitin and diacylchitins in the literature [12] and that of chitosan was similar to that of the trifluoroacetic acid salt of chitosan reported [16]. Chitin and the two chlorodeoxychitins of ds 0.51 and 0.91 gave a signal of $COCH_3$ (methyl) around 23 ppm. The intensity of this signal relative to that of the C-1 signal was almost equal among the three samples. This implies that neither $N \rightarrow O$ acetyl migration took place during chlorination nor amide exchange between *N*-acetyl and trifluoroacetic acid during trifluoroacetylation and dissolution into trifluoroacetic acid. GlcNAc was trifluoroacetylated under the conditions used for derivatization of chitin and other chlorodeoxychitins for ^{13}C NMR measurements and analyzed by GLC. The peak area of *N,O*-TFA derivative of GlcN was negligibly small as compared to that of *O*-TFA derivative of GlcNAc, confirming that amide exchange did not take place appreciably.

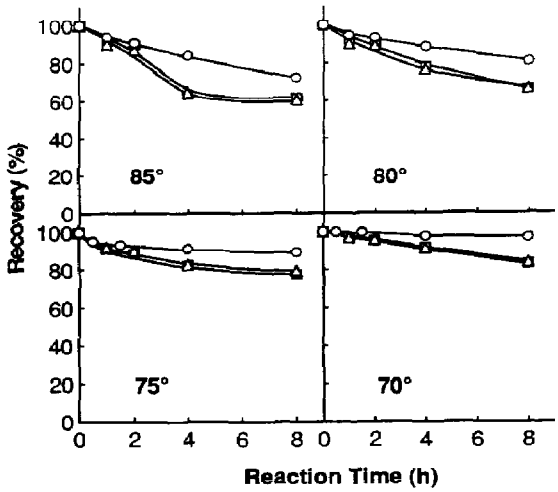

Fig. 3. Recoveries of chlorodeoxychitins prepared under various reaction conditions: [NCS·TPP]/[PRU]; 5 (○), 8 (△), and 10 (□).

The signals of C-6 carbons of chitin and chitosan appeared at ca. 69 ppm. This signal became weaker for chlorodeoxychitin of ds 0.49 and almost disappeared for that of ds 0.91. A new signal appeared at 44 ppm for chlorinated chitins whose intensity increased with increasing ds values. The chemical shift of this new signal was close to that reported for the C-6 carbon of 6-chloro-6-deoxycellulose [17]. These results clearly indicate that chlorination took place at C-6. The signals of C-2 carbons appeared around 58 ppm and were shifted to a slightly higher field by chlorination. The C-3, -4, and -5 signals appeared in the range between 70 and 80 ppm. The C-1 signal around 100 ppm was shifted to lower field by chlorination.

Chain scission during chlorination.—Fig. 3 shows the recoveries of chlorodeoxychitins obtained under various chlorination conditions. When the reaction was performed at a high reagent molar ratio ([NCS·TPP]/[PRU] = 8 or 10), the recovery decreased with increasing reaction time, even after the ds leveled off, and the extent of decrease was remarkable at 85°C. When chitin was chlorinated at a lower molar ratio of reagents, 5 at 70°C, the recovery did not drop with prolongation of the reaction time. The drop in recovery under more-severe reaction conditions showed that low-molecular-weight products were formed through molecular chain-scission during the treatment, and they were lost during isolation and purification treatments.

Reduced viscosities of chlorodeoxychitins were measured in 5% (w/v) LiCl–DMA. Fig. 4(a) shows that the viscosity of chitin decreased sharply at the beginning of chlorination, the extent of the decrease depending on the reaction conditions. When high molar ratios (8 and 10) of reagents were used, the viscosity fell to below one fifth of the original value for chitin in 1 h. When the chlorination was carried out at a lower molar ratio of reagents, 5, at a lower temperature, 70°C, the viscosity decrease was less significant, but it was still as high as about one half of the original value in 1.5 h. Substitution of C-6 hydroxyl groups by chlorine atoms may lead to a conformational change of the originally semi-rigid chitin in solution because of breakdown of the hydrogen-bonded structure in which C-6 hydroxyl groups are involved. The decrease in viscosity partially arises from the change of the polymer conformation, as earlier discussed for chloro- [18] and bromodeoxycelluloses [19].

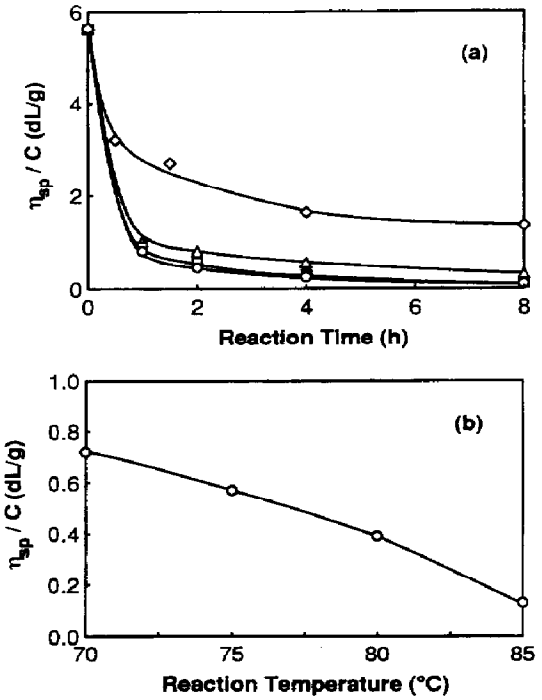

Fig. 4. Reduced viscosities of 0.1% (w/v) solutions of chlorodeoxychitins measured in 5% (w/v) LiCl–DMA at 40°C: (a) chlorination conditions ([NCS·TPP]/[PRU], temperature): 5, 70°C (◇); 8, 75°C (△); 10, 80°C (□); 10, 85°C (○), and (b) chlorinated at [NCS·TPP]/[PRU] of 8 for 4 h.

Fig. 4(b) shows the effect of reaction temperature on the viscosity when chlorination was effected at a reagent molar ratio of 8 for 4 h. Chlorodeoxychitins prepared at higher temperatures showed lower viscosities, although the ds values of all the chlorodeoxychitins were about the same (ds, 0.93–0.98).

Samples were analyzed by gel-permeation chromatography (GPC) with an 0.08% (w/v) solution of LiBr in dimethylformamide (DMF) as a solvent and with monodisperse polystyrene standards for molecular-weight calibration. Fig. 5 shows that the apparent dp distribution curve of the chlorodeoxychitin prepared at a reagent molar ratio of 10 at 85°C

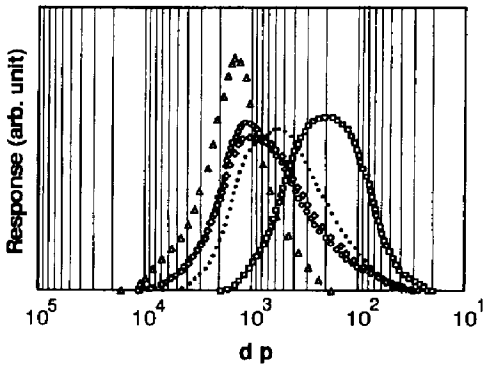

Fig. 5. Calculated dp distributions of chitin and chlorodeoxychitins: chitin (△) and chlorodeoxychitins prepared under the conditions ([NCS·TPP]/[PRU], temperature, time): 8, 75°C, 4 h (◇); 10, 85°C, 1 h (○); 10, 85°C, 2 h (●); 10, 85°C, 8 h (□).

shifted to a lower dp with increasing reaction time. Chlorodeoxychitins prepared at the reagent molar ratio of 8 at 75°C for 4 h and at the reagent molar ratio of 10 at 85°C for 1 h gave distribution curves similar to each other, and the peak positions of these two samples were nearly equal to that of the original chitin. However, the chlorodeoxychitins had more fractions of lower dp and fewer fractions of higher dp.

These results indicate that the molecular degradation took place during chlorination. Both cellulose [8] and chitin [5] were reported to be stable in LiCl–DMA. Reactions of chitin in this solvent system with acetic anhydride, tosyl chloride, and phenyl isocyanate gave highly *O*-substituted chitins without apparent chain scission [6]. Probably the observed molecular degradation in the present study is due to the oxidative degradation at higher temperatures because of the excess NCS present.

3. Experimental

Materials.—Chitin samples used (degree of *N*-deacetylation, 21%; PRU = 194.45) were obtained from commercial chitin (Tokyo Kasei Kogyo Co., Ltd.) by purification according to the method of Tokura [20]. Some of the purified chitin was deacetylated to chitosan (degree of *N*-deacetylation, 90%) with 50% NaOH for 3 h at 135°C. The degree of *N*-deacetylation was determined by titration with a pH meter [21]. NCS and TPP were purified by recrystallization from distilled water and EtOH, respectively. Lithium chloride was dried over 185°C under diminished pressure. DMA was distilled over CaH_2 at diminished pressure under N_2 and stored over Linde type 4A molecular sieves. All other chemicals were used without further purification.

Instruments and procedures.—Transparent films of chitin and chlorodeoxychitins for IR measurement were obtained by casting 2% (w/v) solution of products in 5% (w/v) LiCl–DMA followed by coagulation in EtOH. The spectra were recorded on a Jasco FT/IR-3 spectrophotometer.

A sample of a chitin derivative (5 mg) was dissolved in 3 mL of 35% HCl, degassed, and kept for 6 h at 80°C in a Pierce hydrolysis-tube. The solution was transferred to a flask and evaporated to dryness. The HCl was removed from the solid by five cycles of dilution with 40 mL of water and evaporation. The saccharides were converted into *N,O*-TFA derivatives in a Pierce Reacti-Vial with 200 μL of trifluoroacetic anhydride in 200 μL of CH_2Cl_2 for 10 min at 110°C and dried under a stream of N_2. After two cycles of this procedure, 600 μL of CH_2Cl_2 was added to the vial under N_2.

A Shimadzu GC-4BM dual-column gas chromatograph equipped with two flame-ionization detectors was used for GLC analysis, and a Shimadzu Chromatopac C-E1B was used for the data analysis. A glass column (3 m×3 mm i.d.) packed with 3% Silicone SE-30 on Gas Chrom Q (100–120 mesh) was used. The column temperature was raised from 60 to 250°C at 3°C/min, and the flow rate of the carrier gas (N_2) was 60 mL/min. GLC–MS analysis was performed on a Shimadzu LKB-9000 gas chromatograph–mass spectrometer and a Shimadzu GC-MSPAC 300 data processor. The mass spectra were measured at an ionization voltage of 70 eV. The temperatures of the ion source and the separator were 290 and 280°C, respectively. The flow rate of the carrier gas (He) was 30 mL/min, and other conditions for analysis were the same as those for the GLC analysis.

A mixture of 30 mg of chlorodeoxychitin and 200 μL of trifluoroacetic anhydride in 200 μL of CH_2Cl_2 was heated at 110°C for 20 min in a Pierce Reacti-Vial, and dried at 60°C under a stream of N_2. After three cycles of this procedure, 600 μL of trifluoroacetic acid-*d* and 40 μL of Me_4Si as the internal reference were added to the vial under N_2. This mixture was transferred to an NMR tube by a liquid-handling pipette under N_2, and the top of the tube was sealed. Chitosan could not be trifluoroacetylated in a similar way because of gelation in the presence of trifluoroacetic anhydride. Therefore, it was directly dissolved in trifluoroacetic acid-*d* for NMR measurements. ^{13}C NMR spectra were recorded on a Jeol JNM-FX90Q spectrometer at 35°C at 22.53 MHz.

A mixture of 4 mg of chlorodeoxychitin and 200 μL of trifluoroacetic anhydride in 200 μL of CH_2Cl_2 was heated for 20 min at 110°C in a Pierce Reacti-Vial, and dried at 60°C under N_2. After two cycles of this procedure, 4 mL of 0.08% (w/v) LiBr–DMF solution were added under N_2. Molecular-weight distributions of chlorodeoxychitins after trifluoroacetylation were determined with polystyrene standards, with a Shodex DS-3 gel permeation chromatograph (Showa Denko Co.) on Shodex GPC AD-80M/S, GPC AD-80M/S, and GPC AD-803/S columns connected with a Shodex RI SE-51 detector with 0.08% (w/v) LiBr–DMF as eluent. Molecular-weight-distribution curves were converted to dp distribution curves with the use of the ds of the samples. Peak areas were normalized for all samples.

Chlorination of chitin.—All procedures for the dissolution and chlorination of chitin were carried out under N_2. To a flask containing 35 mL of DMA, 2.5 g of LiCl was added and the mixture was heated for 1 h at 70°C with stirring. The mixture was then cooled to room temperature and 0.25 g of purified chitin was added. The temperature was raised to 70°C again and kept for 12 h with stirring, then lowered to 0–5°C and held at this temperature for 2 h with stirring. The temperature of the mixture was finally raised to and kept at 70°C with stirring, and a clear homogeneous solution was obtained within several hours.

In a typical chlorination, given amounts of NCS and TPP (both dissolved in appropriate volumes of DMA) were added quickly in this order to the solution mentioned above under cooling with ice–water. The final volume of DMA was 50 mL. The solution was then stirred at room temperature for ca. 15 min and held at a given reaction temperature for a required time with stirring. The resulting brown solution was poured into 300 mL of acetone. Precipitates were collected by centrifugation, washed with acetone and with MeOH, and treated with a solution of Na_2CO_3 (pH 11.4) at room temperature overnight. They were washed with water and dialyzed against distilled water for 3 days. After lyophilization, chlorodeoxychitin was obtained as light-tan powders. The chlorine content was determined by an oxygen-flask combustion method [22] from which the ds of chlorodeoxychitin was calculated as follows:

$$ds = (A \times B) / (3534 - 18.44 \times A) \quad (1)$$

where A and B is the chlorine content %(w/w) and mass of PRU of the purified chitin (= 194.5), respectively. The recovery of chlorodeoxychitin was calculated from the yield as follows:

$$\text{Recovery}(\%) = 400 \times Y \times B / (B + 18.44 \times D) \quad (2)$$

where Y is the yield (g) of chlorodeoxychitin from 0.25 g of purified chitin and D is the ds of chlorodeoxychitin. Chlorodeoxychitins were not soluble in 40% (w/w) NaOH and the degree of deacetylation could not be determined by the method [21] used for chitin and chitosan. IR spectrum (film) of chlorodeoxychitin of ds 0.91: 3458 (ν_{O-H}), 1660 (amide I), 1556 (amide II), 753 and 723 (ν_{C-Cl}) cm^{-1}. ^{13}C NMR (CF_3CO_2D) of chlorodeoxychitin of ds 0.51: 23.1 ($COCH_3$, methyl), 44.3 (C-6^{Cl}), 58.2 (C-2), 67.6 (C-6), 74–78 (C-3,4,5), 101.1 (C-1), and 178.1 ($COCH_3$, carbonyl); $[\alpha]_D^{25}$ −23°(*c* 1.0, formic acid) for chlorodeoxychitin of ds 0.90. Elemental analysis of chlorodeoxychitin of ds 1.0: Calcd. for $(C_8H_{12}NO_4Cl)_{0.79}(C_6H_{10}NO_3Cl)_{0.21}\cdot(H_2O)_{1.15}$: C, 38.90; H, 5.97; Cl, 15.44; N, 5.98. Found: C, 38.47; H, 5.63; Cl, 15.72; N, 5.82.

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