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The *N*-acylation of chitosan fibre and the *N*-deacetylation of chitin fibre and chitin–cellulose blended fibre at a solid state

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Abstract

Not only the filament surface of chitosan fibre but also its inside was *N*-acylated at a solid state by treatment with a series of carboxylic anhydrides in methanol at room temperature, and novel *N*-acylchitosan fibres (d.s. 0.8–1.1 for NAc) were obtained. Their filament tenacity and elongation values were little influenced by the *N*-acylation. Each of chitin fibre and chitin–cellulose blended fibre was treated with aq. 40% NaOH at 95–100°C for 4 h at a suspension state, and a chitosan fibre and a novel cellulose–chitosan blended fibre were obtained. The relative hydrolysis rate of *N*-acetyl, *N*-propionyl and *N*-butyrylchitosan fibres by an egg-white lysozyme were 1.0:0.3:0.0 at a suspension state. A SEM observation revealed a scaly pattern on the surface of the *N*-octanoylchitosan filament.

Keywords: Cellulose–chitosan blended fibre; Chitosan fibre; *N*-acylation; *N*-acylchitosan fibres; *N*-deacetylation

1. Introduction

Chitin is a (1 → 4)-linked *N*-acetyl-β-D-glucosaminan, and its *N*-deacetylation gives rise to chitosan. These biopolymers are biorenewable, biodegradable and biofunctional (Hirano, 1996; Shigemasa & Minami, 1995). Chitin is structurally similar to that of cellulose which is still extensively used in the viscose rayon industry.

Chitin fibre has been prepared by a wet-spinning method from (1) an aqueous alkaline solution of the sodium xanthates (Agboh & Qin, 1996; Balassa & Prudden, 1978; Hirano, Usutani & Midorikawa, 1997; Nakajima, Atsumi & Kifune, 1984; Noguchi, Wada, Seo, Tokura & Nishi, 1973; Tokura, Nishi & Noguchi, 1979), (2) an aqueous alkaline solution of the sodium salts (Hirano and Midorikawa, 1998), and (3) a LiCL-*N*,*N*-dimethylacetamide solution of chitin (Nakajima et al., 1984) as the dope. In our laboratory, *N*-propionylchitosan fibre was prepared by spinning an aqueous alkaline solution of each sodium *N*-propionylchitosan salt (Hirano & Midorikawa, 1998) and sodium *N*-propionylchitosan xanthate in aq. 14% NaOH (Hirano et al., 1997). However, these spinning methods could not be applied to the preparation of *N*-higher fatty-acyl (>C4)-chitosan fibres e.g. *N*-hexanoyl and *N*-octanoylchitosan fibres), because these derivatives are insoluble in these spinning solutions.

Chitin filament has been clinically used as an absorbable surgical suture (Kifune, 1995; Nakajima et al., 1984), and chitin sponge sheets and membranes have been used as a wound-dressing material in medical and veterinary fields (Kifune, 1995; Shigemasa & Minami, 1995). The novel *N*-higher carbon acylchitosan fibres are usable as a biocompatible and functional material: *N*-hexanoyl and *N*-octanoylchitosans are antithrombogenic and resistant to hydrolysis by lysozyme (Hirano & Noishiki, 1985).

It has been essential to develop a new method for the preparation of *N*-higher-carbon-acyl chitosan fibres. The present paper reports (1) the preparation of novel *N*-higher-carbon-acylchitosan fibres by a post-treatment of chitosan fibre with a series of carboxylic anhydrides in methanol at room temperature, (2) the chemical *N*-deacetylation of each chitin fibre and its blended fibre with cellulose at a solid state, and (3) their hydrolyses by an egg-white lysozyme at a fibre state.

2. Experimental

2.1. Materials

A purified sample of crab shell chitosan (Katakura Chikkarin Co., Ltd. Tokyo) was used in the present study and had

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the following analytical data: 1000 cps (MW 24×10^4) and $[\alpha]_D^{23}$ − 7° (*c* 0.5, aq. 2% acetic acid). Anal. Calc. for $[C_6H_{11}NO_4 \cdot 0.70H_2O]_n$: C, 41.47; H, 7.14: N, 8.06. Found: C, 41.76; H, 7.00; N, 7.78. The elemental analysis data indicate the degree of substitution (d.s.) 0.1 for NAc. A sample (Grade III) of lysozyme (EC 3.2.1.17) from hen egg whites was a product from Sigma Co., USA. The Chitin–cellulose (41:59, w/w) blended filament used in the present study was prepared from sodium chitin salt (alkaline chitin) and sodium cellulose xanthate as reported (Hirano & Midorikawa, 1998).

2.2. Methods

Filament titre (dtex) values were measured on a Vibroscop Micro (Lenzing Technic Instrument Co. Ltd., Austria), and filament tenacity (g/dtex) and elongation (%) values on a Vibrodyn 400 (Lenzing Technic Instrument Co, Ltd., Austria) at a dry condition in an air-conditioned room. FTIR spectra were recorded on a Jasco FTIR 5300 spectrometer (Jasco Co., Ltd., Tokyo), and specific rotations on a Jasco Dip-181 polarimeter (Jasco Co., Ltd., Tokyo). Elemental analyses were performed at the Micro-analytical Center of Kyoto University, Kyoto. The d.s. values for *N*-acyl groups were calculated from the *C*/*N* ratio of the elemental analysis data. Scanning Electron Microscopic (SEM) analyses were performed on a scanning electron microscope (JSM-6301F), Jeol. Ltd., Tokyo.

2.3. Chitosan fibre

Chitosan fibre was prepared from a chitosan solution (ca. 3%) in aq. 2% acetic acid–methanol (2:1, v/v) by spinning at a rate of 8.0 m per minute through a viscose-type spinneret into an aq. 10% NaOH solution containing aq. 30% sodium acetate (1.5 m in length) at 30–40°C as reported (Hirano, Nagamura, Zhang, Kim & Chung, 1999). The filaments were stretched at 1.2–1.4 times in an ethyleneglycol solution containing aq. 2% NaOH (1.5 m in length) by adjusting the rotation speed of the second rotor at room temperature, and cut into about 25 cm-length. The staple fibres thus prepared were kept in a mixed solution of aq. 10% NaOH and 30% sodium acetate for one day, washed several times with deionised water, and dried by the following treatments A and B. (1) The drying treatment A: one portion of the above chitosan fibre was suspended in 100% methanol at room temperature overnight, mechanically pressed, and air-dried to afford opaque chitosan staple fibre, which had 6.30–9.02 dtex for the titre, 0.62–1.20 g/dtex for the tenacity, and 6.2–8.7% for the elongation. (2) The drying treatment B: the other portion of the chitosan fibre was suspended in aq. 30% methanol and then aq. 50% methanol overnight successively, and air-dried by clipping both the ends of the stable fibre with weak stretching at room temperature to afford transparent chitosan staple fibre, which had 3.50–5.00 dtex for the titre, 1.10–1.33 g/dtex for the tenacity, and 9.2–13.2% for the elongation.

These data indicate that the drying treatment B gives slightly stronger and high quality fibres than the drying treatment A.

2.4. *N*-acylchitosan fibres

A portion (0.3 g) of the above dry chitosan staple fibre (B) was suspended in 20 ml of methanol, and each (5 mol/GlcN) of acetic, propionic, butyric, hexanoic, octanoic and succinic anhydrides was added. The mixture was stirred for a few minutes to remove air bubbles on the surface of these fibres and then kept at room temperature overnight. The thus treated fibre was washed several times with 100% methanol, and air-dried.

2.4.1. *N*-acetylchitosan (chitin) fibre (**1**)

ν_{max}^{KBr} 1657 and 1553 (C=O and NH of *N*-acetyl), and 1082 (C–O) (cm^{-1}). Anal. Calc. for $[C_6H_{10}NO_4N(C_2H_3O)_{1.00} \cdot 0.87H_2O]_n$: C, 43.90; H, 6.74; N, 6.40. Found: C, 43.93; H, 6.71; N, 6.22.

2.4.2. *N*-propionylchitosan fibre (**2**)

ν_{max}^{KBr} 2889 (C–H), 1650 and 1550 (C=O and NH of *N*-propionyl), and ~1050 (C–O) cm^{-1}. Anal. Calc. for $[C_6H_{10}NO_4(C_3H_5O)_{1.00} \cdot 0.84H_2O]_n$: C, 46.53: H, 7.19: N, 6.03. Found: C, 46.71; H, 7.20; N, 5.95.

2.4.3. *N*-butyrylchitosan fibre (**3**)

ν_{max}^{KBr} 2889 (C–H), 1657 and 1553 (C=O and NH of *N*-butyryl), and 1082 (C–O) cm^{-1}. Anal. Calc. for $[C_6H_{10}NO_4(C_4H_7O)_{1.00} \cdot 0.78H_2O]_n$: C, 48.97; H, 7.59; N, 5.71. Found: C, 48.86; H, 7.58; N, 5.70.

2.4.4. *N*-hexanoylchitosan fibre (**4**)

ν_{max}^{KBr} 2927 and 2860 (C–H), 1657 and 1553 (C=O and NH of *N*-hexanoyl), and ~1070 (C–O) cm^{-1}. Anal. Calc. for $[C_6H_{10}NO_4(C_6H_{11}O)_{1.00} \cdot 0.90H_2O]_n$: C, 52.51; H, 7.95; N, 5.11. Found: C, 52.50; H, 7.96; N, 5.24.

2.4.5. *N*-octanoylchitosan fibre (**5**)

ν_{max}^{KBr} 2934 and 2872 (C–H), 1657 and 1553 (C=O and NH of *N*-octanoyl), and ~1070 (C–O) cm^{-1}. Anal. Calc. for $[C_6H_{10}NO_4(C_8H_{15}O)_{1.00} \cdot 0.81H_2O]_n$: C, 55.71; H, 8.83; N, 4.64. Found: C, 55.61; H, 8.83; N, 4.63.

2.4.6. *N*-benzoylchitosan fibre (**6**)

ν_{max}^{KBr} 2889 (C–H), 1650 and 1555 (C=O and NH for *N*-benzoyl), 750 and 700 (mono subst. phenyl), and ~1050 (C–O) cm^{-1}. Anal. Calc. for $[C_6H_{10}NO_4(C_7H_5O)_{0.97}(H)_{0.03} \cdot 0.80H_2O]_n$: C, 55.55; H, 5.96; N, 5.07. Found: C, 55.31.; H, 5.80; N, 5.04.

2.4.7. *N*-succinylchitosan fibre (**7**)

ν_{max}^{KBr} 1734 (COOH of *N*-succinyl), 1653 and 1558 (C=O and NH of *N*-succinyl), and 1069 (C–O) cm^{-1} Anal. Calc.

Table 1
Effect of the molecular weight of chitosan on the mechanical properties of chitin filaments prepared by the post *N*-aceylation (A) and by direct spinning (B)

Molecular weight ($\times 10^4$) for chitosan	Chitin filaments[a]	Titre (dtex)	Tenacity (g/dtex)	Elongation (%)
14	A	3.88	0.80	11.1
	B	3.91	0.79	11.7
18	A	2.80	0.90	10.0
	B	5.44	0.84	10.4
19	A	4.73	0.82	10.9
	B	4.44	0.98	9.4
24	A	5.44	1.19	10.6
	B	5.00	1.10	11.2

[a] A, Chitin filaments prepared by the post *N*-acetylation of chitosan filament; B, chitin filaments prepared by direct spinning (Hirano et al., 1997).

for $[C_6H_{10}NO_4(C_4H_5O_3)_{0.81}(H)_{0.19}\cdot 1.17H_2O]_n$: C, 42.15; H, 6.30; N, 5.32. Found: C, 42.10; H, 6.30; N, 5.31.

2.5. *N*-deacetylation of chitin fibre

The chitin fibre (0.54 g) obtained above were treated at a suspension state in 50 ml of aq. 40% NaOH at 95–100°C for 4 h. The mixture was allowed to cool at room temperature and then diluted with ice water. The fibre thus treated was washed with distilled water several times, soaked in distilled water for a few hours, and air-dried to afford slightly yellow chitosan fibre (**8**) in 0.40 g yield. No absorptions of *N*-acetyl group were detected at ~1650 and ~1550–1620 cm^{-1} in the FTIR spectrum.

Anal. Calc. for $[C_6H_{10}NO_4N(C_2H_3O)_{0.20}(H)_{0.80}\cdot 0.74H_2O]_n$: C, 42.03; H, 7.05; N, 7.66. Found: C, 42.04; H, 7.04; N, 7.64.

2.6. *N*-deacetylation of chitin–cellulose fibre

The chitin–cellulose blended fibre (0.50 g) containing 41% chitin (Hirano & Midorikawa, 1998) was similarly treated with aq. 40% NaOH as described above, and novel chitosan–cellulose fibre (**9**) was obtained in 0.42 g yield. No absorptions of *N*-acetyl group were detected at ~1650 and ~1550 cm^{-1} in the FTIR spectrum. Anal. Found: N, 3.00%.

3. Results and discussion

3.1. *N*-acylation at a fibre state

Almost all the free amino groups of not only the surface of chitosan fibre but also its inside were selectively *N*-acylated even at a solid state by treatment with a series of carboxylic anhydrides in methanol at room temperature. Although the d.s. for *N*-acyl groups calculated from the *C*/*N* ratio of the elemental analyses were in the range of 0.8–1.1, no *O*-acylation was detected by their FTIR spectral analysis. *N*-Acetyl-, *N*-propionyl-*N*-butyryl-, *N*-hexanoyl-, and *N*-octanoyl-chitosan fibres were insoluble in water, aq. alkaline solutions and acidic solutions, but the *N*-succinylchitosan fibre was soluble in aq. alkaline solutions. In our previous study, a chitosan hydrogel has been *N*-acetylated by treatment with acetic anhydride (Hirano, Yamaguchi,

Table 2
Some mechanical properties of the *N*-acylchitosan filaments, and the *N*-deacetylated filament (the chitosan filament was prepared by the drying treatment B, and showed 4.16 dtex for titre value, 1.13 g/dtex for tenacity and 11.0% for elongation)

Filaments[a]	d.s. for *N*-acyl[b]	Titre (dtex)	Tenacity (g/dtex)	Elongation (%)
1	1.1	5.44	1.19	10.6
2	1.1	5.34	1.44	12.6
3	1.0	5.67	1.56	11.4
4	1.0	4.98	1.13	12.0
5	1.0	5.21	1.31	13.0
6	1.0	6.80	1.10	12.5
7	0.8	6.10	1.26	13.0
8	0.2	3.26	0.72	18.9
9	n.d.	3.60	0.87	21.4
10[c]	1.0	3.20	1.28	29.8

[a] 1, *N*-acetylchitosan (chitin) filament; 2, *N*-propionylchitosan filament; 3, *N*-butyrylchitosan filament; 4, *N*-hexanoylchitosan filament; 5, *N*-octanoylchitosan filament; 6, *N*-benzoylchitosan filament; 7, *N*-succinylchitosan filament; 8, chitosan filament prepared from *N*-acetylchitosan filament (**1**) by treatment with aq. 40% NaOH at 95–100°C for 4 h; 9, chitosan–cellulose filament prepared form chitin–cellulose filament (**10**) by treatment with aq. 40% NaOH at 95–100°C for 4 h.

[b] Calculated from the *C*/*N* ratio of the elemental analyses.

[c] See Hirano and Midorikawa (1998).

Table 3
Reducing-sugar values in the hydrolyses of some *N*-acylchitosan fibres by an egg-white lysozyme (Prepared from the chitosan fibre dealt with the drying treatment A)

Filaments[a]	Increase in reducing-sugar value (μ mol/ 5 h)[b]	Relative rate
1	1.55 ± 0.25	1
2	0.40 ± 0.15	0.3
3	Trace	0.0

[a] 1, *N*-acetylchitosan; 2, *N*-propionylchitosan; 3, *N*-butyrylchitosan.
[b] Expressed as μ mol of *N*-acetyl-D-glucosamine.

Fukui & Iwata, 1990) and chitosan fibre was also *N*-modified by treatment with aldehydes under the same present conditions (Hirano et al., 1999). These data indicate that chitosan can be selectively *N*-modified not only at its solution state (Hirano, Ohe & Ono, 1976) but also at its solid state by treatment with carboxylic anhydride and aldehydes in methanol at room temperature.

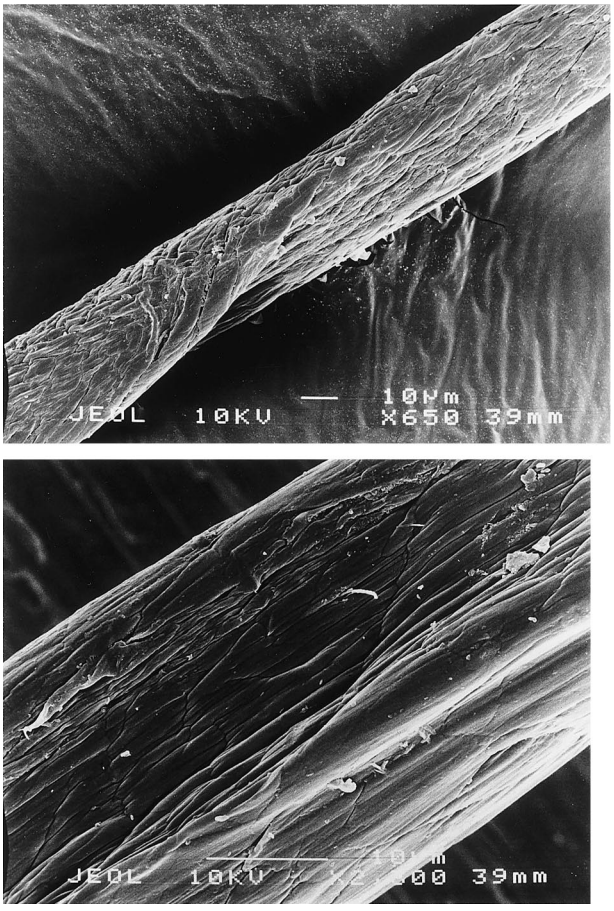

Fig. 1. SEM photographs of *N*-octanoylchitosan. The filament has 33 μm in diameter (—:10 μm) and 5.21 dtex for the titre, and a scaly pattern on the surface.

3.2. Some properties of *N*-acylchitosan fibres

The chitosan fibres prepared from the different molecular weights (14×10^4–24×10^4) of chitosan were *N*-acetylated, and their properties are shown in Table 1. Its tenacity and elongation values were essentially similar to those of the original chitosan filament. These data indicate that the original fibril arrangement in the chitosan filaments is kept even after the post *N*-acetylation.

As shown in Table 2, the *N*-acylchitosan filaments obtained in the present method have slightly higher tenacity values (1.10–1.56 g/dtex) and lower elongation values (10.6–13.0%) than those (0.52–0.79 g/dtex and 25.9–27.1%) of the *N*-acylchitosan filaments which had been prepared by the direct spinning from their sodium salts and sodium xanthates (Hirano & Midorikawa, 1998; Hirano et al., 1997). These *N*-acylchitosan fibres kept their tenacity and elongation values even after storage for three months at room temperature. The *N*-hexanoyl- and *N*-octanoyl-chitosans have an antithrombogenic function (Hirano & Noishiki, 1985).

3.3. *N*-deacetylation at a fibre state

The *N*-acetyl group in both the chitin fibre and the chitin–cellulose blended fibre was removed by treatment once or twice with aq. 40% NaOH at 95–100°C for 4 h. Their FTIR spectra revealed the absence of *N*-acetyl group at ~1665 and ~1650 cm^{-1}. The d.s. 0.2 for *N*-acetyl group was calculated from the *C*/*N* ratio of the elemental analyses, and (**8**) was swelled in aq. 2% acetic acid and slowly solubilised within two days at room temperature, although the chitosan fibre (d.s. 0.1 for Nac), which was directly spun from a chitosan solution in aq. 2% acetic acid–methanol (1:1, v/v) into aq. 10% NaOH-30% NaOAc, was instantly solubilised in aq. 2% acetic acid at room temperature (Hirano et al., 1999). The obtained chitosan–cellulose fibre was only swelled in aq. 2% acetic acid at room temperature, but it was insoluble. Their tenacity and elongation values decreased slightly probably due to partial destruction of glycosidic linkages during treatment with conc. alkali. The present study give us a novel method for the preparation of novel chitosan–cellulose blended fibre via chitin–cellulose fibre by *N*-deacetylation.

3.4. Hydrolyses of *N*-acylchitosan fibres by lysozyme

A portion (20 mg) of the several *N*-acylchitosan fibres were tested for the hydrolysis by lysozyme at a suspension state (Hirano & Midorikawa, 1998; Hirano and Yagi, 1989). As shown in Table 3, chitin fibre was accessible to lysozyme, and *N*-propionylchitosan fibre was slightly assessable at the solid state (Hirano & Yagi, 1989), however, the *N*-hexanoyl, *N*-octanoyl, *N*-benzoyl and *N*-succinylchitosan fibres were not accessible under the present conditions. These results are in good agreement with our previous

data on the enzymatic hydrolyses of the *N*-acylchitosans at the fine powder state (Hirano & Yagi, 1989).

3.5. SEM observation

Fig. 1 shows a scaly structure on the surface pattern of the *N*-octanoylchitosan filament, which was prepared from chitosan filament by *N*-acylation with *n*-octanoic anhydride. The filament has 33 μm in diameter and 5.21 dtex for the titre. Essentially the same surface structural pattern was observed with the other *N*-acylchitosan filaments prepared in the present study. The scaly pattern on the filament surface is probably produced during the neutralization and dehydration processes of the original chitosan fibre (Hirano et al., 1999).

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