ELSEVIER

Carbohydrate Polymers 47 (2002) 121–124

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Wet-spun blend biofibers of cellulose–silk fibroin and cellulose–chitin–silk fibroin

Shigehiro Hirano*, Tamayo Nakahira, Min Zhang, Masuo Nakagawa, Masatoshi Yoshikawa, Takehiko Midorikawa

Chitin/Chitosan R and D Center, 445-Sakuradani, Tottori-shi, Tottori 680-0853, Japan

Received 22 September 2000; accepted 30 October 2000

Abstract

Each spinning solution of silk fibroin–sodium cellulose xanthate (viscose) in aqueous NaOH, and silk fibroin–viscose–sodium *N*-acetylchitosan salt (alkaline chitin) in aqueous NaOH was spun at room temperature through a spinneret (0.1 mm in hole diameter) into a 10% aqueous sulfuric acid solution containing 40–43% ammonium sulfate. Novel white blend biofilaments were obtained in over 95% yield. The cellulose–silk fibroin filaments containing less than 10% silk fibroin had 4.9–9.9 denier for the titer value, 1.08–1.20 g/denier for the tenacity value and 29.7–35.0% for the elongation value. The cellulose–chitin–silk fibroin filaments containing less than 43% silk fibroin had 3.9–5.0 denier for the titer value, 0.70–0.93 g/denier for the tenacity value and 20.6–28.6% for the elongation value.

Keywords: Alkaline chitin; Biofibers; Cellulose–silk fibroin fiber; Cellulose–chitin–silk fibroin fiber; Viscose

1. Introduction

Silk fibroin is a linear protein consisting of Gly, Ala and Ser as main amino acid residues. Cellulose, chitin and chitosan are a linear (1 → 4)- linked beta-D-glycopyranan. Cellulose fiber is well known as 'rayon' (a regenerated cellulose) in the textile field. Chitin fiber and chitosan fibers (Agboh & Qin, 1996; Hudson, 1997; Rathke & Hudson, 1994; Szosland, 1996; Urbanczyk, 1997) are partly commercialized as biocompatible materials in the clinical (Kifune, 1995; Nakajima, Atsumi & Kifune, 1984), veterinary (Shigemasa & Minami, 1995), and medical and hygienic (Dutkiewicz, 2000) fields.

As artificial blend biofibers, cellulose–chitin fiber (Hirano & Midorikawa, 1998; Noguchi, Wada, Seo, Tokura & Nishi, 1973), silk fibroin–chitin fiber (Hirano, Nakahira, Nakazawa & Kim, 1999b), chitosan–silk fibroin fiber (Park, Oh, Yoo & Shin, 2000) and chitosan–tropocollagen fiber (Hirano, Zhang, Nakagawa & Miyata, 2000) are reported, and the cellulose–chitin fiber is commercialized as 'crabyon' in the textile field (Yoshikawa, 1999). However, little is known about blend biofibers of cellulose–silk fibroin, and cellulose–chitin–silk fibroin. These artificial biofibers as well as natural fibers including wool, cotton and silk are ecological and environmental friendly on the earth.

The present study aims to prepare the novel blend biofibers of cellulose–silk fibroin and cellulose–chitin–silk fibroin.

2. Experimental

2.1. Materials

Crab shell chitosan (MW 25×10^4, Nihonkayaku Co., Tokyo, Japan) was selectively *N*-acetylated by treatment with acetic anhydride in an aqueous acetic acid-methanol solution to give rise to *N*-acetylchitosan (a regenerated chitin, d.s. 1.0 for NAc) (Hirano, Ohe & Ono, 1976). Cellulose was a commercial product (Lot MBE7547, Nacalai Tesque Company, Kyoto, Japan). Cocoons of silk worms (*Bombyx mori*) were supplied from the Department of Agronomy in Tottori University, Tottori, Japan.

A 9% sodium cellulose xanthate (viscose) solution in 5% aqueous NaOH (Hirano & Midorikawa, 1998) and an 8% sodium *N*-acetylchitosan salt (alkaline chitin) solution in 14% aqueous NaOH (Hirano & Midorikawa, 1998; Thor & Henderson, 1940) were prepared by the conventional methods.

* Corresponding author.

0144-8617/02/$ - see front matter
PII: S0144-8617(01)00171-0

2.2. Methods

A viscose-type spinneret ($12.5 \times 18.0 \times 10.0$ mm^3 in size, Japan Nozzle Company, Kobe, Japan) was used in the present study, and the spinneret had 300 holes diameter of 12.5 mm and thickness of 0.30 mm along its length. Each hole had diameters of 0.50 mm at the entrance and 0.10 mm at the exit. FTIR spectra (KBr disks) were recorded on a Jasco FTIR 5300 spectrometer (Jasco Co., Tokyo, Japan). The filament titer values were analyzed on a Vibroscope Micro (Lenzing Technomic Instrument Co., Ltd., Austria), and expressed as denier for the weight (g) of a filament of 9000 m in length. The filament tenacity (g/denier) and elongation (%) values in the dry state were analyzed on a Vibron 400 (Lenzing Technomic Instrument Co., Ltd., Austria). These values were expressed at an average value of 3–5 measurements. The SEM analyses for the filaments were performed on a scanning electron microscope JSM-6301F, Jeol Co., Ltd., Tokyo, Japan.

2.3. Aqueous silk fibroin solution

Each of the cocoons (72 g) was cut into four to five pieces by a knife, and the pieces were soaked in a 0.2% aqueous soap solution (2.0 l) containing sodium carbonate (10 g). The mixture was boiled for 20 min with stirring to remove sericin. The insoluble precipitate was washed with water several times, pressed, and air-dried. The dry precipitate was soaked in a mixed solution (750 ml) of chloroform and methanol (2:1, v/v) with occasional stirring at room temperature for 70 h, and lipids were removed. The residue was pressed and air-dried to give rise to white silk fibroin in 50 g (69% yield). A portion (6.0 g) of the silk fibroin was suspended in a 10 M LiBr H_2O solution (60 ml), and the suspension mixture was retained at room temperature overnight (Ambrose, Bamford, Elliot & Hanby, 1951). The produced solution was dialyzed through a dialysis membrane tube against running water for two days, and the tube was hanged in the air in an air-ventilated room at room temperature for a few days to give to give rise to a concentrated aqueous silk fibroin solution (ca. 60 ml-volume). The solution was filtered through a glass filter to remove a small amount of insoluble materials to give rise to about 10% aqueous silk fibroin solution.

2.4. Spinning

Spinning solutions (dopes) A and B were prepared as follows. Dope A: a 10% aqueous silk fibroin solution and a 9% viscose solution in 5% aqueous NaOH were mixed in the ratios of 50:50, 29:71, 9:91, 5:95 and 2:98 (v/v) at room temperature. Dope B: a 10% aqueous silk fibroin solution, a 9% viscose solution in 5% aqueous NaOH and an 8% alkaline chitin solution in aqueous 14% NaOH were mixed in the ratios of 10:10:80, 40:42:18 and 10:10:80 (v/v/v) at room temperature.

Each 25-ml portion of the above dopes was taken, air-bubbles present in the dopes were removed under reduced pressure, and the clear supernatant solution was used for spinning at an extruding rate of 8 m/min. A 10% aqueous H_2SO_4 solution containing 40–43% $(NH_4)_2SO_4$ at room temperature was used as the coagulating solution. The extruded wet filaments were cut into about 25-cm length, and dipped in the same coagulating solution at room temperature overnight. The fiber was washed several times with aqueous 30% methanol, and dipped in aqueous 60% methanol and in 100% methanol at room temperature for a few hours, respectively. The product was pressed and air-dried on mechanical stretching to give rise to the corresponding white filaments in over 95% yield.

Cellulose–silk fibroin filaments (**1**–**5**). νmax (KBr): ~3447 (OH), 1651 (amide I for fibroin), 1539 (amide II for fibroin), ~1065 (C–O for cellulose) cm^{-1}.

Cellulose–chitin–silk fibroin filaments (**7**–**9**). νmax (KBr): ~3422 (OH), 1653 (amide I for fibroin and NAc), 1529 (amide II for fibroin and NAc), ~1064 (C–O for cellulose and chitin) cm^{-1}.

3. Results and discussion

3.1. Spinning and coagulating solutions

The aqueous silk fibroin solution was mixed with the viscose solution in aqueous NaOH (dope A), and dope A was mixed additionally with the alkaline chitin solution in aqueous NaOH (dope B). The dopes A and B were stable at room temperature for at least one hour, but a hydrogel was produced after standing at room temperature for several hours. The blends of cellulose–silk fibroin and cellulose–chitin–silk fibroin were essentially insoluble in the present coagulating solution. The aqueous ammonium sulfate solutions at various concentrations are widely used in the biochemistry field for the salting-out of enzyme proteins because of their little denaturation, high solubility effectiveness (Gree & Hughes, 1955).

3.2. Filaments and their properties

Silk fibroin content in the spun filaments was at 2–53% for **1**–**5** and at 11–43% for **7**–**9** (Table 1). These spun filaments were white, mechanically soft, insoluble in cold and boiling water, stable on heating at up to 200°C, and turned to brown color on heating at over 230°C. Filaments **3**–**5**, which contained less than 10% silk fibroin, had relatively good mechanical properties: 4.9–9.9 denier for the titer value, 1.08–1.20 g/denier for the tenacity value and 29.7–35.0% for the elongation value. The tenacity and elongation values of **4** were larger than those (1.05 g/denier for the tenacity value and 8.44% for the elongation value) of the silk fibroin–chitin (94:6, w/w) filament (Hirano et al., 1999b), but the tenacity value was slightly smaller than 1.27 g/denier of cellulose and 1.11 g/denier of chitin. With an increase in silk fibroin contents from 31 to

Table 1
Some properties of blend fibers of cellulose–silk fibroin and cellulose–chitin–silk fibroin

Filament	Cellulose (%)	Silk fibroin (%)	Chitin (%)	Titer (denier)	Tenacity (g/denier)	Elongation (%)
1	47	53	0	19.7	0.15	0.8
2	69	31	0	7.5	0.63	30.7
3	90	10	0	9.9	1.08	35.0
4	94	6	0	5.8	1.15	29.7
5	98	2	0	4.9	1.20	33.8
6[a]	100	0	0	4.1	1.27	39.2
7	80	11	9	5.0	0.93	25.0
8	41	43	16	4.8	0.70	20.6
9	11	12	77	3.9	0.85	28.6
10[a]	62	0	38	3.2	0.68	29.8
11	0	0	100	4.0	1.11	13.4

53%, the titer values increased from 7.5 to 19.7 denier, and the tenacity values decreased drastically from 0.63 to 0.15 g/denier mainly due to a filament shrinking to about 2/3 in the length during the air-drying process. The filament shrinking was partly prevented by mixing of up to 27% chitin as observed with **7**–**9**, which had 3.87–4.96 denier for the titer value. The filament shrinking phenomenon was also observed with the chitosan filament (Hirano, Nagamura, Zhang, Kim, Chung, Yoshikawa & Midorikawa, 1999a), but not with the chitin–silk fibroin filament (**10**) (Hirano et al., 1999b). The filament shrinking was also prevented by mechanical stretching of the filaments during the air-drying process. A commercial application of **3** and **4** as a textile material for fabrics is progressing.

In the silk fibroin–cellulose–chitin filaments, chitin content (up to 77%) effected on a decrease in the tenacity value (0.70–0.93 g/denier) but little on the elongation value (20.6–28.6%). The spinning of silk fibroin filaments was unsuccessful under the present conditions.

3.3. Scanning electron microscopic analyses

As shown in Fig. 1, fine vertical striped patterns appeared on the surface of 1 (55 μm in diameter). These fine striped pattern on the filament surface was similar to those of silk yarns and the silk fibroin–chitin (33:67, w/w) filament (Hirano et al., 1999b), but differed from a rough striped pattern of rayon (Kawai & Tagawa, 1983).

3.4. Blend organization in the filaments

Table 2 shows the diameter, titer and density of the blend and non-blend filaments, which were spun through the same spinneret of 0.1 mm in hole diameter under the same conditions. The filament diameter, titer and density values were increased by the blending with each of silk fibroin (Hirano et al., 1999b) and tropocollagen (Hirano et al., 2000), but not with each of heparin and hyaluronate (Hirano & Zhang, 2000). Filament **1** and the chitin–silk fibroin filament (Hirano et al., 1999b) had 55 and 46 μm diameter, and 19.7 and 18.5 denier for the titer value. Cellulose and chitin filaments (Hirano & Midorikawa, 1998) had 29–31 μm diameter and 4.10–5.44 denier for the titer value. The filament densities calculated as (denier/μm in diameter) were 0.36 and 0.46 for **1** and the silk fibroin–chitin filament, which were larger than those (0.14–0.18) of chitin and cellulose filaments.

These data indicate that (1) some molecular interactions

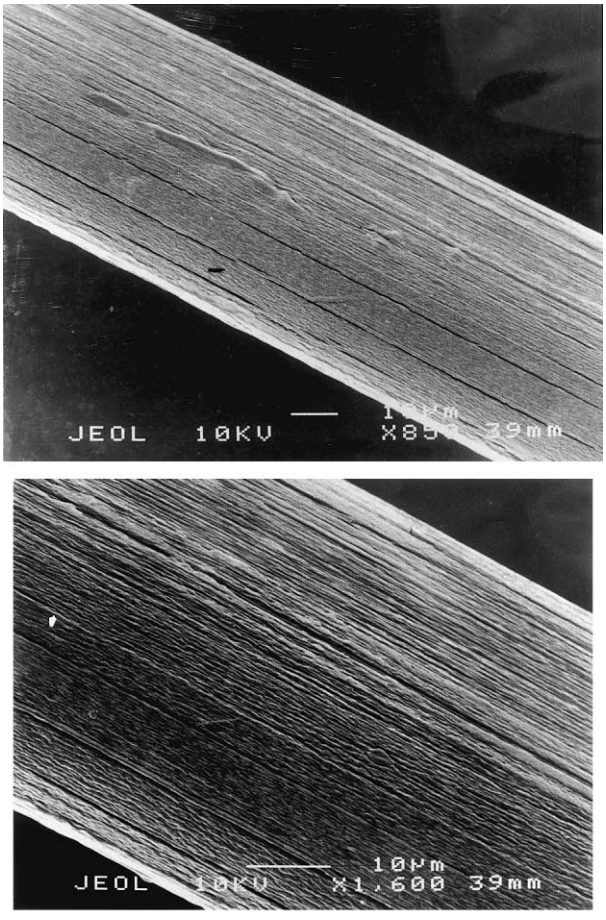

Fig. 1. Scanning electron microscopic (SEM) photographs of the cellulose–silk fibroin (47:53, w/w) filament (**1**, 55 μm in diameter). A bar (—) in the photograph is equivalent to 10 μm in the length.

Table 2
A comparison of the diameters, titers and densities of some biofilaments

Filaments[a]	Filament diameter (μm)	Titer (denier)	Density (denier/μm in filament diameter)	Ref.
Cellulose–silk fibroin (47:53, w/w) (1)	55	19.7	0.36	This work
Chitin–silk fibroin (67:33, w/w)	46	18.5	0.40	Hirano et al., 1999b
Chitosan–tropocollagen (50:50, w/w)	36	17.7	0.49	Hirano et al., 2000
Cellulose–heparin (77:23, w/w)	24	7.36	0.30	Hirano and Zhang, 2000
Cellulose–hyaluronate (78:22, w/w)	19	4.61	0.24	Hirano and Zhang, 2000
Cellulose–chitin (51:49, w/w)	25	2.79	0.11	Hirano and Midorikawa, 1998
Chitosan	31	5.44	0.18	Hirano et al., 1999a
Chitin (11)	30	5.14	0.17	Hirano and Midorikawa, 1998
Cellulose (6)	29	4.10	0.14	Hirano and Midorikawa, 1998

[a] Spun through the same spinneret (0.1 mm in the hole diameter) under the same conditions.

exist in the filaments between cellulose and chitin because of their similar chain structures. (2) Only weak molecular interactions except some physically entangled interactions exist between silk fibroin and cellulose or chitin. 3) The cellulose and chitin fibrils in the filaments play a function as a skeleton, and the silk fibroin plays a function as filling material among the skeletons of cellulose and/or chitin as observed with acidic glycosaminoglycans portions in the cellulose–acidic glycosaminoglycans fibers (Hirano and Zhang, 2000).

References

Agboh, O. C., & Qin, Y. (1996). Chitin and chitosan fibers. *Polymer Advances in Technology*, *8*, 355–365.

Ambrose, E. J., Bamford, C. H., Elliot, A., & Hanby, E. (1951). Water-soluble silk, an alpha-protein. *Nature*, *167*, 264–265.

Dutkiewicz, J. (2000). *Advances in Polymer Materials for Medicine and Hygiene*. lodz, Poland: Polish Academy of Science.

Green, A. A., & Hughes, W. L. (1955). Protein fractionation on the basis of solubility in aq. solutions of salts and organic solvents. *Methods in Enzymology*, *1*, 67–90.

Hirano, S., & Midorikawa, T. (1998). Novel method of the preparation of *N*-acylchitosan fiber and *N*-acylchitosan-cellulose fiber. *Biomaterials*, *19*, 293–297.

Hirano, S., & Zhang, M. (2000). Cellulose–acidic glycosaminoglycan blend fibers releasing a portion of the glycosaminoglycans in water. *Carbohydrates Polymer*, *43*, 281–284.

Hirano, S., Ohe, Y., & Ono, H. (1976). Selective *N*-acylation of chitosan. *Carbohydrate Research*, *47*, 315–320.

Hirano, S., Nagamura, K., Zhang, M., Kim, S. K., Chung, B. G., Yoshikawa, M., & Midorikawa, T. (1999a). Chitosan staple fibers and their chemical modification with some aldehydes. *Carbohydrates Polymer*, *38*, 293–298.

Hirano, S., Nakahira, T., Nakazawa, M., & Kim, S. K. (1999b). The preparation and applications of functional fibers from crab shell chitin. *Journal of Biotechnology*, *70*, 373–377.

Hirano, S., Zhang, M., Nakagawa, M., & Miyata, T. (2000). Wet-spun chitosan–collagen fibers, their chemical *N*-modifications, and blood compatibility. *Biomaterials*, *21*, 997–1003.

Hudson, S. M. (1997). Applications of chitin and chitosan as fiber and textile chemicals. *Advances in Chitin Science*, *2*, 590–599.

Kawai, H., & Tagawa, T. (1983). *Fiber morphological illustration*. Tokyo: Asakura.

Kifune, K. (1995). Biomedical materials. In Chitin/Chitosan Handbook, Japn Soc. (Ed.), Chitin/Chitosan (pp. 323–354). Tokyo: Gihodo.

Nakajima, M., Atsumi, K., & Kifune, K. (1984). Development of absorbable sutures form chitin. In J. P. Zikkakis, *Chitin, Chitosan and Related Enzymes* (pp. 407–410). New York: Academic Press.

Noguchi, J., Wada, O., Seo, H., Tokura, S., & Nishi, N. (1973). Chitin and chitin–cellulose fibers. *Kobunshi Kagaku*, *39*, 320–326.

Park, K. H., Oh, S. Y., Yoo, D. I., & Shin, Y. (2000). Preparation of silk fibroin/chitosan fiber. *Advances in Chitin Science*, *4*, 122–127.

Rathke, T.D., & Hudson, S.M. (1994). Review of chitin and chitosan as fiber and film forms. *Journal of Macromolecule Science, Review of Macromolecule Chemistry and Physics*, *C34(3)*, 375–437.

Shigemasa, R., & Minami, S. (1995). Applications of chitin and chitosan for biomaterials. *Biotechnology Gene Engineering Review*, *13*, 383–420.

Szosland, l. (1996). A simple method for the production of chitin materials from the chitin ester derivatives. *Advances in Chitin Science*, *1*, 297–302.

Thor, C. J. P., & Henderson, W. F. (1940). Alkali chitin. *American Dye Report*, *29*, 461–463.

Urbanczyk, G. W. (1997). Fine structure and properties of filaments prepared from chitin derivatives. In M. F. A. Goosen, *Applications of chitin and chitosan* (pp. 281–296). lancaster: Technomic.

Yoshikawa, M. (1999). Crabyon. *Kagaku (kyoto)*, *54*, 34–36.