

Preparation of Polyion Complex Fibers and Capsules of DNA and Chitosan at an Aqueous Interface

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Hybrid fiber and capsule creation based on the electrostatic polynucleotide–polysaccharide interaction has been studied. The fibrous form is made from single (SS)- and double (DS)-stranded DNAs and chitosan via polyion complex (PIC) formation. When DNA solutions with and without NaCl are added to an aqueous chitosan solution without mixing, PIC films are formed at the interface. When these PIC films are withdrawn from the interface, fiber lines form in the wet states. After the intact wet fibers are dried in air, SS- and DSDNA–chitosan fibers form. The tensile strength of the strongest DSDNA–chitosan fiber is 84 MPa. The strength and the strain of the fibers depend on the NaCl concentrations of the DNA solutions. Stable SS- and DSDNA–chitosan PIC capsules can also be prepared.

DNA is the most important genetic material of living organisms, and is a highly charged polyelectrolyte with a negative charge at the phosphate group of each nucleotide. Although DNA is readily purified from salmon milts or shellfish gonads, large amounts of these DNA-enriched materials have been discarded as waste by industry. DNA is highly water-soluble and biochemically unstable. These properties have been making it difficult to utilize them as industrial and/or functional materials. For overcoming this weak point, DNA as a molecular material has recently been studied regarding complexation with other molecular materials and the UV-irradiation of DNA.[1–6]

Chitosan is readily prepared from crystalline chitin, which has been produced from the epidermis of shellfish, via N-deacetylation with alkali. A part of this material is used as a coagulant for sewage treatment, and others are discarded due to a limited understanding of their use.[7] The conversion of these discarded DNAs and chitosans into useful materials would be beneficial in order to utilize these unique natural resources.

Polyion complexes (PICs) are formed by the reaction of a polyelectrolyte with an oppositely charged polyelectrolyte in aqueous solution. PICs have numerous applications, such as hybrid membranes, fibers, gels, and capsules and microcapsules, and have been widely studied.[8–11] During the course of our continuing work on the creation of biomimetic hybrid materials,[12,13] we found that the interaction between anionic and cationic polyelectrolytes produces characteristic structures at the interface between aqueous solutions. In this article, we report on the different characteristic surface structures, such as fibers and capsules, between anionic DNA with phosphoric functional groups and cationic chitosan composed of [(1,4)-2-amino-2-deoxy-β-D-glucan] with amino functional groups, via PIC formation at the aqueous interface.

Experimental

Materials and Methods. The chitosans (chitosan 10, M_w = 210000; chitosan 100, M_w = 1310000; chitosan 500, M_w = 1580000; chitosan 1000, M_w = 1800000) were purchased from Wako Pure Chemical Ind., Japan. The viscosity average molecular weights of the chitosans are in the 210000–1800000 range, based on the viscosity equation.[14] Double-stranded DNA (DSDNA) (Na salt from salmon milt; M_w = 6600000) and single-stranded DNA (SSDNA) (Na salt from salmon milt; M_w = 1000000) were obtained from Yuki Fine Chemical Co., Ltd., Japan. These chitosans and DNAs were used without further purification. A 1% DSDNA solution was degassed before use.

Preparation of DNA Fiber. Based on earlier articles,[15,16] DSDNA fiber was prepared in a precipitating liquid (73% ethyl alcohol containing 0.4 M NaCl). This method, however, was not appropriate for the DSDNA used here, due to the difference in the kinds or molecular weights of the DNA. Therefore, we prepared the DSDNA fiber by a modified method, in which a 1% DSDNA solution in 0.4 M NaCl was pushed onto a Teflon board immersed in the precipitating liquid (73% ethyl alcohol containing 0.4 M NaCl) using a syringe with a 0.9 mm diameter needle. The fiber was then soaked in 73% ethyl alcohol without NaCl. After drying it in a desiccator with a few grams of silica gel, a DSDNA fiber was obtained.

Preparation of PIC Fibers and Capsules. The chitosan samples were dissolved in 0.15 M acetic acid. DSDNA was dissolved in 0 (distilled water), 0.001, 0.01, 0.1, and 0.4 M NaCl solutions, and SSDNA in the 0 (distilled water) and 0.1 M NaCl solutions. When DSDNA solutions are added to the chitosan solutions and the interfaces are withdrawn, long fiber lines form at the interfaces in the wet states. Wet reactive spinning in water can be done using a roll-up apparatus after being dehydrated in ethyl alcohol (Fig. 1). SS- and DSDNA PIC capsules were prepared using a similar method reported in our earlier articles.[10,11]

Characterization of Fibers. The sodium contents were measured using an atomic absorption spectrophotometer (Shimadzu AA-6200). Scanning electron microscopy (SEM) was performed using a Hitachi S-5000 microscope to observe the surfaces of the fibers (accelerating voltage, 1.5 kV). The diameters of the fibers were measured both by an optical microscope and SEM. The stress/strain curves of a single thread of the fibers were meas-

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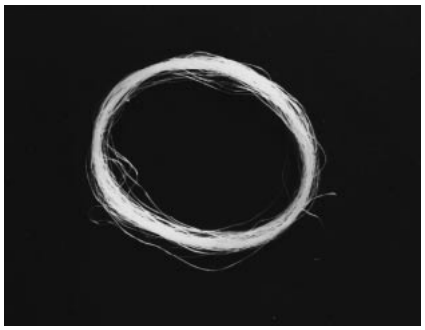

Fig. 1. Photograph of DSDNA (in distilled water)–chitosan polyion complex (PIC) fiber.

ured using a Tensilon (STA-1150, Orientec Co.). The initial gauge length was 20 mm, and the drawing speed was 20 mm/min. The average stress/strain curves were determined from 10 independent measurements.

Results and Discussion

DSDNA–Chitosan PIC Fibers. When an aqueous DSDNA solution was carefully added to aqueous chitosan 10–1000 solutions at pH 5 and room temperature without mixing, a film of PIC was formed at the interface. When this PIC film was withdrawn from the interface, a fiber line formed in the wet state. The spinnable conditions are listed in Table 1. The chitosan with a low molecular weight (chitosan 10) had a better spinnability than those with higher molecular weights. DSDNA–chitosan PIC fiber made under optimal conditions, i.e., the concentrations of DSDNA were 0.5–1 wt % and those of chitosan 10 were 1–2 wt %, could be continuously spun. Subsequent experiments were carried out under the 1 wt % DSDNA and 2 wt % chitosan 10 condition. After the intact wet fiber was dried in air after being dehydrated in ethyl alcohol, a strong fiber formed as a monofilament (Fig. 1). This fiber had a kind of double counter ion structure; that is, the outside layer was chitosan and the inside layer was DSDNA. Thus, as reported in our earlier articles,[12] a water-insoluble DSDNA PIC fiber with a strong tensile strength was created by a counter-charged self-assembly. The stoichiometrical amounts between DNA and chitosan in the DSDNA–chitosan fibers were determined in hydrochloric acid according to an earlier report.[17] The DNA amounts in the DSDNA–chitosan fibers were 87:13 (%) in 0–0.001 M NaCl, 85:15 (%) in 0.1 M NaCl, 82:18 (%) in 0.4 M NaCl, and 79:21 (%) in 0.6 M NaCl (not shown), respectively. At least 13% chitosan is sufficient to create DNA–chitosan fibers.

The sodium contents in the DNA–chitosan PIC fibers were determined by atomic absorption spectrometry. The starting DSDNA contained 7.00% sodium (theoretically 6.93%), which means that stoichiometrical electrostatic ionic salts exist between anionic phosphate groups and counter cation Na^+. The PIC fibers contained sodium in the range of 5.76% (prepared in 0–0.001 M NaCl), 6.00% (in 0.1 M NaCl), and 6.02% (in 0.4 M NaCl) (refer DSDNA contents in the PIC fibers). From these sodium contents, the electrostatic interaction between anionic DNA and cationic chitosan was fundamentally little influenced by the presence of NaCl. However, small amounts of NaCl were contained in the PIC fibers.

Stress/strain curves of the PIC fibers are shown in Fig. 2. When a DSDNA solution was pushed onto a Teflon board in 73% ethyl alcohol containing 0.4 M NaCl, a ribbon-like fiber was obtained after slow drying. This DSDNA fiber was used for a comparison with the DSDNA–chitosan PIC fiber newly prepared in the present study. In this DSDNA fiber, the ribbon width was not constant, and was easily soluble in water. The

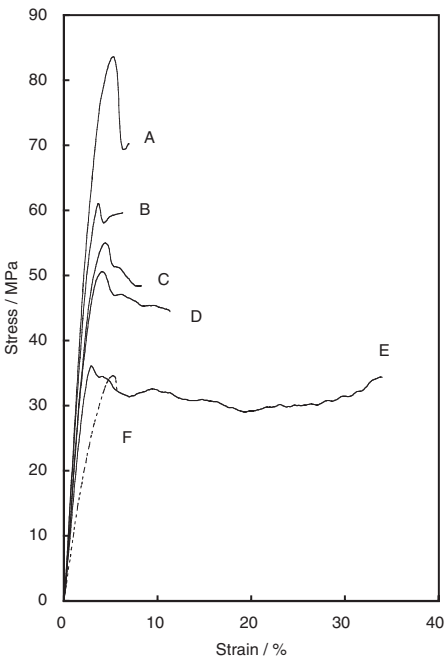

Fig. 2. Stress/strain curves of the DSDNA–chitosan PIC fibers, whose inside is DSDNA and whose outside is chitosan. A, DSDNA in 0 M NaCl solution (distilled water) (S.D. ±15 MPa); B, 0.001 M NaCl (S.D. ±14 MPa); C, 0.01 M NaCl (S.D. ±8.8 MPa); D, 0.1 M NaCl (S.D. ±6.9 MPa); E, 0.4 M NaCl (S.D. ±6.7 MPa); F, DSDNA fiber in distilled water (S.D. ±6.0 MPa).

Table 1. Spinning Conditions for DSDNA–Chitosan PIC Fiber Production[a)]

	Chitosan									
	10		100			500		1000		
DSDNA	1[b)]	2	0.5	1	2	0.5	1	0.1	0.5	1
0.5[b)]	◎[c)]	◎	◎	○	×	×	×	×	△	×
1	◎	◎	◎	◎	○	△	△	×	△	×

a) The DSDNA solutions were added into the chitosan solutions. b) Concentration of solutions, wt %. c) Symbols; ◎, good spinnability; ○, spinnable; △, spinnable, but short; ×, not spinnable.

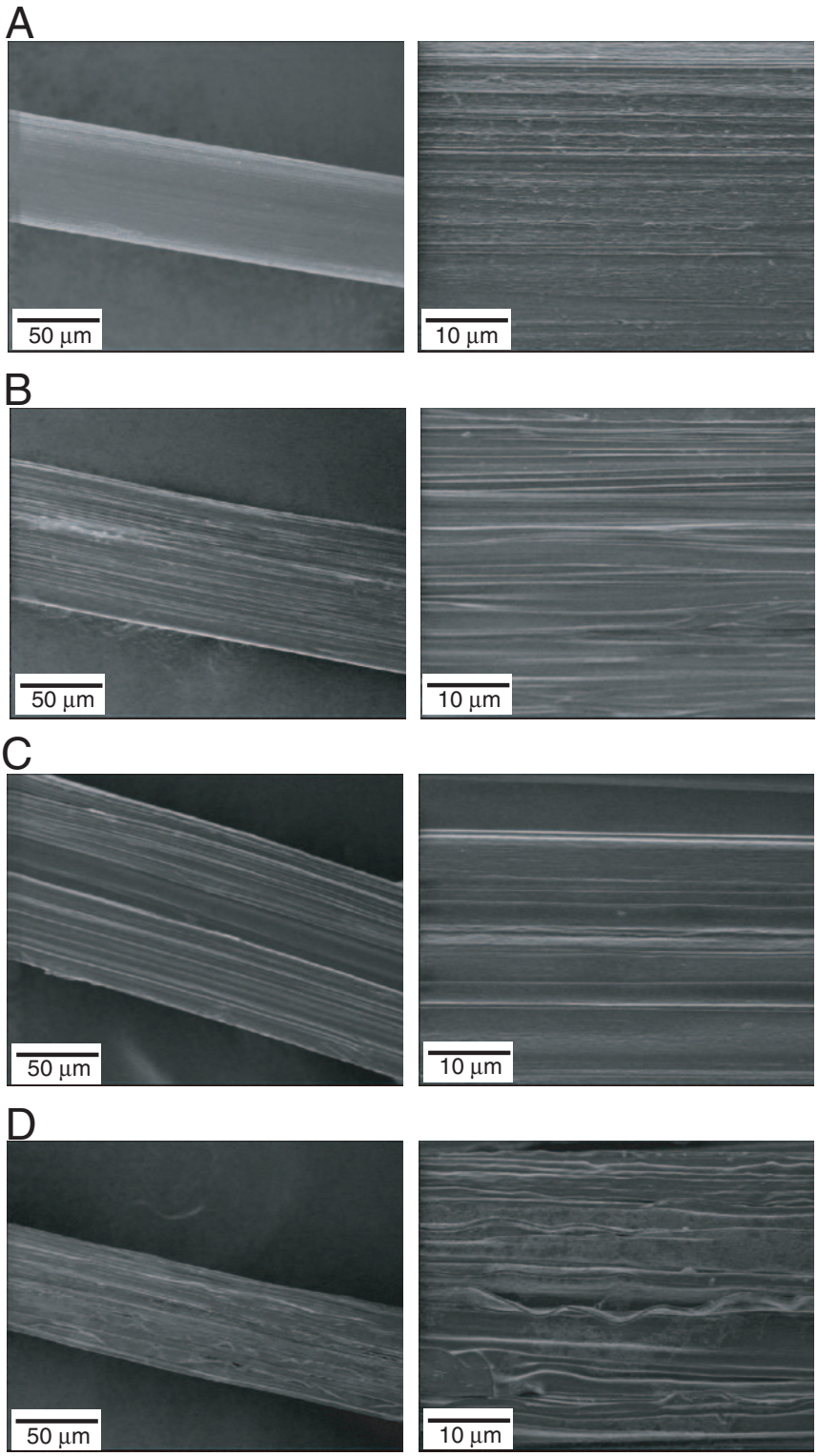

Fig. 3. SEM images of DSDNA–chitosan PIC fibers. On left side, magnification ×1000; on right side, ×2500: A, DSDNA in 0 M NaCl solution (distilled water); B, 0.001 M NaCl; C, 0.1 M NaCl; D, 0.4 M NaCl.

DSDNA fiber showed a 34 MPa tensile strength and 6% strain (broken line in Fig. 2F). The DSDNA (0 M NaCl, in distilled water)–chitosan PIC fiber was the strongest fiber, having an 84 MPa tensile strength and 7% strain. This PIC fiber was stronger than the DSDNA fiber, which had a 34 MPa tensile strength and 6% strain (broken line in Fig. 2F). DSDNA (in 0.001, 0.01, 0.1, and 0.4 M NaCl solutions)–chitosan PIC fibers had 61, 55, 50, and 35 MPa tensile strengths and 6, 8, 11, and 34% strains, respectively. As the concentration of the NaCl in the DSDNA solutions increased, the tensile strengths decreased and the strains increased, compared with the DSDNA (in 0 M NaCl)–chitosan fiber. The tensile strengths of the PIC fibers

depend on the concentration of NaCl. As described in a quantitative Na analysis, a little NaCl was contained in the PIC fibers. Since Na cations interact with the anions of the phosphoryl moieties of DSDNA, when NaCl was added, some cations of chitosan had no interaction with the DSDNA. Therefore, the number of electrostatic interactions between the anionic DNA and cationic chitosan decreased and the tensile strengths of the formed PIC fibers decreased, while the strains of the fibers increased after the yield points. This suggests that an excess of chitosan may be formed in the DSDNA backbone. SEM images of the PIC fibers are shown in Fig. 3. The diameters of the DSDNA–chitosan fibers were in the 70–120 micron range, and the fibril structures of the all fibers were aligned (Figs. 3A to D). Wrinkles in the fibrils were observed with an increase in the concentration of the NaCl. This SEM image well supports the above strain results in Fig. 2, together with the excess of chitosan explanation given above.

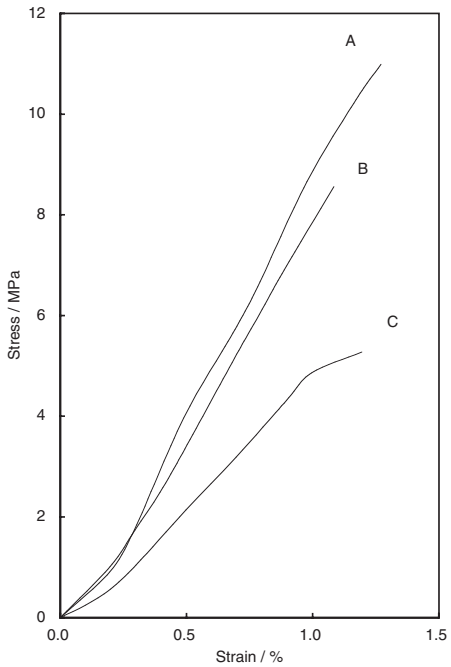

Fig. 4. Stress/strain curves of the SSDNA–chitosan PIC fibers, whose inside is chitosan and whose outside is SSDNA. A, 2.5 wt % SSDNA in 0 M NaCl (S.D. ±3.0 MPa); B, 5 wt % SSDNA in 0 M NaCl (S.D. ±3.1 MPa); C, 5 wt % SSDNA in 0.1 M NaCl (S.D. ±1.8 MPa).

SSDNA–Chitosan PIC Fibers. SSDNA–chitosan PIC fibers were created by the same method as described for the preparation of the DSDNA PIC fiber using a roll-up apparatus. The SSDNA–chitosan PIC fibers, whose inside is chitosan and whose outside is SSDNA, can be made by adding an aqueous chitosan solution to an aqueous SSDNA solution. Stress/strain curves of the SSDNA–chitosan PIC fibers are shown in Fig. 4. The stress and the strain of the PIC fibers decreased more than those of the DSDNA–chitosan fibers, exhibiting 11 MPa and 1.2% (at 2.5 wt % SSDNA in distilled water; Fig. 4A) and 8.3 MPa and 1% (at 5 wt % SSDNA in distilled water; Fig. 4B), respectively. When SSDNA was dissolved at 5 wt % in a 0.1 M NaCl solution, the stress and the strain of the PIC fiber created by the same method were 5 MPa and 1%. These results suggested that, like the DSDNA and chitosan interaction in the NaCl solution (Fig. 2), the interaction numbers of the SSDNA and chitosan decreased with the addition of NaCl. Since the strain values of the SSDNA–chitosan PIC fibers were low (1–1.2%), the effect of NaCl is not clear.

DNA PIC Capsules. Chitosan 1000 was dissolved at 1 wt % in 0.15 M (AcOH). DSDNA was dissolved at 0.5 wt % in distilled water. The chitosan solution was dropped into the DSDNA solution through a syringe and gently stirred. PIC capsules having spherical shapes were immediately formed after this dropwise addition; after a 30-min reaction, the capsules were transferred to distilled water, and washed for a few seconds (Fig. 5A). Capsules, whose inside was chitosan and whose outside was DSDNA, were formed. The diameters of the capsules were variable by using syringes having various diameters for controlling the size of the droplets. The use of a syringe with a needle internal diameter of 0.8 mm produced capsules having a diameter of approximately 6.0 mm. The capsule swelled to a diameter of 10.6 mm after being immersed for 2 h in distilled water. When immersed in a NaCl solution, the degree of swelling of the DSDNA–chitosan PIC capsule decreased. As an example, for the PIC capsule immersed in a 1 M NaCl solution for 24 h, the diameter of capsule was 7.3 mm. When the NaCl concentration was over 1.3 M, the capsule became turbid.

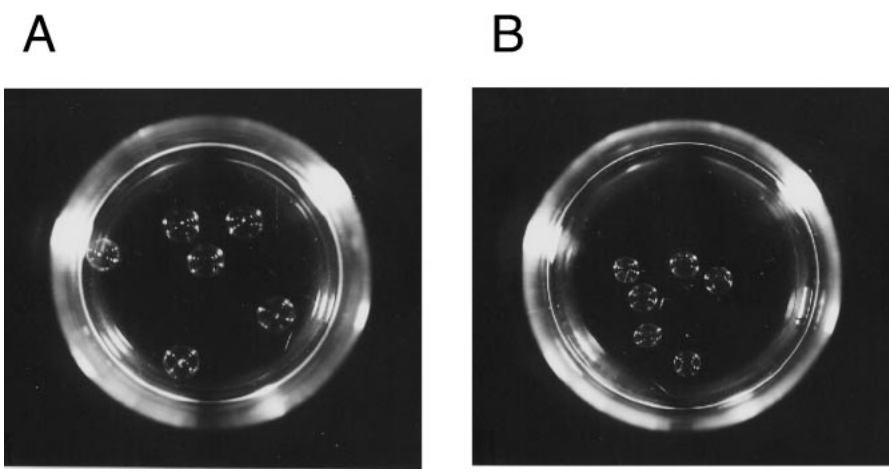

Fig. 5. Photographs of SS- and DSDNA–chitosan PIC capsules. A, DSDNA–chirosan; B, SSDNA–chitosan.

Spherical capsules were obtained starting from SSDNA and chitosan 1000 using the same method. First SSDNA was dissolved at 5 wt % in distilled water, and then a chitosan 1000 solution at 1 wt % in 0.15 M AcOH was added dropwise into the SSDNA solution. The capsules had a diameter of approximately 4.5 mm (Fig. 5B). They swelled to 9.3 mm after being immersed for 2 h in distilled water. The SSDNA capsule was turbid when immersed in a NaCl solution at over 1 M.

Thus, strong fibers and stable capsules could be prepared via this PIC between SS- and DSDNA and chitosan. Although DNA–chitosan PIC fiber could be constantly prepared, the reproducibility of the stress/strain curve measurements gave a rather wide variation. The results themselves are scientifically interesting, and might offer additional knowledge toward understanding the biological counter-ion interaction, which undoubtedly includes the charged nucleic acid and/or polysaccharides interaction process, together with biodegradation by microorganisms and enzymes[18] involved in keeping both the land and sea ecology clean.

Since Watson and Crick found the double helical structure of DNA in 1953, DNA investigations and material science became active, starting from genetic life science to biomacromolecule material development, together with genetic engineering. During this time period, for a waste material utilizing purpose, DNA as an intercalator has been studied to remove some heavy metals.[19] Most recently, monolayer DNA and bilayer DNA–chitosan hybrid materials have been investigated as novel molecular devices[4] and as a bi-functional biomedical adhesive.[20]

In our PIC hybrid material science studies, we first created fibers between SS- and DSDNA and chitosan. We can prepare a rigid and strong fiber and/or an elastic PIC fiber by controlling the NaCl concentration that dissolves the DNA molecules. We also created stable true spherical capsules between SS- and DSDNA and chitosan. These findings may also have the prospect of developing new biomimetic materials under aqueous circumstances. In addition, the applications toward the material science of wastes, such as DNA and chitosan, have a possibility for developments in environmental, medical, and fiber fields.

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References

1 K. Iwata, T. Sawadaishi, S. Nishimura, S. Tokura, and N. Nishi, *Int. J. Biol. Macromol.*, **18**, 149 (1996).

2 H. Kitamura, C. Iwamoto, N. Sakairi, S. Tokura, and N. Nishi, *Int. J. Biol. Macromol.*, **20**, 241 (1997).

3 K. Tanaka and Y. Okahata, *J. Am. Chem. Soc.*, **118**, 10679 (1996).

4 M. Shimomura, R. Mitamura, J. Matsumoto, and K. Ijiro, *Synth. Met.*, **133**, 473 (2003).

5 M. Yamada, K. Kato, K. Shindo, M. Nomizu, M. Haruki, N. Sakairi, K. Ohkawa, H. Yamamoto, and N. Nishi, *Biomaterials*, **22**, 3121 (2001).

6 M. Yamada, K. Kato, M. Nomizu, N. Sakairi, K. Ohkawa, H. Yamamoto, and N. Nishi, *Chem.—Eur. J.*, **8**, 1407 (2002).

7 "Applications of Chitin and Chitosan (Kitin, Kitosan no Ouyou)," ed by M. Yabuki, Gihoudo Press, Tokyo (1990).

8 T. Sakiyama, C.-H. Chu, T. Fujii, and T. Yano, *J. Appl. Polym. Sci.*, **50**, 2021 (1993).

9 K. Y. Lee, W. H. Park, and W. S. Ha, *J. Appl. Polym. Sci.*, **63**, 425 (1997).

10 H. Yamamoto, K. Ohkawa, E. Nakamura, K. Miyamoto, and T. Komai, *Bull. Chem. Soc. Jpn.*, **76**, 2053 (2003).

11 K. Ohkawa, T. Kitagawa, and H. Yamamoto, *Macromol. Mater. Eng.*, **289**, 33 (2004).

12 a) M. Amaike, Y. Senoo, and H. Yamamoto, *Macromol. Rapid Commun.*, **19**, 287 (1998). b) H. Yamamoto and Y. Senoo, *Macromol. Chem. Phys.*, **201**, 84 (2000). c) H. Yamamoto, C. Horita, Y. Senoo, A. Nishida, and K. Ohkawa, *J. Appl. Polym. Sci.*, **79**, 437 (2001). d) K. Ohkawa, Y. Takahashi, M. Yamada, and H. Yamamoto, *Macromol. Mater. Eng.*, **286**, 168 (2001). e) Y. Takahashi, M. Hachisu, K. Ohkawa, and H. Yamamoto, *Macromol. Rapid Commun.*, **23**, 540 (2002). f) M. Hachisu, K. Ohkawa, and H. Yamamoto, *Macromol. Biosci.*, **3**, 92 (2003). g) Y. Kuboe, H. Tonegawa, K. Ohkawa, and H. Yamamoto, *Biomacromolecules*, **5**, 348 (2004).

13 H. Yamamoto and K. Ohkawa, "Encyclopedia of Surface and Colloid Science," ed by A. Hubbard, Dekker, New York (2002), p. 4242.

14 G. A. F. Roberts and J. G. Domszy, *Int. J. Biol. Macromol.*, **4**, 374 (1982).

15 A. Rupprecht, *Biochem. Biophys. Res. Commun.*, **12**, 163 (1963).

16 A. Rupprecht, *Acta Chem. Scand.*, **20**, 494 (1966).

17 J. D. Smith and R. Markham, *Biochem. J.*, **46**, 509 (1950).

18 a) H. Yamamoto, M. Amaike, and H. Saitoh, *Biomimetics*, **3**, 123 (1995). b) H. Yamamoto and M. Amaike, *Macromolecules*, **30**, 3936 (1997). c) K. Ohkawa, M. Yamada, A. Nishida, N. Nishi, and H. Yamamoto, *J. Polym. Environ.*, **8**, 59 (2000).

19 M. Yamada, K. Kato, M. Nomizu, M. Haruki, K. Ohkawa, H. Yamamoto, and N. Nishi, *Bull. Chem. Soc. Jpn.*, **75**, 1627 (2002).

20 S. Rikimaru, Y. Wakabayashi, M. Nomizu, and N. Nishi, *Polym. J.*, **35**, 255 (2003).