Synthesis and assembly of novel chitin derivatives having amphiphilic polyoxazoline block copolymer as a side chain

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SUMMARY: The first synthesis of chitin derivatives with well-defined block copolymer side chains, i.e., chitin-*graft*-[poly(2-methyl-2-oxazoline)-*block*-poly(2-phenyl-2-oxazoline)] (**5**), chitin-*graft*-[poly(2-methyl-2-oxazoline)-*block*-poly(2-butyl-2-oxazoline)] (**6**), and chitin-*graft*-[poly(2-methyl-2-oxazoline)-*block*-poly(2-*tert*-butyl-2-oxazoline)] (**7**), was achieved by the reaction of partially deacetylated chitin (**1**) with living polyoxazoline block copolymers **2**–**4**. The graft copolymers **5**–**7** are associated into micelles above the critical micelle concentration (CMC). CMCs of **5** (0.01–0.02 wt.-%) are smaller than those (0.32–0.50 wt.-%) of ω-hydroxyl-terminated poly(2-phenyl-2-oxazoline)-*block*-poly(2-methyl-2-oxazoline) (**2**-OH), which is a model block copolymer of the side chain segment of **5**. The self-aggregates of **5**–**7** are capable of forming a complex with hydrophobic low molecular weight substances such as pyrene and magnesium 1-anilinonaphthalene-8-sulfonate (ANS). Cryo-transmission electron microscopy showed that the graft copolymer **5** forms globular particles (diameter: 40 nm) and larger cylindrical aggregates (diameter: 40 nm, length: 80–200 nm). The average radius of gyration of the particles of **5** from the SANS analysis is 36 nm.

Introduction

Chitin, the most abundant natural polymer after cellulose, is found in a variety of biosystems including fungal cell walls, the exo-skeleton of crustaceans, and the integument of insects. An estimated 10^{11} tons of chitin is produced annually in the biosphere[1]. The growing interest in chitin led to their uses in a wide variety of fields from basic biological research to developments in chemical industry[2]. However, its actual utilization has been hitherto limited as applications, e.g., for biomedical materials and simple flocculents of wastewater treatment, mainly due to its insolubility in most organic solvents. Therefore, chemical modification of chitin is an important subject for production of biofunctional materials[3]. We have already reported the synthesis of a novel chitin derivative having hydrophilic poly(2-methyl-2-oxazoline) side chains[4] and its miscibility with commodity polymers[5].

Natural polysaccharides, which are biocompatible and biodegradable polyhydroxyl compounds, show unique properties such as formation of hydrogels or liquid crystals. Especially, hydrophobized polysaccharide derivatives[6] have attracted much attention in many fields including pharmacological, cosmetic, and biotechnological applications. Recently, chitin-*graft*-poly(2-methyl-2-oxazoline)/poly(vinyl alcohol) miscible hybrids have been studied as a decay rate-changeable degradable material[7]. Naka et al. have reported that chitin-*graft*-poly(2-methyl-2-oxazoline) is capable of incorporating lipase and catalase in its micelle, and increases the hydrolysis activities[8]. Our interest in biomedical application of the big biomass chitin has prompted us to study the synthesis and solution properties of chitin derivatives having amphiphilic poly(2-oxazoline) block copolymer as a side chain (**5**–**7**).

The point in this study is the application of the reaction between the aminopolysaccharide and living block copolymers to synthesize amphiphilic polysaccharide derivatives. The synthetic strategy of artificial glycoconjugates having monodisperse side chains by living polymerization is important, but only a few examples have been reported until now[4, 9]. Although chitosan-*graft*-[poly(ethylene oxide)-*block*-poly(propylene oxide)-*block*-poly(ethylene oxide)][10] has been synthesized, the present amphiphilic chitin-containing graft copolymer is a first example of polysaccharide having well-defined block copolymer side chains obtained by living polymerization. The amphiphilic AB block-type poly(2-oxazoline) was prepared by a one-pot two-stage copolymerization technique[11], based on living character of cationic ring-opening polymerization of 2-oxazoline[12], in which one of 2-oxazolines (2-phenyl-, 2-butyl-, and 2-*tert*-butyl-2-oxazoline) is first polymerized to give a hydrophobic A block, and then the second monomer (2-methyl-2-oxazoline) is polymerized at the living ends of the first polymerization system to give a hydrophilic B block which functions as a flexible spacer. Although polysaccharide-based amphiphiles are obtained by substitution with hydrophobic groups to the rigid polysaccharide, the present macromolecular design has an advantage of introducing a flexible

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spacer, i.e., poly(2-methyl-2-oxazoline) segment, in a block-type side chain. The spacer length and hydrophilic/lipophilic balance can be easily controlled by molecular design. The influence of the structure and composition of these graft copolymers on the solution properties was evaluated by means of ^{1}H NMR, surface tension, cryo-transmission electron microscopy (cryo-TEM), small-angle neutron scattering (SANS), and fluorescence analyses. Observations of chitin derivatives in an aqueous solution by cryo-TEM have not been hitherto published to the best of our knowledge.

Experimental part

Materials

Chitin from crab shells was purchased from Sigma Chemical Co. (St. Louis). Partially *N*-deacetylated chitin (ca. 50%) was prepared by the method of Kurita et al.[13] 2-Methyl-, 2-phenyl-, 2-butyl-, and 2-*tert*-butyl-2-oxazolines were prepared according to the literature[14]. Methyl trifluoromethanesulfonate (MeOTf) was purchased from Aldrich Chemical Co. and distilled under nitrogen. Dimethyl sulfoxide (DMSO)-d_6 (Aldrich Chemical Co.), D_2O (Aldrich Chemical Co.), and $CDCl_3$ (Janssen Chimica) were used without purification for NMR analysis. Magnesium 1-anilinonaphthalene-8-sulfonate (ANS) (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) and pyrene (Acros, Geel, Belgium) were commercially available.

Synthesis of chitin derivatives having various poly(2-oxazoline) block copolymer side chains (**5**–**7**)

According to our previous work[4], all operations of polymerization of 2-oxazolines and polymer reaction between living block copolyoxazolines **2**–**4** and partially deacetylated chitin (**1**) were performed in test tubes equipped with a three-way stopcock under nitrogen. An experimental procedure of synthesis of chitin-*graft*-[poly(2-methyl-2-oxazoline)-*block*-poly(2-phenyl-2-oxazoline)] (**5a**) is as follows. The stepwise block copolymerization of 2-phenyl-2-oxazoline as the first monomer and 2-methyl-2-oxazoline as the second monomer initiated with MeOTf was carried out in acetonitrile at 70 °C[12a]. After the polymerization was completed, the solution was divided into two portions. In the one portion, the solvent was evaporated under reduced pressure. After reprecipitation with dichloromethane as a solvent and hexane as a non-solvent, the polymeric material was collected and dried in vacuo to give a white powdery material (99.4% yield). Poly(2-phenyl-2-oxazoline)-*block*-poly(2-methyl-2-oxazoline) from this portion was used to determine the degree of polymerization and the molecular weight distribution. From the other portion, 3.99 g (3.96 mmol) of the living block copolymer was obtained, and then dissolved in 10 mL of dry DMSO. This solution was immediately added to the suspension of randomly deacetylated chitin (0.150 g) in DMSO (10 mL) under nitrogen. After stirring vigorously at 27 °C for 70 h, the reaction mixture was filtered. Water was added to the filtrate, and the solution was treated with an excess amount of the ion-exchange resin (Amberlite IRA-68, in basic form) in order to convert the ammonium-type linkage between D-glucosamine and the ω-end of the block copolymer to the amine-form. The aqueous solution was purified by dialysis against water using a seamless cellulose tube (Nacalai Tesque, Inc.; MW cut off 3500), and lyophilized to afford a white powdery product; yield 0.365 g (92.6%).

Chitin-*graft*-[poly(2-methyl-2-oxazoline)-*block*-poly(2-phenyl-2-oxazoline)] (**5**):

IR (KBr disk): 3423 (ν_{O-H}), 3063 (ν_{C-H} (aromatic)), 2926 (ν_{C-H}), 1630 ($\nu_{C=O}$), 1422 (δ_{C-H}), 1072, 1030 cm^{-1} (ν_{C-O-C}).

^{1}H NMR (D_2O/CD_3COOD (95:5, v/v), conc.: 1.0 wt.-%, 90 °C, ref.: sodium 2,2-dimethyl-2-silapentane-5-sulfonate; 270 MHz): δ = 2.05 (CH_3CO), 2.85–3.10 (CH_3—N), 3.16 (H-2 of D-glucosamine units), 3.30–4.05 (H-2 of *N*-acetyl-D-glucosamine units, H-3, 4, 5, 6 of pyranose units, and CH_2 of poly(2-oxazoline)), 4.58 (H-1 of *N*-acetyl-D-glucosamine units), 4.88 (H-1 of D-glucosamine units), 7.00–7.64 (C_6H_5).

^{13}C NMR (D_2O/HCl (1 drop), conc.: 5.0 wt.-%, 40 °C, ref.: sodium 2,2-dimethyl-2-silapentane-5-sulfonate; 100 MHz): δ = 22.8 (*C*H_3CON), 24.7 (*C*H_3CONH of *N*-acetyl-D-glucosamine units), 39.8 (CH_3N), 45.8–52.0 (CH_2 of poly(2-oxazoline)), 58.2 (C-2 of pyranose units), 61.2 (NH*C*H_2CH_2 linked to C-2 of pyranose units), 62.9 (C-6 of pyranose units), 74.2 (C-3 of pyranose units), 77.0 (C-5 of pyranose units), 79.4–81.6 (C-4 of pyranose units), 100.0 (C-1 of D-glucosamine units), 103.8 (C-1 of *N*-acetyl-D-glucosamine units), 128.9 (meta position of aromatic carbons), 131.5 (ortho position of aromatic carbons), 133.1 (para position of aromatic carbons), 175.5–178.7 (carbonyl carbons).

Chitin-*graft*-[poly(2-methyl-2-oxazoline)-*block*-poly(2-butyl-2-oxazoline)] (**6**):

IR (KBr disk): 3444 (ν_{O-H}), 2956 (ν_{C-H}), 1635 ($\nu_{C=O}$), 1422 (δ_{C-H}) 1067, 1034 cm^{-1} (ν_{C-O-C}).

^{1}H NMR (D_2O/CD_3COOD (95:5, v/v), conc.: 1.0 wt.-%, 90 °C, ref.: sodium 2,2-dimethyl-2-silapentane-5-sulfonate; 270 MHz): δ = 0.89 (C*H*$_3$CH$_2$CH$_2$CH$_2$), 1.34 (CH$_3$C*H*$_2$CH$_2$CH$_2$), 1.54 (CH$_3$CH$_2$C*H*$_2$CH$_2$), 2.09 (CH_3CO), 2.34 (CH$_3$CH$_2$CH$_2$C*H*$_2$), 2.86–3.14 (CH_3N), 3.21 (H-2 of D-glucosamine units), 3.29–4.03 (H-2 of *N*-acetyl-D-glucosamine units, H-3, 4, 5, 6 of pyranose units, and CH_2 of poly(2-oxazoline)), 4.60 (H-1 of *N*-acetyl-D-glucosamine units), 4.90 (H-1 of D-glucosamine units).

^{13}C NMR (D_2O/HCl (1 drop), conc.: 5.0 wt.-%, 40 °C, ref.: sodium 2,2-dimethyl-2-silapentane-5-sulfonate; 100 MHz): δ = 15.6 (*C*H$_3$CH$_2$CH$_2$CH$_2$), 22.8 (*C*H_3CON), 24.5 (*C*H_3CONH of *N*-acetyl-D-glucosamine units and CH$_3$*C*H$_2$CH$_2$CH$_2$), 29.8 (CH$_3$CH$_2$*C*H$_2$CH$_2$), 34.8 (CH$_3$CH$_2$CH$_2$*C*H$_2$), 39.5 (CH_3N), 45.7–52.0 (CH_2 of poly(2-oxazoline)), 58.3 (C-2 of pyranose unit), 61.8 (NH*C*H_2CH_2 linked to C-2 of pyranose units), 62.7 (C-6 of pyranose units), 73.2–74.7 (C-3 of pyranose units), 77.0 (C-5 of pyranose units), 79.2–82.0 (C-4 of pyranose units), 100.0 (C-1 of D-glucosamine units), 103.7 (C-1 of *N*-acetyl-D-glucosamine units), 176.8 (carbonyl carbons of *N*-acetyl-D-glucosamine units and poly(2-methyl-2-oxazoline)), 179.2 (carbonyl carbons of poly(2-butyl-2-oxazoline)).

$(C_8H_{13}O_5N)_{0.45}(C_6H_{11}O_4N)_{0.24}[C_6H_{10}O_4N(C_4H_7ON)_{23.5}$

$(C_7H_{13}ON)_{4.2}CH_3]_{0.31} \cdot 2H_2O$ Calc. C 55.83 H 8.67 N 13.74
Found C 55.80 H 8.94 N 13.89

Chitin-*graft*-[poly(2-methyl-2-oxazoline)-*block*-poly(2-*tert*-butyl-2-oxazoline)] (**7**):

IR (KBr disk): 3441 (ν_{O-H}), 2938 (ν_{C-H}), 1631 ($\nu_{C=O}$), 1422 (δ_{C-H}) 1068, 1034 cm^{-1} (ν_{C-O-C}).

^1H NMR (D_2O/CD_3COOD (95:5, v/v), conc.: 1.0 wt.-%, 90 °C, ref.: sodium 2,2-dimethyl-2-silapentane-5-sulfonate; 270 MHz): δ = 1.27 (CH_3C), 2.09 (CH_3CO), 3.08–3.17 (CH_3N and H-2 of D-glucosamine units), 3.28–4.00 (H-2 of *N*-acetyl-D-glucosamine units, H-3, 4, 5, 6 of pyranose units, and CH_2 of poly(2-oxazoline)), 4.63 (H-1 of *N*-acetyl-D-glucosamine units), 4.91 (H-1 of D-glucosamine units).

^{13}C NMR (D_2O/HCl (1 drop), conc.: 5.0 wt.-%, 40 °C, ref.: sodium 2,2-dimethyl-2-silapentane-5-sulfonate; 100 MHz): δ = 22.8 (CH_3CON), 24.9 (CH_3CONH of *N*-acetyl-D-glucosamine units), 29.9–30.3 (CH_3Cof poly(2-*t*-butyl-2-oxazoline)), 40.5 (CH_3N), 41.2–41.6 (CH_3C of poly(2-*t*-butyl-2-oxazoline)), 45.9–52.0 (CH_2 of poly(2-oxazoline)), 58.3 (C-2 of pyranose units), 61.0–62.9 (C-6 of pyranose units and $NHCH_2CH_2$ linked to C-2 of pyranose units), 72.8–74.5 (C-3 of pyranose units), 77.2 (C-5 of pyranose units), 79.3–82.0 (C-4 of pyranose units), 100.0 (C-1 of D-glucosamine units), 104.0 (C-1 of *N*-acetyl-D-glucosamine units), 176.9 (carbonyl carbons of *N*-acetyl-D-glucosamine units and poly(2-methyl-2-oxazoline)), 183.6 (carbonyl carbons of poly(2-*tert*-butyl-2-oxazoline)).

Preparation of ω-hydroxyl-terminated amphiphilic AB block-type poly(2-oxazoline)s

A typical experimental procedure is as follows. A living poly(2-phenyl-2-oxazoline)-*block*-poly(2-methyl-2-oxazoline) (**2a**) (1.00 g, 0.92 mmol) was dissolved in 50 mL of methanol. The solution was treated with 6.5 g of ion-exchange resin (Amberlite IRA-410, in basic form) to convert the onium species to ω-hydroxyl groups (27 °C, 1 h). The solution was filtrated and lyophilized to afford a white powder. The yield was 0.759 g (88.9%).

Measurements

^1H NMR measurement was performed on a JEOL EX-270 spectrometer at 270 MHz, and ^{13}C NMR spectra were taken by a Bruker ARX 400 spectrometer operating at 100 MHz.

Size exclusion chromatography (SEC) was conducted with a Jasco Model PU-980 high performance liquid-chromatograph apparatus (column, Shodex B-805 → B-804; solvent, H_2O; temp., 27 °C), and with a Tosoh HLC-8020 system (column, Tosoh TSK-gel $G3000H_{XL}$ → $G2000H_{XL}$; solvent, chloroform; temp., 38 °C).

The surface tension of aqueous solutions was measured at 25 °C by the drop weight method[15].

The critical micelle concentration (CMC) was defined as a break point on the surface tension curve.

Cryo-TEM observation was carried out on a Hitachi H-800 electron microscope equipped with a cold stage by the technique described by Bellare et al.[16] The specimen was prepared by depositing a small droplet of the solution on a TEM grid coated by a holey carbon film. A thin film of the solution on the grid was formed by removing excess fluid. The specimen was vitrified by plunging into nitrogen slush and imaged at an accelerating voltage of 100 kV.

SANS measurements were carried out using the SANS-U spectrometer of JRR-3 M at the Japan Atomic Energy Research Institute, Tokai. A 7 Å incident neutron beam, taken from the cold neutron source and monochromated by a mechanical velocity selector, was used.

Complexation between the chitin derivatives and hydrophobic guest molecules such as ANS and pyrene was fluorometrically investigated at 25 °C with a Hitachi F-4500 fluorescence spectrophotometer. The concentration of ANS was 2.0×10^{-5} M in water[6b], in which 0.5 mL of the stock aqueous solution of ANS (1.0×10^{-4} M) was mixed with 2.0 mL of the aqueous polymer solution. On the other hand, the concentration of pyrene was 6.0×10^{-7} M because of low solubility in water[17]. To a vial, 1 mL acetone solution (1.8×10^{-6} M) of pyrene was added, and then the solvent was evaporated to form a thin film at the bottom of the vial. An aqueous polymer solution was added to the vial, and the resulting mixture was kept at 25 °C for 1 h with mixing by a magnetic stirrer. Fluorescence emission spectra were taken by excitation at 380 nm for ANS and at 339 nm for pyrene, and the excitation spectra of pyrene were obtained by collecting fluorescence emission at 390 nm.

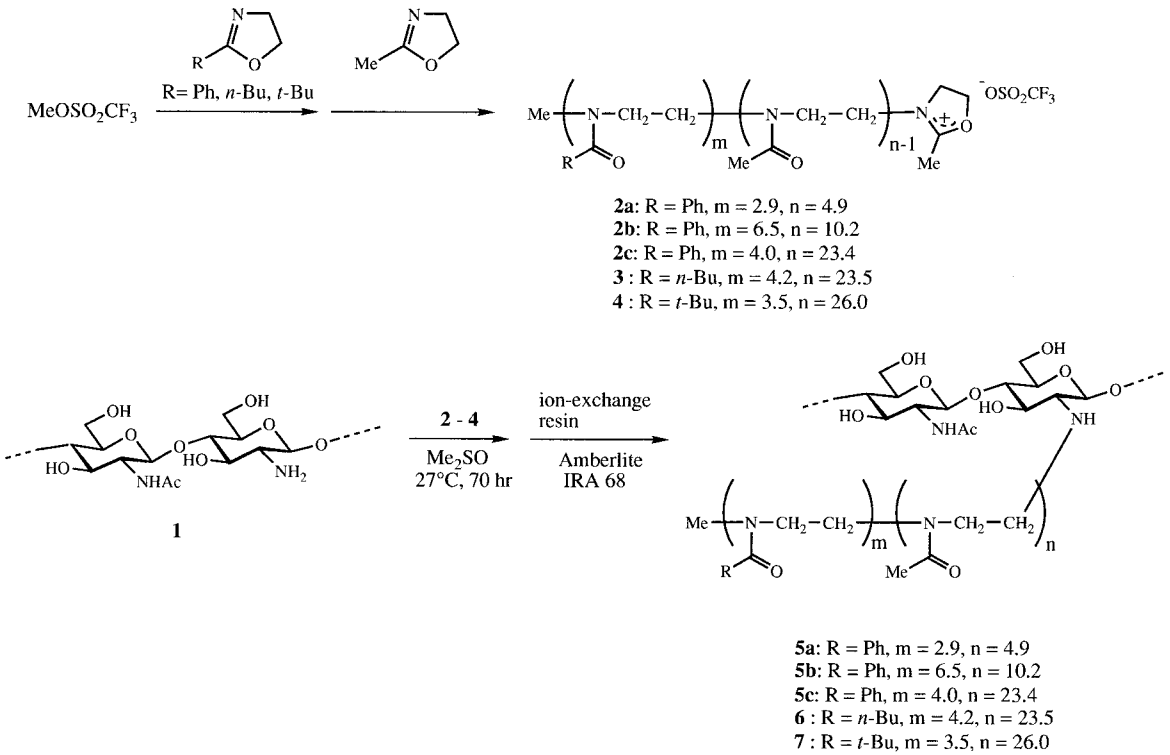
Results and discussion

Synthesis of chitin derivatives having block copolyoxazoline side chains **5**–**7**

According to Scheme 1, chitin-containing graft copolymers **5**–**7** having amphiphilic block copolymer branches were synthesized by termination of living polyoxazoline block copolymer with an amino group of partially deacetylated chitin[4]. In order to control the block length in the block copolymer synthesis by living polymerization with step-wise monomer addition, higher reactivity of an active species generated by the polymerization of the first monomer is required than that of the second monomer. An oxazolinium salt of 2-phenyl-2-oxazoline has a sufficiently higher electrophilic reactivity to form an ordered sequence with subsequent addition of 2-methyl-2-oxazoline[18]. Therefore, desirable amphiphilic graft copolymer, which consists of a hydrophilic main chain and has hydrophobic side chains connected with a hydrophilic flexible spacer segment, is favorably derived from 2-phenyl-2-oxazoline (the first monomer), 2-methyl-2-oxazoline (the second monomer), and the aminopolysaccharide as a terminator.

The results of one-pot two-stage feeding block copolymerization and the termination are summarized in Tab. 1. The structures of the resulting graft copolymers were determined by IR, elemental analysis, and ^1H and ^{13}C NMR spectroscopies. Complete removal of unreacted

Scheme 1:



polyoxazolines was confirmed by size exclusion chromatography (SEC). The main chain of the product graft copolymer consists of randomly linked D-glucosamine and *N*-acetyl-D-glucosamine repeating units. Poly(2-oxazoline) (PROZO) branches are attached to the 2-position of the D-glucosamine units of the main chain regiospecifically, as confirmed by ^{13}C NMR analysis. No termination occurred by a model reaction of *N*-acetyl-D-glucosamine and living poly(2-ethyl-2-oxazoline) under the same reaction conditions[4]. The result supported the poor reactivity of the hydroxyl groups for the termination. In every run of Tab. 1, the side chain length was relatively regulated by the feed ratios of the oxazoline monomers to the initiator. Yields of graft copolymers **5**–**7** were calculated on the basis of the amounts of the chitin main chain. The $[PROZO]_0/[—NH_2]_0$ and $[PROZO]/[—NH_2]$ values in Tab. 1 represent the feed and reacted molar ratios of oxazoline polymer to D-glucosamine units of **1**, respectively. The $[PROZO]/[—NH_2]$ values were determined by ^1H NMR signal intensity ratios of the methyl protons of *N*-acetyl-D-glucosamine units at 1.82 ppm to the methyl protons of poly(2-methyl-2-oxazoline) segment at 1.99 ppm in DMSO-d_6. The $[PROZO]/[—NH_2]$ value was roughly controlled by the feed molar ratios ($[PROZO]_0/[—NH_2]_0$), irrespective of the side chain length.

The amphiphilic structure of graft copolymers having block copoly(2-oxazoline) side chains was supported by the difference of the solubilities of the graft copolymer and each segment. In contrast to the backbone component **1**, all graft copolymers **5**–**7** showed much improved solubilities. They were soluble in water, 5% acetic acid aq., DMSO, *N*,*N*-dimethylformamide, and *N*,*N*-dimethylacetamide, and partially soluble in methanol, acetonitrile, and chloroform. Aqueous solutions of all the graft copolymers **5**–**7** foamed by stirring, which suggested that the graft copolymers were expected to exhibit surface active properties.

Solution property of graft copolymers **5**–**7**

The molecular motion of chitin-*graft*-[poly(2-methyl-2-oxazoline)-*block*-poly(2-phenyl-2-oxazoline)] (**5**) was evaluated by ^1H NMR analysis in D_2O/CD_3COOD (95:5, v/v) (polymer concentration: 1.0 wt.-%). Fig. 1 shows the ^1H NMR spectrum of **5a** at 30 °C. The terminal *N*-methyl proton signals of poly(2-phenyl-2-oxazoline) segment (δ 2.85–3.10 ppm) of **5a** broadened at 50 °C and disappeared at 85 °C. This phenomenon is reasonably interpreted by reducing interaction between water and the oligo(2-phenyl-2-oxazoline) segment in an aqueous solution of **5a** at higher temperature. Moreover, line broadening of the aromatic protons of the poly(2-phenyl-2-oxazoline) segment (δ 7.00–7.64 ppm) was confirmed in D_2O/CD_3COOD (95:5, v/v) solution of **5a** (Fig. 1), compared with that in DMSO-d_6 solution. The reason is that poly(2-

Tab. 1. Synthesis of chitin derivatives having amphiphilic block copoly(2-oxazoline) side chain (**5**–**7**)

Run no.	Poly(2-oxazoline) (PROZO)				Graft copolymer[d]								
	Polymer	1st stage[a]		2nd stage[a]	Polymer	DA[e] in %	m[f]	n[f]	$\bar{M}_w/\bar{M}_n$[g]	$\frac{[PROZO]_0}{[-NH_2]_0}$[h]	Yield[i] in %	$\frac{[PROZO]}{[-NH_2]}$[j]	$\bar{M}_n$[k] $\times 10^{-5}$
		R	$[M_1]/[I_0]$[b]	$[M_2]/[I_0]$[c]									
1	**2a**	Ph	2.9	5.3	**5a**	52	2.9	4.9	1.1_2	10.0	93	0.76	4.0
2	**2b**	Ph	5.9	10.3	**5b**	52	6.5	10.2	1.1_5	10.0	86–92[l]	>0.92[l]	6.0
3	**2c**	Ph	3.6	22.8	**5c**	45	4.0	23.4	1.1_5	0.6	59	0.60	8.8
4	**3**	*n*-Bu	3.8	23.4	**6**	45	4.2	23.5	1.2_5	0.6	73	0.57	10.0
5	**4**	*t*-Bu	3.1[m]	21.2[n]	**7**	45	3.5	26.0	1.1_5	0.6	28	0.58	3.3

a) In acetronitrile, at 70 °C, for 20 h. Oligo(2-oxazoline)s and block cooligo(2-oxazoline)s were obtained in 90–99%.
b) The feed molar ratio of the first monomer to the initiator (methyl trifluoromethanesulfonate).
c) The feed molar ratio of the second monomer (2-methyl-2-oxazoline) to the initiator.
d) Reaction of **1** and **2**–**4** were carried out in dimethyl sulfoxide at 27 °C for 70 h under nitrogen.
e) Degree of *N*-acetylation of **1**, by ^{1}H NMR in D_2O/CD_3COOD (95:5, v/v).
f) DP of the side chain, by ^{1}H NMR in $CDCl_3$ at 27 °C.
g) Polydispersity of the side chain, by SEC in $CHCl_3$ (polystyrene standard).
h) Feed molar ratio of block cooligo(2-oxazoline)s to D-glucosamine unit of **1**.
i) Soluble part of reaction mixture.
j) Molar ratio of poly(2-oxazoline) (PROZO) unit to D-glucosamine unit of **1**, by ^{1}H NMR.
k) By SEC in H_2O (pullulan standard).
l) Estimated by the weight of products.
m) In benzonitrile, at 100 °C, for 20 h.
n) In benzonitrile, at 70 °C, for 20 h.

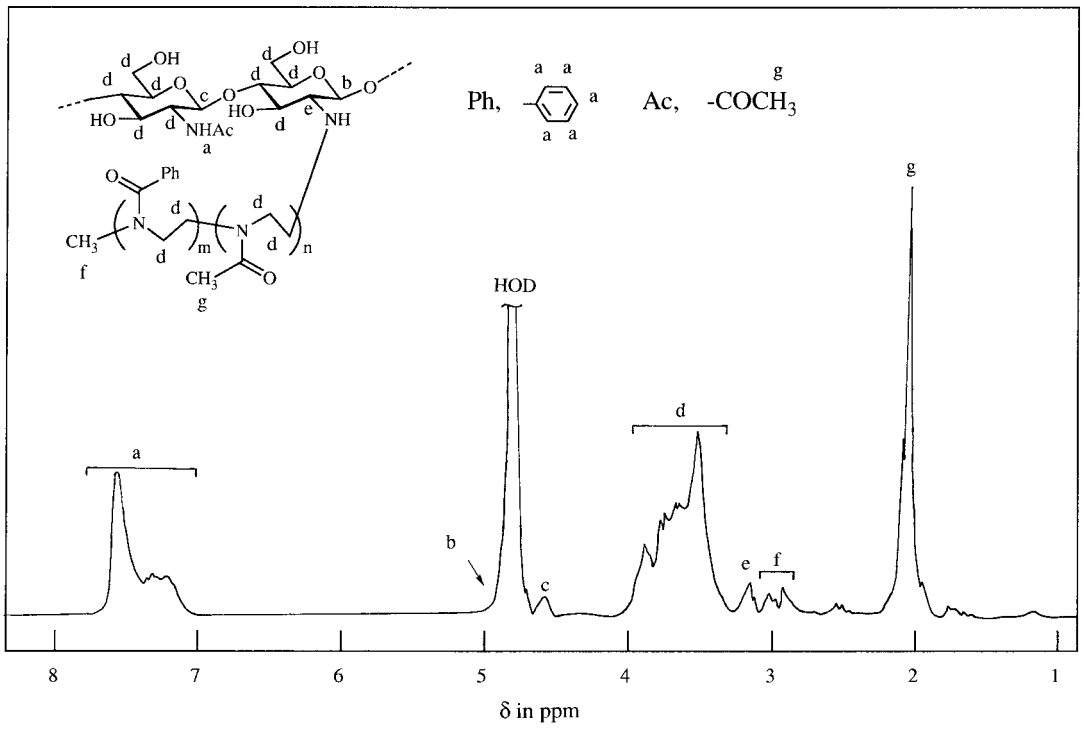


Fig. 1. ^{1}H NMR spectrum of chitin-*graft*-[poly(2-methyl-2-oxazoline)-*block*-poly(2-phenyl-2-oxazoline)] (**5a**) in D_2O/CD_3COOD (95:5, v/v) (temp.: 30 °C, concentration: 1.0 wt.-%)

phenyl-2-oxazoline) is soluble in DMSO-d_6, whereas it is insoluble in D_2O/CD_3COOD (95:5, v/v). The ^{1}H NMR behavior suggests low mobility of the poly(2-phenyl-2-oxazoline) segment.

Surface tension (γ) of aqueous solutions of **5a** measured at 25 °C gradually decreased with increasing concentration (C, wt.-%), and it reached a constant value at the critical micelle concentration (CMC) indicated by the inflection point in Fig. 2. The result of the surface tension measurement suggests that **5a** forms a micelle which is composed of both a flexible hydrophilic moiety and a relatively rigid hydrophobic moiety.

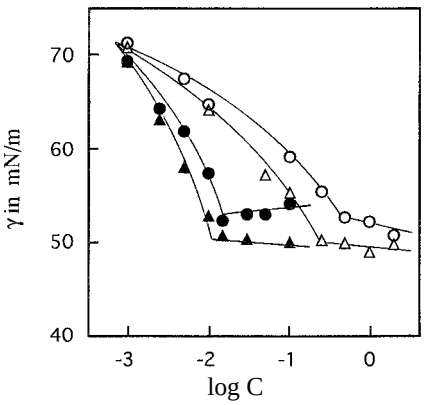


Fig. 2. Surface tension (γ) of aqueous solutions as a function of concentration (C, wt.-%). Temp.: 25 °C. ●: **5a**, ▲: **5b**, ○: **2a**-OH, △: **2b**-OH

Surface tension measurement also indicated that **5**–**7** adsorbed at the air/water interface. The surface excess density Γ (mol/area) at the interface can be calculated through a γ-C plot by using the Gibbs adsorption isotherm (Eq. 1),

$$\Gamma = -(RT)^{-1} \times (\partial\gamma/\partial \ln C) \quad (1)$$

where T is the absolute temperature and R is the gas constant. The Gibbs adsorption isotherm provides values about the area (A) that one molecule occupies at the interface, that is,

$$A = (N_A\Gamma)^{-1} \quad (2)$$

where N_A is Avogadro's number[15, 19a]. For these graft copolymers, the A value corresponds to the area per poly(2-oxazoline) side chain adsorbed at the surface. Tab. 2 summarizes the CMC, γ at CMC (γ_{CMC}), γ at 1.0 wt.-% ($\gamma_{1.0\ wt.-\%}$), and the A values of the graft copolymers **5**, chitin-*graft*-[poly(2-methyl-2-oxazoline)-*block*-poly(2-butyl-2-oxazoline)] (**6**), and chitin-*graft*-[poly(2-methyl-2-oxazoline)-*block*-poly(2-*tert*-butyl-2-oxazoline)] (**7**) as well as those of ω-hydroxyl-terminated block copolyoxazolines, ω-hydroxy-poly(2-phenyl-2-oxazoline)-*block*-poly(2-methyl-2-oxazoline) (**2a**-OH), ω-hydroxy-poly(2-butyl-2-oxazoline)-*block*-poly(2-methyl-2-oxazoline) (**3**-OH), and ω-hydroxy-poly(2-*tert*-butyl-2-oxazoline)-*block*-poly(2-methyl-2-oxazoline) (**4**-OH). The $\gamma_{1.0\ wt.-\%}$ values of **2a**-OH (52 mN/m) and **3**-OH (37 mN/m) were almost identical with those of poly(2-methyl-2-oxazoline)-*block*-poly(2-phenyl-2-oxazoline) (51 mN/m) and poly(2-methyl-2-oxazoline)-*block*-poly(2-butyl-2-oxazoline) (34 mN/m), respectively, which have been reported by Kobayashi et al.[11]

CMC of **5a** (0.02 wt.-%) was significantly smaller than that (0.50 wt.-%) of ω-hydroxyl-terminated block copolyoxazoline **2a**-OH, which is a model block copolymer of the side chain segment of **5a**, although the γ values of **5a** and **2a**-OH above CMC were almost the same. Similarly, CMC of **5b** (0.01 wt.-%) was lower than that (0.32 wt.-%) of the side chain segment **2b**-OH. Although some amphiphilic graft copolymers show better performance as a surfactant than low molecular weight amphiphiles[19], the behavior of such a polymeric surfactant in aqueous solution is very rare. On the other hand, partially deacetylated chitin **1** and chitin-*graft*-poly(2-methyl-2-oxazoline) showed virtually no surface active properties. The lower CMC of the present system is ascribed to that the chitin main chain of graft copolymer **5** presumably allows the poly(2-phenyl-2-oxazoline)-*block*-poly(2-methyl-2-oxazoline) segment attached to chitin to pack more efficiently at the air/water interface. All the mean areas (A values) of the terminal segments of **5a**–**c** were smaller than those of the corresponding **2a**–**c**. These results suggest that chitin backbone does not reach the air/water interface. Interestingly, the facts suggest the chitin main chain does not disturb the assembly of block copolymer side chains, but aids it.

Graft copolymers **5c**, **6**, and **7** have almost the same polyoxazoline content ([PROZO]/[-NH_2]) and chain

Tab. 2. Characterization of micelles of chitin-*graft*-poly(2-oxazoline)

Polymer	Graft polymer				Model compound of side chain				
	CMC[a] in wt.-%	γ_{CMC}[b] in mN/m	$\gamma_{1.0\ wt.-\%}$[c] in mN/m	A[d] in Å^2/molecule	Polymer	CMC[a] in wt.-%	γ_{CMC}[b] in mN/m	$\gamma_{1.0\ wt.-\%}$[c] in mN/m	A[d] in Å^2/molecule
5a	2.0×10^{-2}	52	52	33	**2a**-OH	5.0×10^{-1}	52	52	100
5b	1.0×10^{-2}	51	50	45	**2b**-OH	3.2×10^{-1}	50	49	87
5c	1.3×10^{-1}	54	52	84	**2c**-OH	5.0×10^{-1}	53	53	94
6	1.7×10^{-1}	38	36	49	**3**-OH	1.3×10^{-1}	40	37	33
7	3.5×10^{-1}	47	46	53	**4**-OH	2.0×10^{-1}	51	45	50

[a] Critical micelle concentration, by surface tension measurement at 25 °C.
[b] The surface tension at CMC (25 °C).
[c] The surface tension at 1.0 wt.-% (25 °C). The value is an experimental calculation.
[d] Molecular area per polyoxazoline side chain, calculated according to ref.[15,19a]. The concentration of the graft copolymers was used to calculate the A value.

length (*m* and *n* values). The reduction of the surface tension and the state of packing at air/water interface depend on the substituents of the hydrophobic oligo(2-oxazoline) blocks. Graft copolymer **6** showed the lowest γ and the smallest molecular area, which is due to the character of the pendant groups of the hydrophobic oligo(2-oxazoline) chains. The CMC value of **5c** was the lowest of the three, while the CMC value of **2c**-OH was higher than that of the corresponding side chain model compounds **3**-OH or **4**-OH. Furthermore, the CMCs of **6** and **7** were slightly higher than those of **3**-OH and **4**-OH, although the CMC of **5a** was lower than that of **2a**-OH (*vide supra*). Therefore, the hydrophobic interaction of the phenyl groups of graft copolymer **5** seems to influence the CMC value.

The role of the oligo(2-methyl-2-oxazoline) segment is discussed below on samples of **5a**, **5b**, and **5c**. CMC and *A* values of **5a** and **5b** were smaller than those of **5c**. This tendency is clear in comparison with the case of **2a**, **2b**, and **2c**. The facts imply that the relatively shorter oligo(2-methyl-2-oxazoline) segment acts as a spacer effectively.

Assembled structure of the graft copolymer **5**

Direct observation of the assembled structure of **5b** was carried out by cryo-TEM (in 0.5 wt.-% D_2O solution, at 25 °C). Globular structural units with a diameter of about 40 nm and cylindrical aggregates of globular units (diameter: 40 nm, length: 80–200 nm) were observed (Fig. 3). The size distribution of the assembly estimated by numbering the particles per 8.7 μm^2 in the photograph

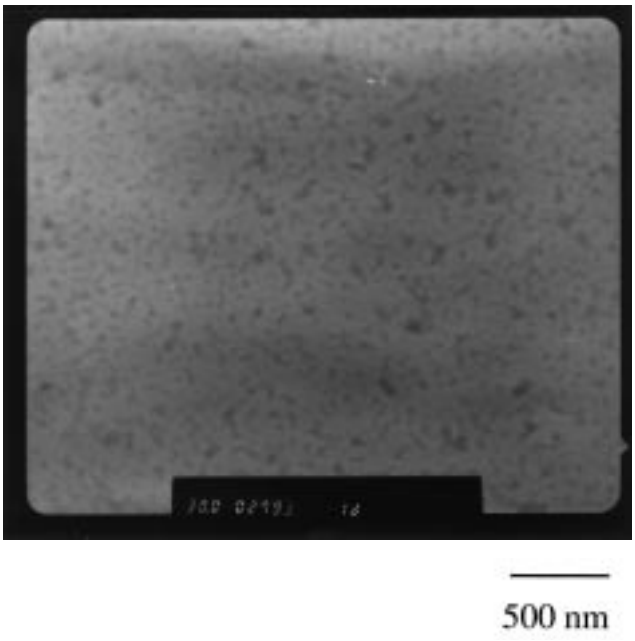


Fig. 3. Cryo-TEM photograph of chitin-*graft*-[poly(2-methyl-2-oxazoline)-*block*-poly(2-phenyl-2-oxazoline) (**5b**) in D_2O (0.5 wt.-%)

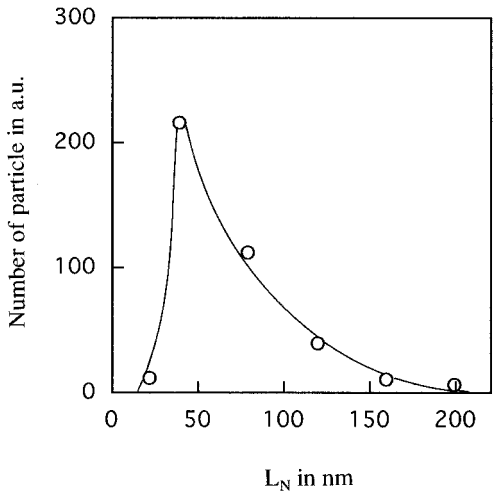


Fig. 4. The size distribution of molecular assembly of chitin-*graft*-[(poly(2-methyl-2-oxazoline)-*block*-poly(2-phenyl-2-oxazoline)] (**5b**) estimated by cryo-TEM analysis

is shown in Fig. 4. Globular micelles with monodispersed volumes should be formed at the concentration of the inflection points in the plots of surface tension values of its aqueous solution versus the logarithm of concentration as shown in Fig. 2. At a higher concentration than the CMCs, the polymers aggregate extensively, and then generate the cylindrical, elongated body. The average radius of gyration of the particle from a Guinier plot[20] in SANS measurement of **5b** (in 0.5 wt.-% D_2O solution, at 25 °C) was 36 nm. The average radius of gyration estimated by cryo-TEM is in agreement with that measured by SANS within the experimental error.

Complexation of the graft copolymers **5**–**7** *with hydrophobic substances*

Binding of magnesium 1-anilinonaphthalene-8-sulfonate (ANS) and pyrene with graft copolymers **5**–**7** was investigated by fluorescence intensity measurement. When ANS was mixed with graft copolymer **5b**, the emission maxima (λ_{max}) shifted to lower wavelength and the relative intensity at 485 nm (I/I_0, where I and I_0 are fluorescence intensities of ANS-containing aqueous solutions with and without **5b**, respectively) increased, as polymer concentration increased. In Fig. 5, λ_{max} and I/I_0 in the presence of **5b** are plotted as a function of concentration (C, wt.-%). Plots of I/I_0 values had a clear break point at 0.02 wt.-%, and this concentration was nearly consistent with the apparent CMC of **5b** (0.01 wt.-%) calculated from surface tension techniques. For pyrene, an increase of the intensity in fluorescence spectra (I/I_0 at 392 nm in the emission spectrum: 6.7) and a shift of the low-energy band in the excitation spectra[17] from 334 to 341 nm occurred when the **5b** content increased to 0.05 wt.-%. These results indicate that the self-aggregate of **5b** com-

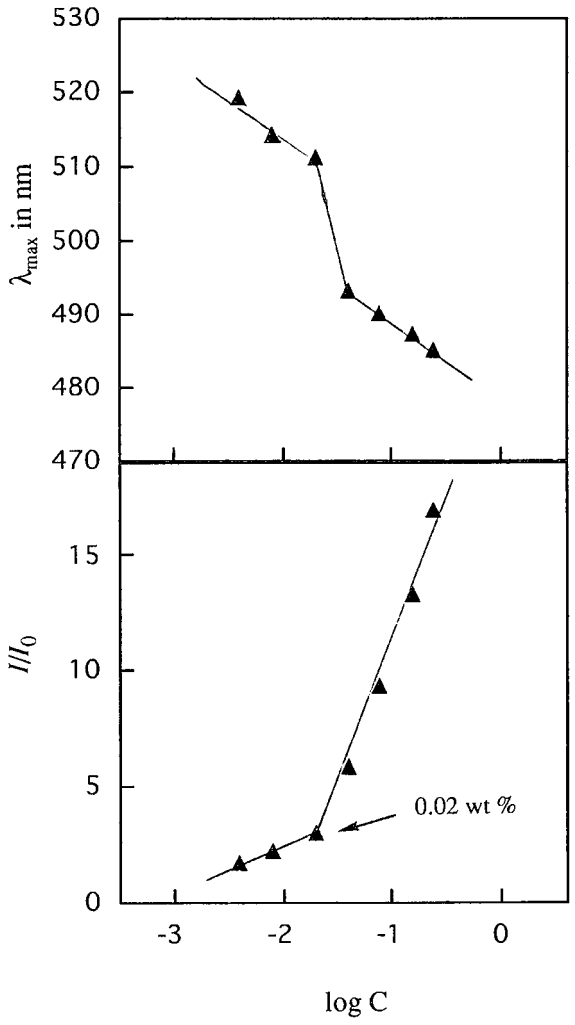


Fig. 5. Emission maximum (λ_{max}) and relative intensity at 485 nm (I/I_0) of ANS as a function of chitin-*graft*-[poly(2-methyl-2-oxazoline)-*block*-poly(2-phenyl-2-oxazoline)] (**5b**) concentration (C, wt.-%) at 25 °C. Concentration of ANS: 2.0×10^{-5} M

plexed with ANS and pyrene, while the data obtained with ANS as a probe give evidence for the formation of polymeric micelles.

Here, the influence of the substituents of the polyoxazoline segment on the guest-binding property is discussed based on the data by fluorescence analysis as well as surface tension measurement (Tab. 2 and 3). Graft copolymer **5c** having poly(2-phenyl-2-oxazoline) side chains as a hydrophobic segment complexed with both ANS and pyrene effectively. This is ascribed to the lowest CMC and stacking effect of the phenyl group of **5c**. Micropolarity around pyrene in the aggregate can be estimated from their relative intensity I_1/I_3 of the vibronic bands[21]. The I_1/I_3 value in 0.4 wt.-% aqueous solution of **5c** was 1.76, which indicated that pyrene located in a domain comparable to the polarity of *N*,*N*-dimethylacetamide ($I_1/I_3 = 1.79$[21b]). The I_1/I_3 values of **6** ($I_1/I_3 = 1.65$) and **7** ($I_1/I_3 = 1.63$) were comparable to the polarity of acetone ($I_1/I_3 = 1.64$[21b]) or ethylene glycol ($I_1/I_3 = 1.64$[21b]). The I_1/I_3 value is averaged over several possible sub-populations of pyrene, located in different parts of the polymer. The values indicated are approximated estimates related to the micropolarities of the possible environments in the copolymers. The results suggest that the hydrophobic microenvironment of the guest-binding site can be controlled by macromolecular design introducing amphiphilic block copolymer segments.

Tab. 3. Complexation of chitin derivatives with fluorescence probes in water[a]

Graft polymer	ANS		Pyrene	
	λ_{em}[b]	I/I_0[c]	I/I_0[d]	I_1/I_3[e]
Control	528	–	–	1.87[e]
5c	467	33	3.2	1.76
6	489	21	3.2	1.65
7	478	27	1.5	1.63

[a] Polymer concentration: 0.4 wt.-%; temperature: 25 °C.
[b] Emission maxima.
[c] Relative fluorescence intensity at 485 nm.
[d] Relative fluorescence intensity at 392 nm.
[e] See ref.[21]

Conclusion

Novel chitin derivatives with monodisperse amphiphilic poly(2-oxazoline) block copolymer side chains were synthesized as a first example of introduction of living block copolymer to a polysaccharide. The micelle formation of the graft copolymers in aqueous solution was investigated by surface tension, cryo-TEM, and fluorescence intensity measurements above the CMC. The surface-active behavior and the complexation with hydrophobic substances depend on the chemical structure, i.e., chain length and substituents of polyoxazolines. Graft copolymer **5** forms cylindrical aggregates (diameter: 40 nm, length: 80–200 nm) in aqueous solution. These results lead us to hope that the size, the shape, the arrangement, and the guest-binding ability of the polysaccharide-based nanoparticles can be controlled by supramolecular design.

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