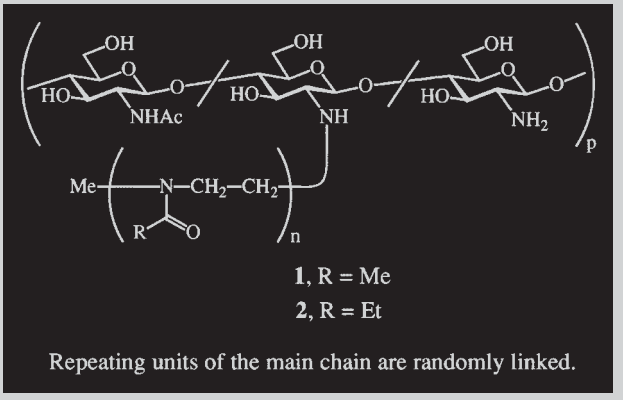
Full Paper: The molecular shapes and the sizes of structures formed by chitin derivatives with monodisperse poly(2-alkyl-2-oxazoline) side chains were investigated using atomic force microscopy (AFM), cryo-transmission electron microscopy (cryo-TEM), and small-angle neutron scattering (SANS) analyses. A ring structure with an outside diameter of 45–60 nm and a cross-sectional diameter of 10–18 nm was observed in the AFM image for chitin-*graft*-poly(2-methyl-2-oxazoline) **1d** (*DP* of the side chain, 8.5; [side chain]/[glucosamine unit], 0.53). From the cryo-TEM observation of the graft copolymer **1d** in 0.5 wt.-% D_2O solution, an average diameter of 40 nm for the particles was determined, with a narrow size distribution. SANS measurements of the 0.5 wt.-% D_2O solution of **1d** revealed that the outside diameter of the particles and the cross-sectional diameter were 57 nm and 8 nm, respectively. The absolute weight average molecular weight of **1d** was determined to be 5.4×10^5 by static light scattering. From these results it was concluded that **1d** can form a unimolecular ring structure in aqueous solution. However, graft copolymers with fewer side chains (**1c**; [side chain]/[glucosamine unit], 0.30) and with more side chains (**1e**; [side chain]/[glucosamine unit], 0.96) did not form rings but instead formed monodisperse unimolecular spherical particles of diameters of 28–36 nm by AFM. A graft copolymer **1f** with relatively long side chains (*DP* of side chain, 19.6; [side chain]/[glucosamine unit], 1.00) was also observed as a spherical particle by AFM (diameter: 30–40 nm by AFM; 40 nm by SANS). On the other hand, an intermolecular aggregate formation (diameter of the aggregate: 36–143 nm) was observed for graft copolymers **1a** and **1b** having short side chains (*DP* of side chains, 5.6; [side chain]/[glucosamine unit], 0.35 and 0.48, respectively), with a spherical molecular particle of diameter 36 nm by the AFM analysis. Chitin-*graft*-poly(2-ethyl-2-oxazoline) (**2**) (*DP* of side chains, 21.7; [side chain]/[glucosamine unit], 0.95) generated larger aggregates of diameter 100–400 nm by AFM. The complexation behavior of graft copolymer **1d** with magnesium 8-anilino-1-naphthalenesulfonate (ANS) and with *N*-phenyl-1-naphthylamine (PNA) was also examined by fluorescence measurement in an aqueous solution. It was found that graft copolymer **1d** complexed with both ANS and PNA, and the binding constants were calculated to be 7.5×10^4 M^{-1} and 5.3×10^4 M^{-1}, respectively.



Chemical structure of chitin-*graft*-poly(2-alkyl-2-oxazoline).

Nano-Scale Molecular Shapes of Water-Soluble Chitin Derivatives Having Monodisperse Poly(2-alkyl-2-oxazoline) Side Chains

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Keywords: atomic force microscopy (AFM); graft copolymer; living polymerization; polysaccharides; ring structure

Introduction

Polysaccharides and their derivatives are expected to be some of the most effective and specific medications in antibacterial and antitumor therapies.[1] Among the polysaccharides, chitin attracts much attention in the pharmacological, biomedical, and biotechnological fields.[2] Chemical modifications of the molecule have been

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developed in order to improve its solubility, a current drawback.[3–7] It has already been reported that introducing poly(2-alkyl-2-oxazoline) side chains into chitin provides not only excellent solubility,[8] but also improved miscibility towards commodity polymers.[9,10] An interesting feature of polyoxazoline chains is the fact that they are regarded as peptoid-type pseudopeptides having good flexibility.[11] It has also been disclosed that chitin-*graft*-poly(2-methyl-2-oxazoline) (**1**) has the capability of incorporating lipase P and catalase and increasing hydrolysis activity compared with the free enzymes.[12] Although the higher order structure of the graft copolymer in the aqueous solution is thought to influence its properties, this has not been demonstrated clearly.

This article is concerned with the investigation of the molecular shape in aqueous solutions of water-soluble chitin derivatives having monodisperse poly(2-alkyl-2-oxazoline) side chains, and the structure-molecular shape relationship. Akiyoshi et al. investigated self-assembly of hydrophobized pullulan[13] and the macromolecular complexation with bovine serum albumin.[14] We reported on the influence of the structure and composition of a chitin derivative on its hydrophobic association behavior, the derivative having amphiphilic polyoxazoline block copolymers as a side chain, i.e., chitin-*graft*-[poly(2-methyl-2-oxazoline)-*block*-poly(2-phenyl-2-oxazoline)].[15] Small-angle neutron scattering (SANS) and small-angle X-ray scattering are powerful tools for investigating the conformation of particles consisting of polysaccharides in their aqueous solution.[16,17] In the present study, we investigated an aqueous solution of chitin-*graft*-oligo(2-alkyl-2-oxazoline) by SANS, cryo-transmission electron microscopy (cryo-TEM), and atomic force microscopy (AFM). It was found that nano-scale structures of the chitin derivatives were highly dependent on the chemical structure.

Experimental Part

Materials

Chitin-*graft*-poly(2-methyl-2-oxazoline) (**1**) and chitin-*graft*-poly(2-ethyl-2-oxazoline) (**2**) were prepared according to our previous studies.[8,11] D_2O (Aldrich Chemical Co.) and CD_3COOD (Janssen Chimica) were used without purification for NMR analysis. Magnesium 8-anilino-1-naphthalenesulfonate (ANS) and *N*-phenyl-1-naphthylamine (PNA) (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) were commercially available. Water used was purified by distillation.

Measurements

^{1}H NMR measurements were performed on a JEOL EX-270 spectrometer at 270 MHz. The surface tension of aqueous solutions was measured at 25 °C by the drop weight method.[18]

Cryo-TEM observation was carried out on a Hitachi H-800 electron microscope equipped with a cold stage, using the technique reported by Bellare et al.[19] Specimens were prepared by depositing a small droplet of the D_2O solution on a TEM grid, coated by a perforated carbon film. A thin film of the solution suspended over the holes of the grid was formed by blotting the grid to remove excess fluid. The specimen was vitrified by plunging it into liquid nitrogen and was then imaged at an accelerating voltage of 100 kV.

AFM measurements were carried out on a Nanoscope III (Digital Instruments) in air. The D_2O solution of the graft copolymer was deposited on a mica plate and the D_2O was evaporated under reduced pressure. Tapping mode analysis was carried out on the plate.

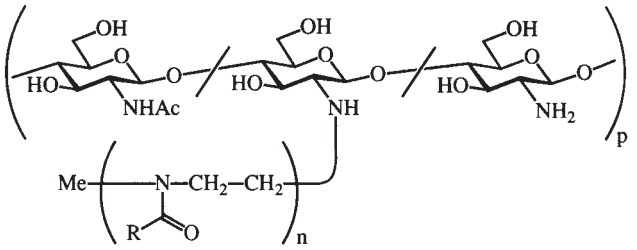
SANS measurements were performed using the SANS-U spectrometer at JRR-3M at the Japan Atomic Energy Research Institute, and the WINK instrument at the High Energy Accelerator Research Organization (KEK). For the SANS-U, a 7 incident neutron beam, taken from the cold neutron source and monochromated by a mechanical velocity selector, was used. For WINK, the instrument was operated at a neutron radiation of 1–16 wavelength at 25 °C. The SANS intensities were obtained as a function of Q $[(4\pi/\lambda)\sin(\theta/2)]$, where λ is the neutron radiation wavelength and θ is the scattering angle. D_2O was employed as a solvent for the SANS measurements.

Static light scattering and specific refractive index increment were measured at 25 °C on a DLS-700 and a RM-102 differential refractometer (Otsuka Electronics), respectively. The $\bar{M}_n$ values of samples were determined by size exclusion chromatography (SEC) (temp., 25 °C; column, Shodex B804 +805; eluent, water; flow rate, 1.0 mL/min). The SEC system (Jasco Model PU-980 high performance liquid-chromatograph apparatus) was calibrated with pullulan standards (Shodex Co. Ltd.).

Complexation between the chitin derivatives and a hydrophobic guest molecule such as ANS and PNA was fluorometrically investigated at 25 °C with a Hitachi F-4500 fluorescence spectrophotometer. The concentrations of ANS and PNA were both 2.0×10^{-5} M in water.[13,20] For ANS, 0.5 mL of the stock aqueous solution of ANS (1.0×10^{-4} M) was mixed with 2.0 mL of the polymer aqueous solution. For PNA, 0.5 mL of acetone solution (1.0×10^{-4} M) of PNA was added to a vial and then the solvent was evaporated to form a thin film at the bottom of the vial. An aqueous solution (2.5 mL) of polymer was added to the vial, and the resulting mixture was kept at 25 °C for 1 h with mixing by a magnetic stirrer. Fluorescence spectra were taken by excitation at 380 nm for ANS and at 340 nm for PNA.

Results and Discussion

Chitin-*graft*-poly(2-methyl-2-oxazoline) (**1**) and chitin-*graft*-poly(2-ethyl-2-oxazoline) (**2**) were synthesized by the reaction of ca. 50% deacetylated chitin with living poly(2-methyl-2-oxazoline) and poly(2-ethyl-2-oxazoline), respectively.[8,11] The chemical structures and characteristics of the graft copolymers **1** and **2** used in this study are shown in Figure 1 and Table 1. The main chain of **1** and **2** was prepared by the alkaline treatment of chitin according to the literature,[21] and consists of randomly linked



1, R = Me
2, R = Et

Repeating units of the main chain are randomly linked.

Figure 1. Chemical structure of chitin-*graft*-poly(2-alkyl-2-oxazoline).

D-glucosamine and *N*-acetyl-D-glucosamine repeating units. Poly(2-alkyl-2-oxazoline) branches, which show a strong affinity toward water,[11,22,23] are attached at the 2-position of the D-glucosamine units of the main chain. The side chain length was regulated by living polymerization of 2-methyl-2-oxazoline or 2-ethyl-2-oxazoline in acetonitrile.[8,11] The molecular weight distributions of the polyoxazolines were estimated by SEC before the graft copolymer formation with the partially deacetylated chitin, and were reasonably narrow. The [side chain]/[–NH_2] values in Table 1 represent molar ratios of oxazoline polymer to D-glucosamine units in the graft copolymers, determined by ^{1}H NMR signal intensity.[8]

The molecular motion of graft copolymer **1** was examined by ^{1}H NMR measurement in D_2O/CD_3COOD (95:5, v/v). In Figure 2, for graft copolymer **1d** the peak intensity ratios of the H-2 proton signals of the D-glucosamine units ($\delta = 3.17$ ppm) to the methyl proton signals of sodium 2,2-dimethyl-2-silapentane-5-sulfonate as the internal reference ($\delta = 0.00$ ppm) and the peak intensity ratios of the terminal *N*-methyl proton signals of the poly-(2-methyl-2-oxazoline) segment ($\delta = 2.85 - 3.10$ ppm) to the same internal reference signal are plotted as a function of temperature. These ratios are represented as ●: $I(\delta = 3.17\ \text{ppm})/I(\delta = 0.00\ \text{ppm})$ and ○: $I(\delta = 2.85 - 3.10\ \text{ppm})/I(\delta = 0.00\ \text{ppm})$, respectively. Although the $I(\delta = 2.85$–$3.10\ \text{ppm})/I(\delta = 0.00\ \text{ppm})$ values were almost constant within the experimental error, the $I(\delta = 3.17\ \text{ppm})/I(\delta = 0.00\ \text{ppm})$ values changed with temperature. The H-2 proton signals of the D-glucosamine units were difficult to detect at 27 °C, so the $I(\delta = 3.17\ \text{ppm})/I(\delta = 0.00\ \text{ppm})$ value at this temperature was nearly zero. However, the signal, and thus the intensity ratio, increased at around 50 °C and was constant at temperatures over 60 °C. The peak intensity change dependent on temperature was reversible. These results indicated that the motion of the main chain was restricted at temperatures below 50 °C, while the motion of the side chain segment was relatively high and independent of temperature.

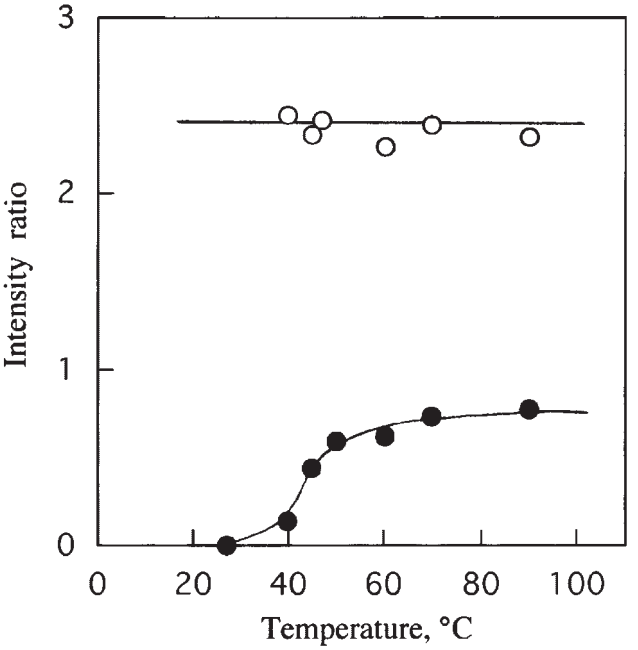


Figure 2. Changes of ^{1}H NMR intensity ratios of the signals at $\delta = 3.17$ ppm (H-2 of D-glucosamine units) and at $\delta = 2.85 - 3.10$ ppm (*N*-methyl protons) of chitin-*graft*-poly(2-methyl-2-oxazoline) (**1d**) to the signal at $\delta = 0.00$ ppm (CH_3Si–) as a function of temperature. Solv.: D_2O/CD_3COOD (95:5, v/v). Conc.: 1 wt.-%. ○: $I(\delta\ 2.85 - 3.10\ \text{ppm})/I(\delta\ 0.00\ \text{ppm})$; ●: $I(\delta\ 3.17\ \text{ppm})/I(\delta\ 0.00\ \text{ppm})$.

Table 1. Chemical structure of chitin-*graft*-poly(2-alkyl-2-oxazoline)s.

Sample	*DA*[a] of chitin	Poly(oxazoline) side chain			[side chain]/[–NH_2][d]	$\bar{M}_n$[e]
	%	R	*DP*[b]	$\bar{M}_w/\bar{M}_n$[c]		10^5
1a	49	Me	5.6	1.2_3	0.35	5.7
1b	49	Me	5.6	1.2_3	0.48	6.2
1c	48	Me	8.6	1.1_1	0.30	2.8
1d	45	Me	8.5	1.1_5	0.53	5.5
1e	48	Me	8.6	1.1_1	0.96	4.1
1f	52	Me	19.6	1.1_2	1.00	3.9
2	45	Et	21.7	1.1_2	0.95	5.9

[a] Degree of *N*-acetylation of partially deacetylated chitin, determined by ^{1}H NMR in D_2O/CD_3COOD (95:5, v/v).
[b] Degree of polymerization of the side chain, determined by ^{1}H NMR in $CDCl_3$ at 27 °C.
[c] Estimated by SEC in $CHCl_3$ at 38 °C (standard: polystyrene).
[d] Molar ratio of polyoxazoline chain to D-glucosamine unit of deacetylated chitin, determined by ^{1}H NMR in D_2O/CD_3COOD (95:5, v/v) at 90 °C.
[e] $\bar{M}_n$ of graft copolymers, estimated by SEC in H_2O at 27 °C (standard: pullulan).

The surface tension of an aqueous solution of graft copolymer **1d** was measured by the drop weight method at 25 °C.[18] In the range of **1d** concentration from 0.001 wt.-%

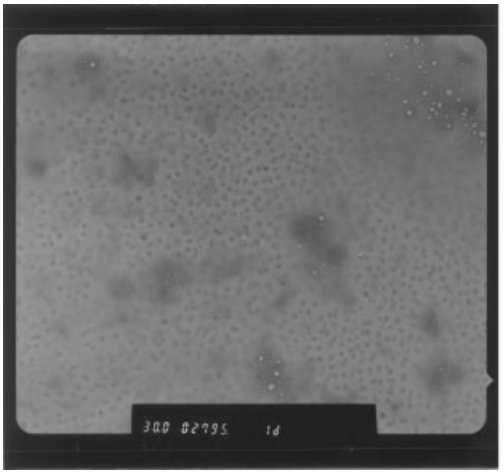


Figure 3. Cryo-TEM photograph of chitin-*graft*-poly(2-methyl-2-oxazoline) (**1d**) in D_2O (0.5 wt.-%).

to 2.0 wt.-%, a significant decrease of the surface tension was not detected. The surface tension at 1.0 wt.-% was 68.1 dyne/cm, which is comparable to water surface tension at 25 °C (72.0 dyne/cm). This result suggests that the graft copolymer has good solubility toward water and that it does not form micelles within the range of the experimental conditions.

The higher order structure of **1d** was observed by cryo-TEM in 0.5 wt.-% D_2O solution. The globular particles were observed as shown in Figure 3. Interestingly, the particles were monodisperse and had an average diameter of about 40 nm.

The particles derived from **1d** were also investigated by AFM. Unexpectedly, a ring structure was observed in the AFM image with good reproducibility, as shown in Figure 4. The outside diameter of the ring particle was 45–60 nm, which was in agreement with the aforementioned cryo-TEM observation. The cross-sectional radius of the particle was estimated to be 5–9 nm by AFM. These results suggest that the molecular shape of graft copolymer **1d** in the aqueous solution was ringed but not spherical. Ring type particles of higher order structure with a diameter of nanometers are rare, although self-assembly of submicrometer ring-like particles has already been reported.[24,25]

Structural analysis of the chitin-based graft copolymer was carried out by SANS. Only a few investigations of the molecular shape or molecular assembly of polysaccharide derivatives have been reported until now.[13–17] SANS analyses were performed on a 0.5 wt.-% D_2O solution of graft copolymer **1d** at 25 °C. The Guinier plot[26,27] obtained is shown in Figure 5. The resulting data fit reasonably well with the Guinier equation of a spherical particle model, and there were two linear slopes in the curve. From these slopes, the radii of gyration R_G were obtained by Equation (1).

$$I(Q) = I(0)\exp(-R_G^2Q^2/3) \quad (1)$$

The R_G calculated from the slope at the lower Q range was 20 nm, and the R_G calculated from the slope at the higher Q range was 3 nm. Therefore, the outside diameter of the ringed particle was evaluated to be 57 nm, and the cross-sectional radius of the particle was 4 nm using $R_G^2 = 3R^2/5$, for a spherical particle. These results were comparable to those obtained from cryo-TEM and AFM analyses. A schematic representation of the ring structure of graft

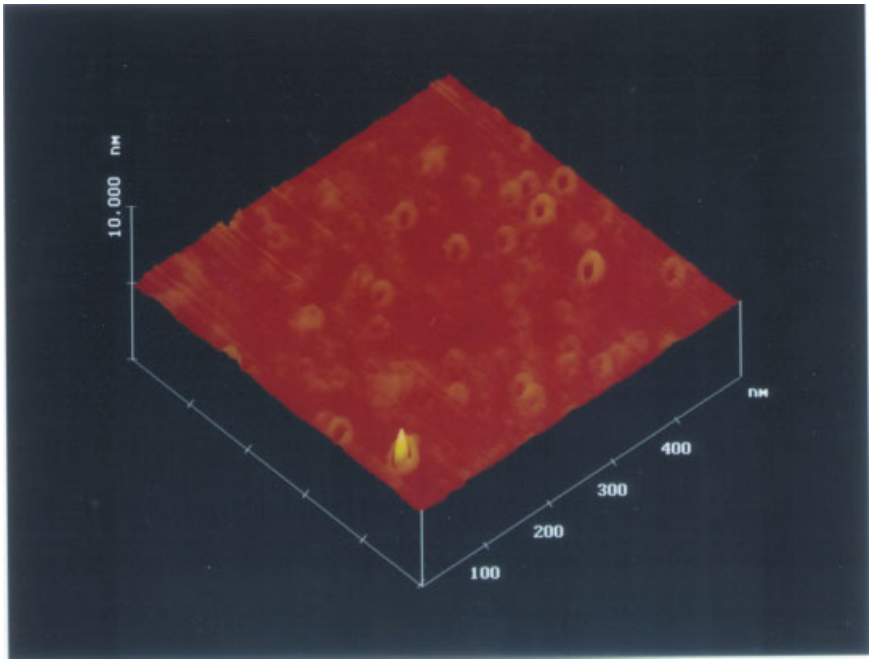


Figure 4. AFM image of the particle of chitin-*graft*-poly(2-methyl-2-oxazoline) (**1d**).

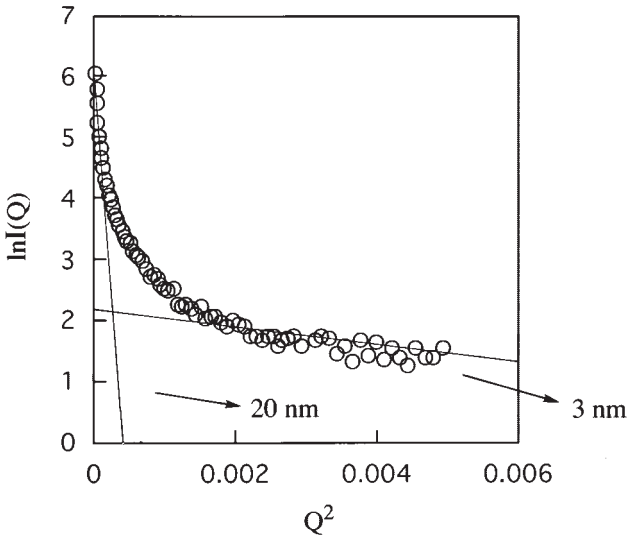


Figure 5. Guinier plot of SANS for chitin-*graft*-poly(2-methyl-2-oxazoline) (**1d**) in D_2O (0.5 wt.-%, 25 °C).

copolymer **1d** is shown in Figure 6. SANS measurements were also carried out for **1d** D_2O solutions of 0.25 wt.-% and 1.0 wt.-%. The radius values calculated from the slopes of the resulting Guinier plots were independent of polymer concentration (0.25–1.0 wt.-%), which suggested that graft copolymer **1d** has the same ring structure in this concentration range.

The absolute weight average molecular weight ($\bar{M}_w$) of the particle was determined by static light scattering. The $\bar{M}_w$ value determined for **1d** was 5.4×10^5. This value was close to the value obtained from SEC measurements using pullulan standards ($\bar{M}_w = 6.3 \times 10^5$). From the result obtained by static light scattering, graft copolymer **1d** appears to form a monodisperse ring structure unimolecularly in the aqueous solution, as represented in Figure 6.

The relationship between chemical structure and molecular shape for chitin-*graft*-poly(2-alkyl-2-oxazoline)s was then investigated. First, in relation to the number of side chains, AFM observation suggested that both **1c** ([PROZO]/[–NH_2], 0.30), with fewer side chains than **1d**, and **1e** ([PROZO]/[–NH_2], 0.96), with more side chains than **1d**, formed not ring but monodisperse spherical molecular shape structures unimolecularly (diameter: 28–36 nm). Next, in order to evaluate the effect of the side chain length, the molecular shapes of graft copolymers having shorter-length side chains, **1a** ($n = 5.6$; [PROZO]/[–NH_2], 0.35) and **1b** ($n = 5.6$; [PROZO]/[–NH_2], 0.48), were observed by AFM. The AFM analyses indicated that **1a** formed a spherical molecular shape (diameter: 36 nm), while graft copolymer **1b** aggregated to form a larger particle (diameter: 36–143 nm). The size distribution of these assemblies was estimated from the number of the particles per 4.00 mm^2 observed in the AFM image, as shown in Figure 7. These results indicate that the chemical structure of chitin derivatives, i.e., the number of side chains and the length of the side chain, has a large influence on the molec-

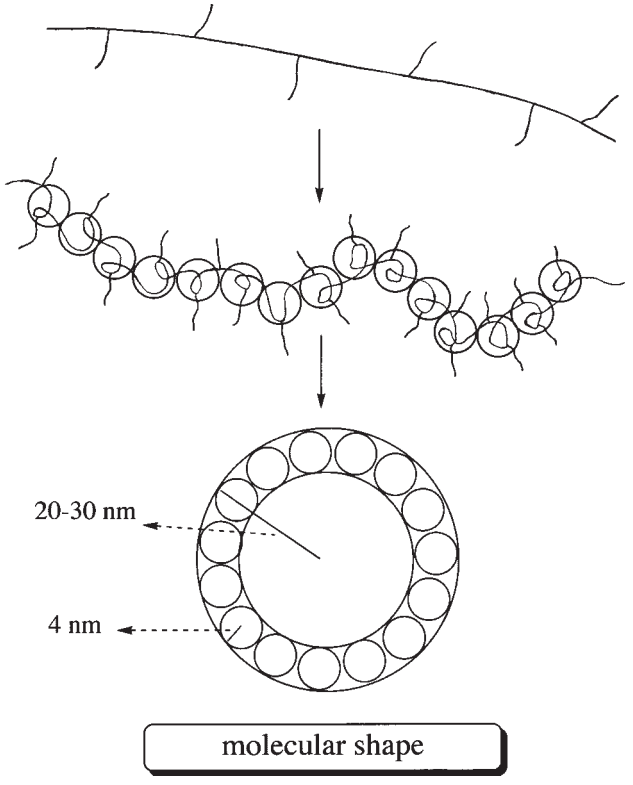


Figure 6. Schematic representation of a particle of chitin-*graft*-poly(2-methyl-2-oxazoline) (**1d**) in D_2O (0.5 wt.-%).

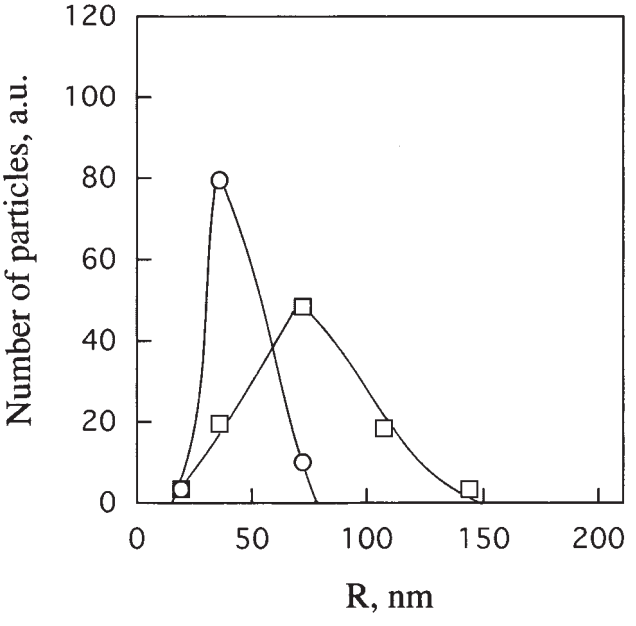


Figure 7. The size distribution of particles of chitin-*graft*-poly(2-methyl-2-oxazoline) (**1a**) (□) and chitin-*graft*-poly(2-methyl-2-oxazoline) (**1b**) (○) estimated by AFM analysis.

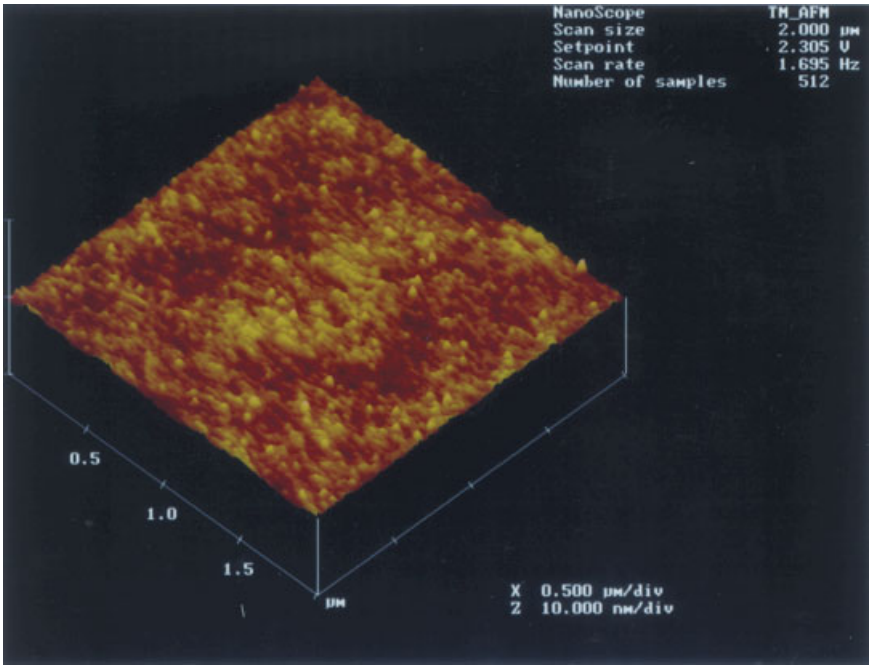


Figure 8. AFM image of a particle of chitin-*graft*-poly(2-methyl-2-oxazoline) (**1f**).

ular shape and the aggregation of the molecule in aqueous solution.

Finally, the effect of substitution of the hydrophilic polyoxazoline side chain was investigated. Graft copolymers **1f** and **2** have almost the same ratios of polyoxazoline segments ([PROZO]/[–NH_2], 1.00 and 0.95, respectively) and chain lengths ($n = 19.6$ and 21.7, respectively). As shown in Figure 8, monodisperse spherical particles of **1f** were observed in the AFM image (diameter: 30–40 nm). SANS measurements for a 1.0 wt.-% D_2O solution of **1f** were also taken. The particle radius calculated using the Guinier equation of a spherical model was 20 nm, which agreed with that from the AFM observation. On the other hand, relatively larger aggregates (diameter: 100–400 nm) were observed in an AFM analysis of graft copolymer **2** (Figure 9). Considering that the hydrophilicity of poly-

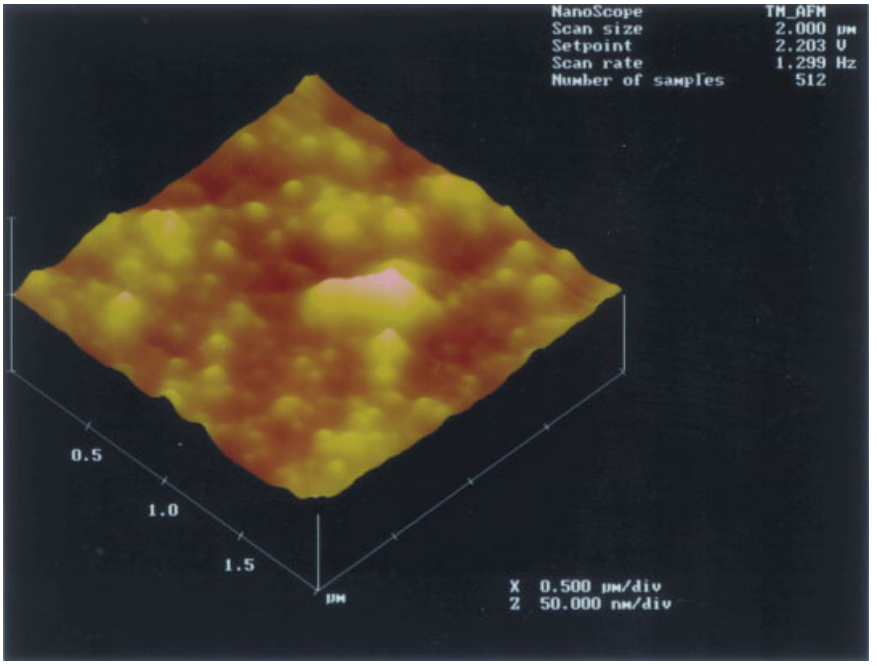


Figure 9. AFM image of a particle of chitin-*graft*-poly(2-ethyl-2-oxazoline) (**2**).

(2-methyl-2-oxazoline) is higher than that of poly(2-ethyl-2-oxazoline),[11,22] it seems that hydrophilicity of the side chains also affects the higher order structure of chitin-based graft copolymers.

Polysaccharides as well as cyclodextrins show unique binding properties, even to hydrophobic substances, because of their amphiphilic properties in water.[28] For this study, complexation of the graft copolymer **1d** with hydrophobic substances was investigated by fluorescence measurement. Binding of ANS and PNA with graft copolymer **1d** was evaluated. The emission maximum (λ_{max}) of ANS in water was 528 nm. When ANS was mixed with an aqueous solution of graft copolymer **1d**, the λ_{max} value shifted toward lower wavelength with an increase of fluorescence intensity. For example, at 0.16 wt.-% of polymer concentration, the λ_{max} value was 489 nm and the relative fluorescence intensity at 487 nm was 8.0. Similarly, the emission maximum of PNA in **1d** aqueous solution shifted to lower wavelength and the relative intensity at 415 nm (I/I_0) increased as a function of polymer concentration (Figure 10). These results suggest that the ringed particle of graft copolymer **1d** formed complexes with both ANS and PNA. The binding constants between the fluorescent probes and the **1d** particles were calculated by the Benesi-Hildebrand relationship.[29] The calculated binding constant of ANS was 7.5×10^4 M^{-1} and that of PNA was 5.3×10^4 M^{-1}. We can infer from these values that the driving force for the complexation of the chitin derivative with ANS derives from both hydrophobic interactions and electrostatic interactions between the ammonium ions of D-glucosamine units and the ANS sulfonate ion, while the driving force for PNA complexation is only from hydrophobic interactions. As shown in Figure 10, there are remarkable inflection points in the λ_{max} and I/I_0 versus log C plots at 0.04 wt.-% and 0.07 wt.-%, respectively. It was speculated that ''ring-to-random coil transitions'' occurred at these concentration ranges (0.04–0.07 wt.-%).

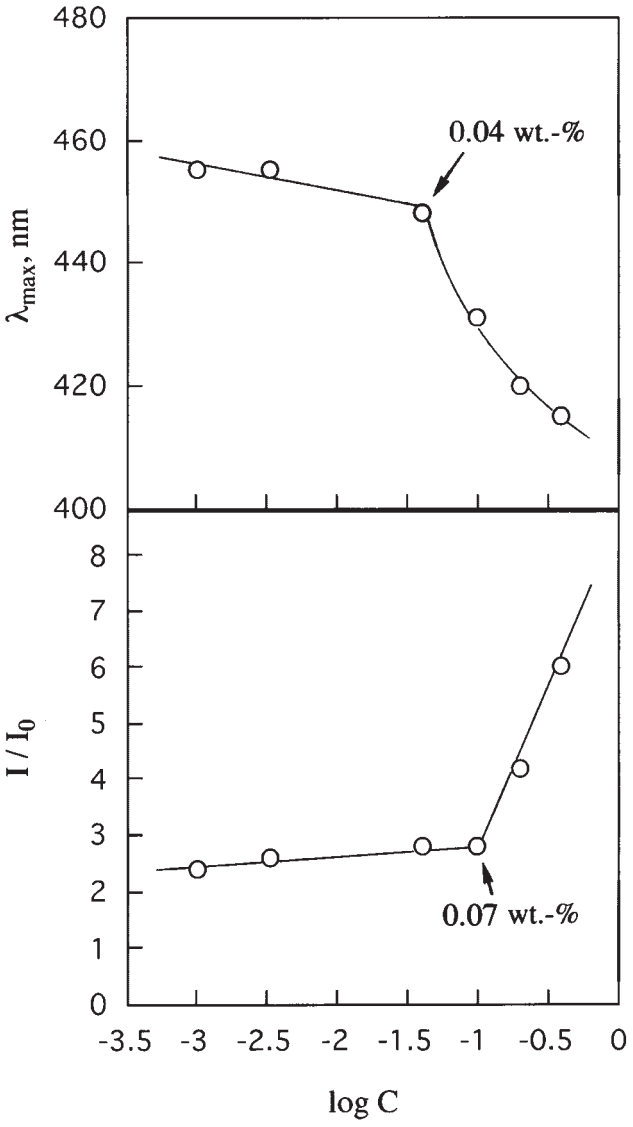


Figure 10. Emission maximum (λ_{max}) and relative intensity at 415 nm (I/I_0) of PNA as a function of concentration of chitin-*graft*-poly(2-methyl-2-oxazoline) (**1d**) at 25 °C. [PNA]: 2.0×10^{-5} M.

In addition, the higher order structure of the chitin derivative had influence on the complexation with PNA. Table 2 summarizes the binding behavior of PNA with various graft copolymers **1** and **2**. Graft copolymers **1a**–**d**, **1f**, and **2** were all complexed with PNA, as evidenced by the decrease in values of λ_{max}. The binding constants of **1f** and **2** for PNA were 1.7×10^4 M^{-1} and 4.2×10^4 M^{-1}, respectively. Interestingly, the ring-shaped graft copolymer **1d** showed the strongest binding with PNA.

Table 2. Complexation of chitin-*graft*-poly(2-alkyl-2-oxazoline)s with PNA in water. Measured at 25 °C.

Polymer	λ_{em} [a]	I/I_0 [b]
Control	468	1.0
1a	431	1.0
1b	431	1.0
1c	434	1.3
1d	413	6.0
1f	424	2.7
2	438	3.5
Chitin[c]	416	2.4
Poly(2-methyl-2-oxazoline)[d]	455	1.8
Poly(2-ethyl-2-oxazoline)[e]	434	1.1

[a] Emission maxima excited at 340 nm (polymer concentration: 0.4 wt.-%).
[b] Relative fluorescence intensity at 415 nm (polymer concentration: 0.4 wt.-%).
[c] Degree of *N*-acetylation: 48%, determined by ^{1}H NMR in D_2O/CD_3COOD (95:5, v/v).
[d] $\overline{M}_n$: 10 200, estimated by SEC in $CHCl_3$ at 38 °C (standard: polystyrene).
[e] $\overline{M}_n$: 10 300, estimated by SEC in $CHCl_3$ at 38 °C (standard: polystyrene).

Conclusions

The molecular shapes of water-soluble chitin derivatives having monodisperse poly(2-alkyl-2-oxazoline) side chains **1** and **2** in aqueous solutions were investigated. Graft copolymer **1d**, having relatively shorter poly(2-methyl-2-oxazoline) side chains ($n = 8.5$), formed a ring structure (diameter: 40–60 nm) unimolecularly, consisting of both a mobile hydrophilic poly(2-methyl-2-oxazoline) side chain shell and a core of relatively restricted chitin main chain in solution. The molecular shape of graft copolymer **1f**, having relatively longer poly(2-methyl-2-oxazoline) side chains ($n = 19.6$), was monodisperse spherical (diameter: 30–40 nm). On the other hand, graft copolymer **2**, with even longer poly(2-ethyl-2-oxazoline) side chains ($n = 21.7$), aggregates intermolecularly and provides larger particles (diameter: 100–400 nm). The binding property of these chitin derivatives with hydrophobic probes as well as their higher order structures were affected by the chemical structure. These results should provide useful information for strategies to regulate by molecular design the molecular shape and the guest-binding property of polysaccharide-based graft copolymers.

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