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Effect of Graft Chain Length and Structure Design on Temperature-Sensitive Hairy Particles

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ABSTRACT: Poly(*N*-isopropylacrylamide) (PNIPAM) chain-grafted particle was prepared to obtain a thermosensitive microsphere. The graft polymerization of NIPAM was carried out by the controlled radical polymerization technique using an iniferter. A photosensitive iniferter was used to conduct the graft polymerization under an ambient condition (at room temperature in water). The particle diameter increased linearly with the conversion. The controllability of this reaction was examined by adding a free initiator species to the medium, and it was found that the polydispersity indices of PNIPAM generated by iniferter-control polymerization were 1.3−2.1 where number-average molecular weights were 64 000−319 000. The graft chain length affected the thermosensitivity, and the longer graft chain brought an abrupt change in diameter around 33 °C. When a small amount of acrylic acid was incorporated into the graft polymer, the particle worked as an adsorbent of protein, and its adsorbability could be controlled by the temperature. The structure design by means of controlling monomer unit distribution in the graft polymer resulted in an improvement of the protein collection.

Introduction

The representative thermosensitive polymer, poly(*N*-isopropylacrylamide) (PNIPAM), has been extensively investigated as an intelligent material in the last 2 decades. Since Tanaka et al. exploited the volume phase transition of PNIPAM gel in water at 33 °C, close to body temperature,[1] there have been considerable efforts to develop temperature-sensitive materials using PNIPAM.[2−8] Some examples of these materials are intelligent sensors,[9,10] which detect the change in surrounding environment by the color change of the materials or the turbidity changes of the solution, membranes[11] or capsules,[12] which change the permeability by the temperature change, reactors[13,14] which control the chemical reaction by the diffusion control inside of PNIPAM gels, soft actuators,[15] and drug carriers,[16,17] which are suitable for controlled releasing, to name a few.[18−20] On the other hand, when PNIPAM-incorporated particles are prepared,[21−23] their characteristics, such as mobility in the electrical field,[24,25] colloidal stability,[26] and hydrophilicity of the surface,[27] also abruptly changed at around 32−33 °C, at which the volume phase transition occurs. These features enable thermosensitive colloids to be used in biomedical applications.[28−31] For example, Okano et al. developed thermosensitive drug carrying micelles which could control the release of the drug loaded inside of the micelle by the temperature change.[16] Kawaguchi et al. showed that a particle having a PNIPAM gel layer on the surface can adsorb proteins by electrostatic and hydrophobic interactions when it shrinks but desorbs them when the surface layer returns to the swollen state.[32] By using the weak interactions between the proteins and a PNIPAM-carrying surface, protein denaturation during the collecting process was less than that of polystyrene surface. Okano et al. also developed an affinity chromatographic system using PNIPAM-immobilized matrixes.[33] The PNIPAM-carrying beads could control the binding capacity between the ligand and the protein by temperature. As these studies have shown, the changes in the PNIPAM layer caused by the temperature change are useful, especially, in the biomedical field.

Characteristics of these environmentally sensitive materials are attractive since they switch their property by a slight change of surrounding physical or chemical environment. However, inhomogeneity of cross-linkage causes a dull response of PNIPAM materials to the temperature change. Thus, one of the major challenges in the synthesis of such materials is to improve the sharpness of volume changes depending on the temperature.[21] Our group proposed hairy particles composed of polystyrene core particles with PNIPAM grafted hair as smart particles that show discontinuous changes in diameter depending on the temperature.[22−23]

In this study, we developed thermosensitive hairy particles by designing PNIPAM hairs using a controlled radical graft polymerization technique. PNIPAM hair was synthesized in an aqueous system at room temperature under its lower critical solution temperature (LCST) so that PNIPAM would grow in the swollen state. The hairy chains were designed in order to investigate the relationship between hairy structure and thermosensitivity and to improve their ability as a protein adsorbent.

Several types of controlled radical polymerizations have been proposed. There are dithiocarbamate-mediated radical polymerization (iniferter),[34] nitroxide mediated radical polymerization (NMP),[35−37] atom transfer radical polymerization (ATRP),[38−43] and reversible additional fragmentation transfer polymerization (RAFT).[44−47] The surface-initiated controlled radical polymerization was also developed using these techniques. The iniferter is the first species proposed to control radical polymerization by Otsu in 1982.[34] Among the iniferters studied since 1982, photosensitive iniferters[48] seems to be a predominant candidate for achieving controlled polymerization under ambient conditions. After the proposal of the iniferter concept, some reports were published which evaluated the iniferter polymerization as a partially living process.[49−53] These studies pointed out that there are side reactions to lead iniferter to decomposition during the polymerization, and part of them lose the controllability of the reaction. Thus, polymers produced by iniferter-mediated radical polymerization have relatively broad distributions of molecular weights.[51−53] However, the possibility of the environmentally friendly polymerization with iniferter that enables the polymerization under ambient condition is attractive for the

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synthesis of thermosensitive polymers, such as PNIPAM in aqueous media. The other advantages of using an iniferter are that (i) an external initiator or catalysis is not required and (ii) the iniferter end group of the final polymer products can be converted into a thiol group (−SH group)[54,55] to functionalize the polymer chain end for further applications.

Herein we present facile synthesis of thermosensitive hairy particles in an aqueous solution at room temperature. The graft polymerization of water-soluble NIPAM monomer or graft copolymerization of NIPAM and acrylic acid (AAc) were carried out from polystyrene particle surface using iniferter-mediated controlled radical polymerization technique. Iniferter-carrying particle was prepared from styrene and commercially available dithioester. The effect of the chain length of the grafted PNIPAM on thermosensitivity of the particle was studied by measuring the particle diameters with dynamic light scattering (DLS). In addition, the hairy particles having different distributions of NIPAM/AAc monomer units in the surface layer were designed. The protein adsorbability of these hairy particles depending on temperature was also investigated.

Experimental Section

Materials. Styrene (Wako Pure Chemicals Co. 99%) and acrylic acid (AAc, Wako Pure Chemicals Co. 98%) were purified by distillation under reduced pressure. Vinyl benzyl chloride (VBC, ACROS CHEMICA N. V. 90%) was washed with 0.5% NaOH aqueous solution. *N*-Isopropylacrylamide (NIPAM, 99%) was kindly given by Kojin Co. and recrystallized from hexane-toluene mixture (1/1 in volume basis, 40 °C). Pottasium persurfate (KPS, Wako Pure Chemicals Co. 95%) was purified by recrystallization from water. Sodium chloroacetate (Wako Pure Chemicals Co. 95%), sodium N, N-diethyldithiocarbamate (NaDC, Wako Pure Chemicals Co. 92%) were used without further purification. Proteins used in this study were lysozyme (LZ, Sigma Chemical Co.), avidin (AV, Wako Pure Chemicals Co), and bovine serum albumin (BSA, Sigma Chemical Co.). Phosphate buffer was prepared from $NaHPO_4 \cdot 12H_2O$ (Wako Pure Chemicals Co. 99%) and NaH_2PO_4 (Wako Pure Chemicals Co. 99%) and adjusted at 10 mM, pH 7.0.

All chemicals were used as received otherwise noted. Water was purified with a Millipore Milli-Q system.

Synthesis of a Water-Soluble Iniferter. A water-soluble iniferter, *N*,*N*-diethyldithiocarbamide acetic acid (DCAA), was synthesized as follows. Sodium chloroacetate (5.17 g, 44.5 mmol) and sodium *N*,*N*-diethyldithiocarbamate (10.0 g, 44.4 mmol) were dissolved in 100 mL of water. This mixture was stirred for 2 days at room temperature. HCl was then added to the solution to precipitate the product. The product was filtered and recrystallized from acetone to obtain the final product, which was a white powder. The yield was 27%. ^{1}H NMR spectrum was recorded on a JEOL Lambda 300 (300 MHz) spectrometer as solution in $CDCl_3$ (Cambridge Isotope Lab. Inc. 99.8%) using tetramethysilane as an internal reference. ^{1}H NMR: δ (ppm) 4.20 (s, 3H, C$\mathbf{H}_2$), 4.02 (q, 2H, C$\mathbf{H}_2$CH$_3$), 3.77 (q, 2H, C$\mathbf{H}_2$CH$_3$), 1.32 (quint, 6H, CH$_2$C$\mathbf{H}_3$).

Preparation of the Core Particle. The core particle was prepared by surfactant-free emulsion polymerization. Styrene (2.85 g) and VBC (0.150 g) were added to the vessel with 75 mL of water. The mixture was heated to 70 °C with thermostat oil bath and nitrogen saturated. KPS (0.100 g) was dissolved in 10 mL of water and added to the reactor for the initiation. After 24 h, the conversion reached to 90%. Then, the colloid dispersion was purified by repeating the centrifugation, decantation and redispersion process. The introduction of the iniferter on the purified latex surface was carried out as follows. An aqueous solution (20 mL) of NaDC (0.740 g, 3.28 mmol) was gently added to the colloid dispersion (2.00 g of particle, which was dispersed in 80 mL of water), and the reaction was continued for 10 h. During this reaction, the methyl chloride residue from the VBC unit was transferred to the iniferter group. The iniferter-carrying particle was purified as the same manner above.

Graft Polymerization of NIPAM with an Iniferter. The graft polymerization of the NIPAM monomer onto the particle surface was carried out in an aqueous dispersion with photoinduced polymerization.

The iniferter-carrying core particle (0.500 g) and NIPAM monomer (2.00 g, 17.7 mmol) was added to 200 mL of water, and the mixture was bubbled with N_2 for 30 min. The reactor was then UV-irradiated with a UV lamp, wavelength 312−577 nm, power 400 W (λ_{max}; 365 nm, UVL-400HA, Riko-Kagaku Sangyo Co. Ltd) for 1 h. The solution was maintained at 25 °C during the reaction. After the UV radiation, the particles were collected by centrifugation and purified as they were redispersed into freshwater. This process was repeated several times.

For the surface-initiated polymerization with the free iniferter, DCAA (2.00×10^{-6}, 1.00×10^{-5}, 1.00×10^{-4} mol) was added with the NIPAM monomer (2.00 g, 1.77 mmol) to the aqueous dispersion (200 mL in total) of the core particle (0.200 g). A small amount of NaOH was added to the solution to dissolve DCAA. The pH of the mixture was 9−10. The mixture was UV-irradiated under the same condition with the surface-initiated polymerization. After the surface graft polymerization, the particle was purified by repeating centrifugation with changing the medium to freshwater. The water phase at the top, which included the free PNIPAM, was collected after the centrifugation and dialyzed against water for 4 days. The free PNIPAM was obtained as white powder by evaporating the water, followed by the precipitation into diethyl ether from acetone. Conversion of NIPAM was calculated by measuring the amount of unreacted monomer in the medium with a gas chromatography (GC-353, GL Sciences, Inc.). The monomer consumption was reached 60−90% after 60 min of reaction.

Block Copolymer Synthesis. After the purification of the PNIPAM-carrying particle, the second block of the graft chain was synthesized by reirradiation of UV with the fresh monomer solution. For designing the particle as an adsorbent of proteins, a particle with a charged graft chain was prepared. The graft copolymerization was carried out with NIPAM and AAc in water for 1 h at 25 °C. NIPAM monomer (0.800 g, 7.08 mmol) and AAc monomer (8.00×10^{-3} g, 0.111 mmol) was added to the core particle dispersion (0.200 g of particles in 200 mL of water). The free iniferter, DCAA (2.00×10^{-6} mol), was added to the solution with NaOH. The obtained particle and polymer were purified and analyzed in the same manner described above. The amount of AAc incorporated to the particle surface was measured by conductometric titration.

Characterization. Hydrodynamic diameters of each particle were determined by dynamic light scattering (DLS) using a laser particle analyzer system (PAR-III, Otsuka Electronic Co.). A diluted aqueous dispersion of each sample was allowed to settle for 15 min at a given temperature before the measurements. For TEM observation, 2 μL of diluted dispersion was dropped on a carbon-coated copper grid (Okenshoji Co., Ltd.). The samples were dried at room temperature and were observed with field emission transmission electron microscopy (FE-TEM, TECNAI F20, Philips Electron Optics Co.). GPC equipment was comprised of pump (MODEL 576, GL Sciences, Inc.), guard column (TSK guardcolumn α, 40 × 6 mm, Tosoh Corporation), a mixed TSKgel column (TSK-GEL α-M, Tosoh Corp., 400 × 6 mm; bead size = 13 μm; molecular weight range = $\sim 1 \times 10^{-7}$ g/mol) differential refractometer (Shodex RI-71, Showa Denko K. K.), and control module to set the temperature at 40 °C. The calibration curve was obtained with different poly(ethylene oxide) standards (Tosoh Corp.). Methanol (HPLC grade, Wako Pure Chemicals Co.) with 10 mM LiBr was used as eluent (1 mL/min).

Adsorption Study. A protein adsorption study was carried out in a phosphate buffer that was adjusted at 10 mM, pH 7.0. A total of 2.50 mg of the particle was dispersed in 200 μL of the buffer and mixed with 0.100 or 0.200 mg of each protein (LZ, AV, or BSA). The mixture was allowed to settle for 1 h at 20 or 37 °C. The amount of the protein adsorbed onto the particles reached an equilibrium within 5 min under this experimental condition. Thus, we assumed that all the data were obtained from equilibrium states. After the incubation, the particle and supernatant, which contained

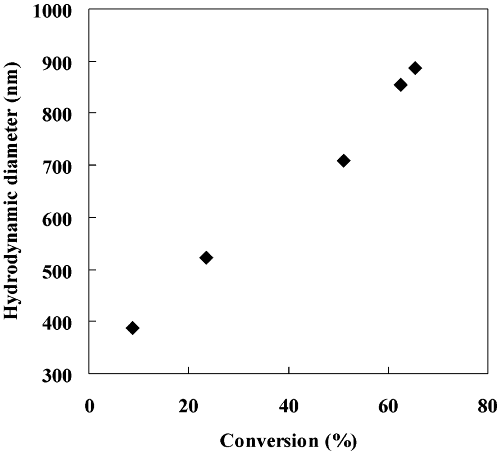

Figure 1. Relationship between the particle diameter and conversion of the graft polymerization of *N*-isopropylacrylamide (NIPAM). The hydrodynamic diameters were measured by dynamic light scattering at 20 °C.

nonadsorbed proteins, were separated by centrifugations at a fixed temperature. The concentration of remaining proteins in the residue was analyzed with the BCA Protein Assay Reagent (PIERCE). The amount of protein adsorbed onto the particle was calculated from the difference between the initial and final concentration of the supernatant. ζ-potential characterization was carried out with Zeta potential analyzer, Zeecom (Microtec Co., Ltd.).

Results and Discussion

Controlled Graft Radical Polymerization of NIPAM from the Core Particle. The core particle was synthesized by soap-free emulsion polymerization of styrene and VBC. A monodispersed core particle was obtained. Its diameter was approximately 400 nm. The fragment from VBC on the core particle surface was reacted with NaDC.[56] Through the reaction, the iniferter fragment, which works as an initiator for the graft polymerization, was immobilized onto the surface of the core particle. The iniferter-attached particle was then dispersed in an aqueous solution of a water-soluble monomer, NIPAM. This mixture was UV-irradiated to carry out controlled radical graft polymerization from the core particle surface at room temperature.

Figure 1 shows the conversion-diameter curve for a typical graft polymerization of NIPAM with the iniferter-carrying core particle. Although the linear increase in the hydrodynamic size of the particle with conversion does not exactly indicate a linear increase in the molecular weight, it does indicate an increase in the length of the grafted chain as the monomer is consumed. It was found that the supernatant was viscous after the graft polymerization, indicating that the polymer was simultaneously produced in solution. The SEC analysis of the polymers in supernatant found that they had high polydispersity indices (PDI $= 3.28$, $M_n = 115\,000$, $M_w = 377\,000$). This kind of problem has often been reported in controlled radical graft polymerization.[40,41,57] In the case of the graft polymerization with iniferter, the polymers in supernatant might be produced by the reactive radicals which were generated from unfavorable side reaction. There have been some reports that suggested the decomposition of iniferter group during the photopolymerization. Tardi et al.[51] and other researchers[52,53] claimed the decomposition of dithioester group of iniferter which leads to the formation of CS_2 and reactive radical formation during the polymerization. The possibility of other bond-breaking of the sulfur−carbon bond that generates active radical was also suggested,[58,59] though there is still no direct evidence for this side reaction. The PDI of the polymer that unfavorably generated in the supernatant in this study was high as 3.28, suggesting the iniferter decomposition followed by the formation of some different active radical.

Thus, the iniferter-mediated polymerization with NIPAM in aqueous media in this study should be regarded as quasi living polymerization process.

Controlled Graft Radical Polymerization with a Free Initiator. To examine the relationship between the molecular weight of grafted PNIPAM and hydrodynamic sizes, a free iniferter which was not attached to the particle was also added in the water phase when graft polymerization was carried out (Scheme 1). For this purpose, the water-soluble iniferter, DCAA, was dissolved in aqueous dispersion of iniferter-carrying particles. The surface chain length was varied by changing the [M]/[I] ratio used in the UV polymerization. The polymerization time was set at 1 h for all cases. The obtained hairy particle and free polymer in the media were separately purified and characterized by GPC for free polymer, DLS measurement, and TEM observation for the hairy particle. The recipes for the polymerization and the results are summarized in Table 1.

The addition of external initiators to carry out solution polymerization in a graft polymerization system is known to increase the control of surface-initiated controlled radical polymerization.[57] Table 1 shows that the molecular weight of the obtained polymer increased as the [M]/[I] ratio in the media increased. From these experimental conditions as shown in Table

Scheme 1. Iniferter-Mediated Controlled Radical Polymerization

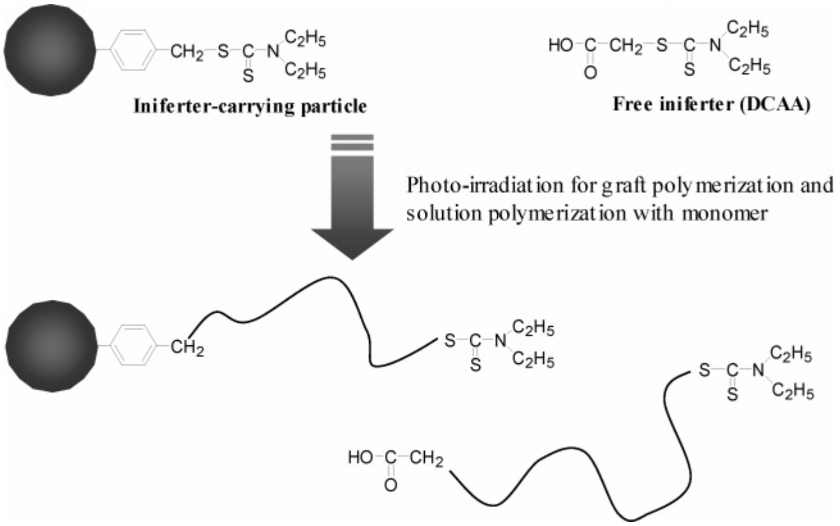

Table 1. Recipe and Characterization for Polymers Produced by Controlled Radical Polymerization of *N*-Isopropylacrylamide (NIPAM) with Photoiniferter in Water[c]

	recipe				free polymer			particle	
sample name	[M] (mol/10^{-2})	[I] (mol/10^{-4})	conversion (%)	theoretical $M_n/10^4$	$M_n/10^4$	$M_n/10^4$	PDI[a] (M_w/M_n)	hydrodynamic diameter (nm)	PDI[b] (D_w/D_n)
SV-N1	1.77	1.00	91.6	1.8	6.4	8.6	1.34	496	1.01
SV-N2	1.77	1.00E−01	80.5	16.1	19.2	34.2	1.78	745	1.02
SV-N3	1.77	2.00E−02	59.3	59.3	31.9	68.3	2.14	956	1.03

[a] Determined by GPC measurement. [b] Determined by DLS measurement at 20 °C. The graft polymerization of NIPAM from iniferter-immobilized polystyrene particle was carried out by UV irradiation in water at room temperature for 60 min. [c] [M], monomer (NIPAM); [I], iniferter (*N*,*N*-diethyldithiocarbamide acetic acid).

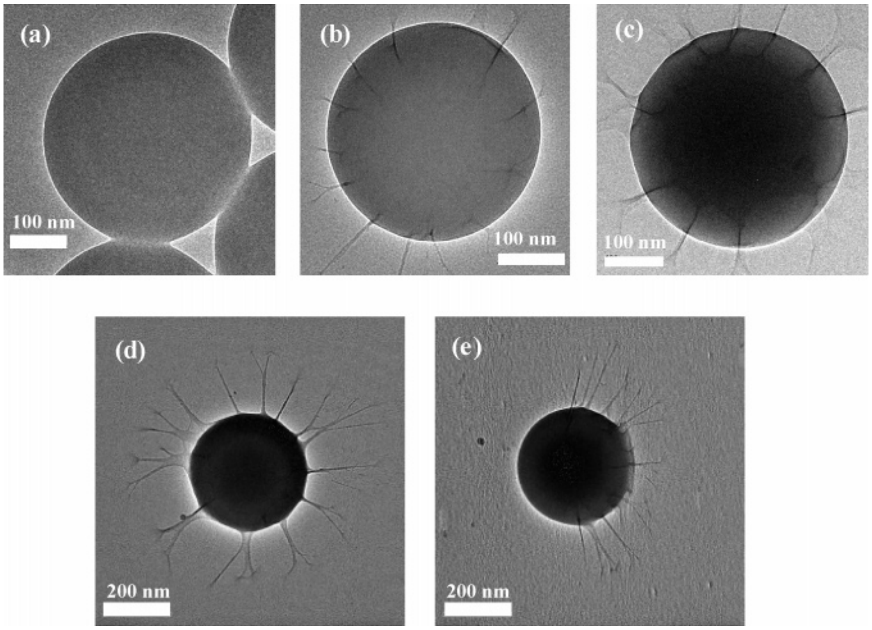

Figure 2. TEM observation of poly(*N*-isopropylacrlyamide) (PNIPAM)-introduced hairy particles. A corona-like structure was observed when the length of the graft chain increased. (a) SV-N1, (b) SV-N2, (c) SV-N3, and (d and e) the SV-N3 particle observed from the top and at an angle of 60°, respectively.

1, iniferter-controlled polymers with a molecular weight range of $M_n = 64\,000-319\,000$ and $\mathrm{PDI_{GPC}} = 1.34-2.14$ were obtained. The graft polymer and free polymer are expected to have similar molecular weights and polydispersities, as is often observed in a controlled radical graft polymerization system.[40,57] Although these values for the graft polymer and free polymer cannot be said to coincide accurately with each other until the graft polymer is directly analyzed and compared to the free polymer, the characteristics of a free polymer generated in the same system under the same iniferter-controlled polymerization can be regarded to be similar to those of a graft polymer. The hydrodynamic sizes of the PNIPAM-grafted particles increased as the molecular weight of the free polymer produced in the media increased. The distribution of the hydrodynamic diameters of PNIPAM-carrying particles was very narrow in all cases ($\mathrm{PDI_{DLS}} = 1.01-1.03$), indicating that the monodisperse particles were obtained. Although the produced free polymer chain shows a particular distribution of molecular weight, the overall extension of the surface layer of the particle appears to be homogeneous in order to give a monodisperse particle.

Figure 2 shows the TEM observation of PNIPAM-carrying particles having different graft chain lengths. A graft polymer that was tethered onto the polystyrene particle was observed as bundles of polymer chains. The PNIPAM layers stretched from the surface of the core particle to the substrate, as can be seen from Figure 2, parts d and e. The surface polymer cannot be seen from the SV-N1 particle. Since the amount of the grafted polymer chain of the SV-N1 particle is little due to the short chain length, the surface layer does not yield a good contrast for the TEM observation.

The chain density of SV-N particles was obtained by measuring the weight increase of a grafted particle compared to a core particle and the molecular weight listed in Table 1. The mass of the free polymer in the media was measured gravimetrically, and was subtracted from the monomer conversion to determine the amount of the polymer that generated on the particle surface. The increase of particle mass after the PNIPAM incorporation was divided by molecular weight and the surface area of core particle. As a result, the PNIPAM chain density on the particle surface was calculated as 0.0235 chain/nm^2. This value indicates that the grafted PNIPAM is in a brushy state. The grafted chains prepared by iniferter-controlled radical polymerization in this study have higher density than the tethered chains prepared by the conventional graft radical polymerization of NIPAM from polystyrene particle in water with redox-initiation system (0.0044 chain/nm^2).[22] In the conventional radical graft polymerization process, the number of grafted chain on the surface increases as the monomer consumed. Therefore, the PNIPAM chain produced in the early stage of the polymerization could inhibit the further diffusion of a monomer to reaction sites near the surface. Since all the chains grow approximately at the same rate from the surface in the controlled graft radical polymerization, such a hindrance does not occur. Well-defined, high-density polymer grafted surfaces have been prepared using controlled radical polymerization techniques, such as RAFT and ATRP, as reported by several groups.[40,60]

The linear PNIPAM is well-known as a thermosensitive polymer in which coil-to-globule transition occurs at around 32 °C in water. For the case with PNIPAM gel, the volume phase transition happens at 33−34 °C, when it is heated in pure

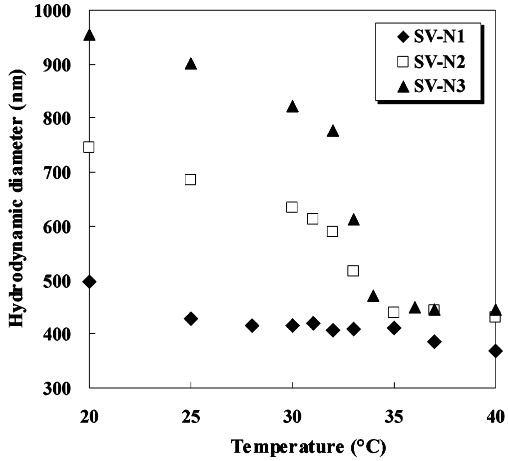

Figure 3. Change in the hydrodynamic diameters of poly(*N*-isopropylacrylamide) (PNIPAM) grafted polystyrene particles depending on the temperature. Each particle has a different graft chain length, as listed in Table 1.

water.[1] The PNIPAM chain grafted on polystyrene particles in this study is supposed to change the degree of extension depending on the temperature. The change in hydrodynamic size of the hairy particle depending on temperature was examined by dynamic light scattering. The results are shown in Figure 3. As can be seen, SV-N2 and SV-N3 particle changed their diameters at around 33 °C, and shrunk to the size close to that of the core particle (400 nm) above 35 °C. On the other hand, one might notice that SV-N1 particle that has the shortest graft chain gradually deswelled between 20 and 28 °C to reach to 400 nm size below 30 °C, and there is no more significant change around 33 °C, at which coil-to-globule transition was supposed to occur.

It is known that the lower critical solution temperature (LCST) or volume phase transition of *N*-acrylates depends on molecular weight, concentration, polymer end group and additives.[61−63] The effect of molecular weight on the phase transition of linear PNIPAM was well-investigated by Stöver et al.[63] They reported that as molecular weight increases, the phase transition temperature decreases to approach 31−32 °C using well-controlled PNIPAM. However, the results in Figure 3 shows when the chain length is long (SV-N2 and SV-N3 particle), the coil-to-globule transition occurs around 32−34 °C, while shorter chain grafted particle (SV-N1 particle) changes the diameter at the lower temperature. The grafted chains in this study are long enough, as they are composed of more than 560 monomer units, to neglect the end group effect. Thus, for the case with grafted PNIPAM, the effect of the molecular weight on phase transition temperature seems to be different from that of linear PNIPAM in solution.

The contraction of shell layer of the particle at a temperature lower than 32 °C seems to be able to be explained with another idea. When PNIPAM is deposited on a solid surface, the coil-to-globule transition might be affected by the density of the NIPAM unit. de Gennes[64] and Zhu and Napper[65] carefully studied the interaction between grafted polymer chains. Zhu and Napper observed that the PNIPAM chain anchored onto a surface has two types of coil-to-globule transition, while the free PNIPAM chain in solution does not have such a transition; they explained this phenomenon by means of the *n*-cluster concept. Interfacial PNIPAM shows weakly attracted interactions in the inner region of the grafted layer below the transition temperature, while the outer layer remains in the coil state. This is due to the difference in polymer density between the inner region and the outer dilute region.

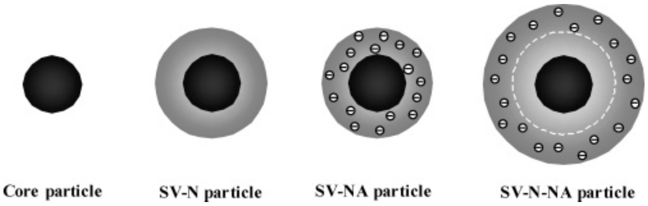

Figure 4. Structure of the particles prepared for the adsorption study. They were bare polystyrene particle, polystyrene particles with poly(*N*-isopropylacrylamide) (PNIPAM) chain, poly(NIPAM-*ran*-acrylic acid) chain, and PNIPAM-*b*-poly(NIPAM-*ran*-acrylic acid) chain.

The effect of the molecular weight of surface PNIPAM on thermosensitivity observed in this study coincides well with the explanation by Zhu and Napper. Thus, when the number of the chain per particle is fixed, the chain length affects on the thermosensitivity. Most of the transition seemed to be attributed to the formation of *n*-clusters when the grafted polymer was short (SV-N1; $M_n = 64\,000$). The change in size at around 33 °C became abrupt as the molecular weight of the grafted chain increased (SV-N2, SV-N3; $M_n > 192\,000$). Addition to the *n*-cluster concept, there may be effects of hydrophobic character of polystyrene surface. The coil-to-globule transition of PNIPAM in water is sensitive to the hydrophilicity of the polymer vicinity when there are comonomers or additives that can be adsorbed by the polymer chain. Since PNIPAM is chemically bonded onto polystyrene surface, the hydrophobic environment near the solid surface can lower the transition temperature. Thus, we think that extended brush on the surface, that is most part of the graft chain, does not affect with each other or with solid surface is needed for the sharp response.

Various types of thermosensitive particles composed of PNIPAM have been prepared so far. They include PNIPAM microgel particle,[3,8] core−shell particle,[5,21,31] and core−hair particle.[7,22−24] Gel layer tends to have inhomogeneous cross-linking distribution, which causes dull response. In contrast, core−hair structure is expected to show sharp response. However, the particles having core−hair structure do not always sharply respond if they do not have enough chain length and chain density.[66] The extended graft chain is necessary to avoid polymer−polymer interaction or polymer−surface interaction that causes dull response to temperature change.

In this study, we demonstrated that the chain length with a fixed number of chains per particle affects the temperature response and that a particle with high molecular weight responds more sharply to the temperature which coincided with the experimental result by Napper et al.[65] On the other hand, when the length of the hairy chain is fixed to a given value, the chain density affects on the responsiveness.[66] When the grafted chains were sparse on the surface, the size-temperature curve became dull compared to that of dense chain-carrying particles.

Adsorption Study. Four types of particles were used for the adsorption study to characterize the surface property in terms of the interaction with proteins: a bare core particle, mainly composed of polystyrene and having SO^{4-} groups originated from the initiator (KPS) of emulsion polymerization; an SV-N particle, a homopoly(NIPAM) chain incorporated particle; an SV-NA particle, a random copolymer, i.e., a poly(NIPAM-*ran*-AAc) chain carrying particle; and an SV-N-NA particle, a block copolymer, i.e., a (PNIPAM-*b*-poly(NIPAM-*ran*-AAc)) chain incorporated particle (Figure 4). The molecular weight of the PNIPAM chain or PNIPAM block is 700 000, and that of the random copolymer, i.e., poly(NIPAM-*ran*-AAc) chain, or block is 160 000. The amount of AAc unit incorporated into the graft layer, 5.0×10^{-8} (mol per mg particle), was measured by

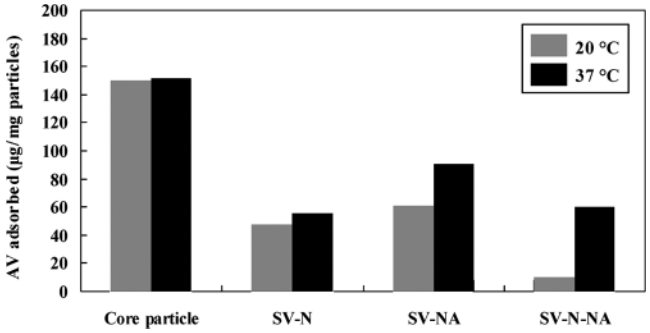

Figure 5. Characterization of particles in terms of cationic protein adsorption. The amount of avidin (AV) onto each particle was measured. The experiments were carried out in a 10 mM phosphate buffer at pH 7. The temperature was set at 20 or 37 °C.

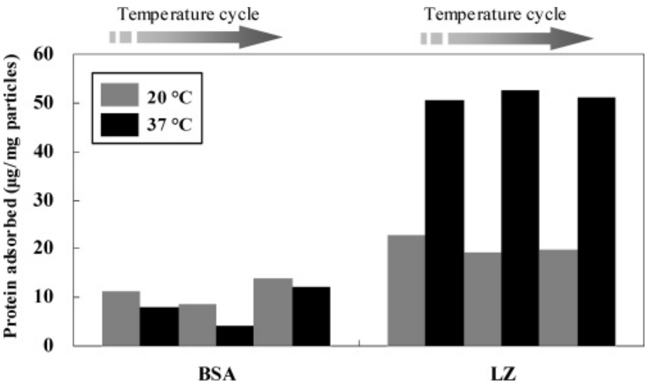

Figure 6. Amount of proteins (bovine serum albumin, BSA, and lysozyme, LZ) adsorbed onto the SV-N-NA particle when the temperature in the surrounding media was cyclically changed.

conductometric titration. All the graft polymerization and graft copolymerization were stopped when the conversion reached to around 50% in this study. Regarding the reactivity ratio of the NIPAM(1)/AAc(2) couple ($r_1 = 14.0$, $r_2 = 0.07$),[67] NIPAM is supposed to be predominantly consumed in the initial stage of the polymerization. The monomer mol ratio in the medium was changed continuously from $f_1^\circ:f_2^\circ = 0.985:0.015$ to $f_1:f_2 = 0.979:0.021$ during the copolymerization. Thus, the AAc unit distribution of the poly(NIPAM-*ran*-AAc) block can be regarded as homogeneous.

The incorporation of the charged molecules into the PNIPAM chain affects its thermosensitivity, and it is known that only 1% of AAc units with a PNIPAM-carrying particle causes a coil-to-globule transition at around 37 °C, which is approximately 4 °C higher than the transition temperature of homo PNIPAM.[68] However, since adsorption experiments were carried out in a 10 mM phosphate buffer, all of the graft chains prepared in this study caused a transition in the range 30−35 °C in the medium. This was followed by measuring the hydrodynamic sizes depending on the temperature in the same buffer.

The coil-to-globule transition of the PNIPAM chain was triggered due to the balance between hydration of the segments and association by hydrophobic interaction. Thus, the particle surface which is covered by the PNIPAM graft chain changes from a hydrophilic to a more hydrophobic characteristic. A fluorescence analysis indicated that the surface of PNIPAM microspheres became hydrophobic above its volume phase transition temperature.[69,70] We previously reported that, when small hydrophobic substances, such as dye, were used as a target molecule, the particle changed the adsorbability depending on the temperature.[68] The amount of dye adsorbed onto the PNIPAM grafted particle changed at around 33 and 37 °C, at which point the hydrodynamic size also changed.

All the particles used for the adsorption study have a negatively charged surface. Figure 5 shows the adsorption of avidin (AV) onto the prepared particles measured above and below the transition temperature. AV is positively charged under the experimental condition. The bare polystyrene particle had the highest amount of adsorbed AV, and its adsorbability was independent of the temperature. The protein may be strongly attracted to the core particle due to the electrostatic and hydrophobic interaction. As seen from Figure 5, the introduction of a hydrophilic polymer on the polystyrene surface resulted in the prevention of protein adsorption. The amount of AV adsorbed onto the SV-N particle increased slightly as the temperature was elevated. Although the PNIPAM layer of the SV-N particle does not contain charged groups, there were initiator residues, SO^{4-} groups, on the core particle surface. The shrinkage of the graft layer caused an increment of the zeta potential of the particle (Supporting Information, Figure S1). Therefore, electrostatic and hydrophobic interaction contributed to the increase in protein adsorption above the transition temperatures.

The introduction of carboxylic groups on the surface layer gave rise to an increase in the adsorbability at both temperatures. In addition, the difference in adsorption between 20 and 37 °C became larger by the introduction of the AAc unit (SV-NA particle). The density of carboxylic units in a grafted layer is diluted when the polymer is in an extended state, but their condensation occurred by the collapse of the surface layer at higher temperatures. The density of the charged units and the dehydration of the surface layer above the transition temperature may cause an increase in protein adsorption. It is noteworthy that the copolymerization of AAc with NIPAM only in the outer part of the graft chain (SV-N-NA particle), which is expected to give a larger difference in surface property between 20 and 37 °C, resulted in the most efficient adsorbent for basic proteins. These results indicated that a proper surface design is important to improve the ability of the surface as an adsorbent.

We also demonstrated the repeatability of protein adsorption/desorption by cycling the heating/cooling process. The temperature dependence on the adsorption of acidic (BSA) proteins and cationic (LZ) onto the SV-N-NA particle is illustrated in Figure 6. This figure clearly shows that the amount of LZ adsorbed was reproducibly changed by simply controlling the temperature. On the other hand, the adsorption of an acidic protein, BSA, was somewhat independent of the temperature. Thus, it seems that electrostatic interaction is a dominant factor for surface−protein interaction.

The protein adsorption/desorption process on PNIPAM-carrying surface is expected to be relatively ambient, since dehydrated PNIPAM still contains water even at an elevated temperature. Fujimoto et al. investigated protein adsorption onto a PNIPAM microgel particle and reported that the amount of protein desorbed from the surface as temperature changed was larger for a PNIPAM-carrying particle than for a polystyrene particle.[71] They also reported that the adsorption/desorption process using such a surface prevented the denaturation of the protein.

Conclusions

A thermosensitive particle was prepared under iniferter-controlled radical graft polymerization. The hydrodynamic diameter of the grafted particle increased linearly as the polymerization proceeded. The length of the grafted chain was controlled by varying [M]/[I] ratio in the reaction. The molecular weight of the grafted thermosensitive polymer affected its coil-to-globule transition. The particle with a graft chain having a number-average molecular weight of 319 000 sharply changed its diameter, while a particle with a chain having a number-average molecular weight of 64 000 did not show a clear transition and resulted in a dull response.

The coverage of the hydrophilic layer on the polystyrene surface prevented the protein adsorption onto the particle surface. However, incorporating a small amount of charged units in the graft layer resulted in the temperature controllability of adsorption/desorption. The designed particle reproducibly adsorbed and desorbed proteins as the temperature was cycled above and below the transition temperature.

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Supporting Information Available: Figure S1. Electrophoretic mobility of polystyrene particle (core particle), SV-N particle (polystyrene particle with poly(*N*-isopropylacrylamide) (PNIPAM) chain), SV-NA particle (polystyrene particle with poly(NIPAM-*ran*-acrylic acid) chain, and SV-N-NA particle (polystyrene particle with PNIPAM-*b*-poly(NIPAM-*ran*-acrylic acid) chain. This material is available free of charge via the Internet at http://pubs.acs.org.

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