

Salt Effect on the Heat-Induced Association Behavior of Gold Nanoparticles Coated with Poly(*N*-isopropylacrylamide) Prepared via Reversible Addition–Fragmentation Chain Transfer (RAFT) Radical Polymerization

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Poly(*N*-isopropylacrylamide) (PNIPAM) with a narrow molecular weight distribution was prepared by reversible addition–fragmentation chain transfer (RAFT) radical polymerization. A dithioester group at the chain end of PNIPAM thus prepared was cleaved by treating with 2-ethanolamine to provide thiol-terminated PNIPAM with which gold nanoparticles were coated via reactions of the terminal thiol with gold. The thermoresponsive nature of the maximum wavelength of the surface plasmon band and hydrodynamic radius (R_h) for the PNIPAM-coated gold nanoparticles were found to be sensitively affected by added salt. In pure water, R_h for the PNIPAM-coated gold nanoparticles at 40 °C (>lower critical solution temperature (LCST)) was smaller than that at 25 °C (<LCST) due to the shrinkage of the PNIPAM chains on the gold surface. The maximum wavelength of the plasmon band in pure water was almost independent of the temperature, the color of the solution remaining pink regardless of temperature. On the other hand, in the presence of 50 mM NaCl, the plasmon band at 40 °C was red shifted compared with that at 25 °C because of interparticle associations, the pink color at 25 °C changing to blue-purple at 40 °C. Furthermore, R_h for the PNIPAM-coated gold nanoparticles at 40 °C was significantly larger than that at 25 °C. Heat-induced association and dissociation for the PNIPAM-coated gold nanoparticles were completely reversible in 50 mM NaCl aqueous solutions, which is responsible for the reversible thermoresponsive color change.

Introduction

Functional gold nanoparticles have been a focus of considerable interest because of their potential applications for catalysis, diagnosis, and photoelectronic devices. Highly dispersed gold nanoparticles solutions exhibit a pink color with an absorption band around 520 nm due to the surface plasmon resonance by the collective oscillation of electron density. The association of the gold nanoparticles in the dispersed solution induces a color change from pink to blue-purple, which would be applied to colloidal sensors.^{1–3} However, further association of the gold nanoparticles creates insoluble aggregates that cannot be re-dispersed. To improve their dispersibility, the gold nanoparticles may be coated with a protective low molecular weight stabilizer or with a water-soluble polymer having a functionality to react with gold.

Polymers prepared by reversible addition–fragmentation chain transfer (RAFT) radical polymerization with use of a dithioester

chain transfer agent (CTA) have a well-controlled molecular weight and narrow molecular weight distribution. Another advantage of the RAFT polymerization is that polymers bearing a dithioester group at the chain end can be obtained. The terminal dithioester group can be converted to a thiol terminal functionality, which can be used for various reactions including “grafting” of a polymer on the surface of gold.

It is well known that an aqueous solution of poly(*N*-isopropylacrylamide) (PNIPAM) undergoes a thermally reversible phase separation.^{4,5} PNIPAM dissolves in water assuming a random coil conformation at room temperature, but it separates from the aqueous phase when heated above 31–32 °C, a lower critical solution temperature (LCST). Recently, well-defined PNIPAM has been synthesized by RAFT radical polymerization by several groups.^{6–10}

McCormick et al. reported that water-soluble polymers prepared by RAFT radical polymerization can stabilize gold nanoparticles by reduction of dithioester end groups to thiol groups in the presence of HAuCl₄ in water.¹¹ Li et al.¹² reported

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(1) Mirkin, C. A.; Letsinger, R. L.; Mucic, R. C.; Storhoff, J. J. *Nature* **1996**, 382, 607–609.

(2) Storhoff, J. J.; Lazarides, A. A.; Mucic, R. C.; Mirkin, C. A.; Letsinger, R. L.; Schatz, G. C. *J. Am. Chem. Soc.* **2000**, 122, 4640–4650.

(3) Ishii, T.; Otsuka, H.; Kataoka, K.; Nagasaki, Y. *Langmuir* **2004**, 20, 561–564.

(4) Fujishige, S.; Kubota, K.; Ando, I. *J. Phys. Chem.* **1989**, 93, 3311–3313.

(5) Kratz, K.; Hellweg, T.; Eimer, W. *Polymer* **2001**, 42, 6631–6639.

(6) Ganachaud, F.; Monteiro, M. J.; Gilbert, R. G.; Dourges, M.-A.; Thang, S. H.; Rizzardo, E. *Macromolecules* **2000**, 33, 6738–6745.

(7) Schilli, C.; Lanzendorfer, M. G.; Muller, A. H. E. *Macromolecules* **2002**, 35, 6819–6827.

(8) Ray, B.; Isobe, Y.; Morioka, K.; Habaue, S.; Okamoto, Y.; Kamigaito, M.; Sawamoto, M. *Macromolecules* **2003**, 36, 543–545.

(9) Convertine, A. J.; Ayres, N.; Scales, C. W.; Lowe, A. B.; McCormick, C. L. *Biomacromolecules* **2004**, 5, 1177–1180.

(10) Kujawa, P.; Segui, F.; Shaban, S.; Diab, C.; Okada, Y.; Tanaka, F.; Winnik, F. M. *Macromolecules* **2006**, 39, 341–348.

the synthesis of gold nanoparticles coated with thiol-terminated PNIPAM prepared by RAFT radical polymerization. The thermoresponsive gold nanoparticles indicate a sharp clear-to-opaque transition in water between 25 and 30 °C. Recently, more detailed reports on the preparation and thermoresponsive behavior of PNIPAM-coated gold nanoparticles were released by Tenhu and co-workers.^{13–15} A solution property of nonionic PNIPAM in water is sensitively influenced by added salt because salt can alter the polymer–water interaction.¹⁶ Furthermore, the stability of aqueous dispersion of gold nanoparticles depends on the ionic strength. Thus, we anticipated that the behavior of the aqueous dispersion of PNIPAM-coated gold nanoparticles would be sensitive to the concentration of salt added. To the best of our knowledge, the salt effect on the thermoresponsive behavior of PNIPAM-coated gold nanoparticles has not so far been reported. In this paper, we focus on the effect of added salt on the thermoresponsive behavior of PNIPAM-coated gold nanoparticles.

Experimental Section

Reagents. 1-Phenylethyl phenyldithioacetate (PEPD) was synthesized according to the method reported by Rizzardo and co-workers.¹⁷ *N*-Isopropylacrylamide (NIPAM) from Aldrich was purified by recrystallization from a mixture of benzene and *n*-hexane (3/7, v/v). Hydrogen tetrachloroaurate(III) tetrahydrate (HAuCl₄·4H₂O) (99.0%) from Kanto Chemical was used as received. Methanol and tetrahydrofuran (THF) were dried over 4 Å molecular sieves and distilled. Water was purified with a Millipore Milli-Q system. Other reagents were used as received.

Preparation of PNIPAM by RAFT Polymerization. NIPAM (30.0 g, 265 mmol) was dissolved in methanol (263 mL), and then PEPD (506 mg, 1.86 mmol) and 4,4'-azobis(4-cyanopentanoic acid) (V-501) (98.9 mg, 0.353 mmol) were added to the solution. The methanol solution was degassed by purging with Ar gas for 30 min, and the solution was refluxed at 70 °C for 7.5 h. After cooling the reaction mixture with an ice bath, the solvent was removed by evaporation, and then the crude product was dissolved in a small amount of acetone. The solution was poured into a large excess of *n*-hexane. The precipitate was dissolved in acetone again, and the solution was poured into a large excess of diethyl ether. The polymer obtained was dried in a vacuum oven at 30 °C for 12 h (16.4 g, 54.7% conversion). The number-average molecular weight (M_n) and molecular weight distribution (M_w/M_n) were 1.14×10^4 and 1.07, respectively, measured by gel-permeation chromatography (GPC).

Preparation of Thiol-Terminated PNIPAM (PNIPAM-SH). PNIPAM (1.00 g, 87.7 μmol) was dissolved in THF (18.9 mL), and then 2-ethanolamine (0.173 g, 2.83 mmol) and a trace of tris(2-carboxyethyl)phosphine hydrochloride were added to the solution. The solution was stirred at room temperature for 1 h. The reaction mixture was poured into a large excess of diethyl ether to precipitate thiol-terminated PNIPAM (PNIPAM-SH). PNIPAM-SH was purified by reprecipitating from methanol into a large excess of diethyl ether two times. The polymer obtained was dried in a vacuum oven at 30 °C for 12 h (0.818 g, 81.8%). M_n and M_w/M_n were 1.22×10^4 and 1.08, respectively, measured by GPC. From the M_n value the number-average degree of polymerization (DP_n) was calculated to be 98.

Preparation of PNIPAM-Coated Gold Nanoparticles. Gold nanoparticles were prepared in the presence of sodium citrate according to the literature procedure.¹⁸ The mean hydrodynamic radius (R_h) was determined to be 9.24 nm by quasi-elastic light scattering (QELS) measurement. The R_h value was almost the same as that estimated by transmission electron microscopy (Supporting Information Figure S1). A mixture of PNIPAM-SH and a solution of gold nanoparticles was kept at room temperature for 24 h in the dark to prevent light-induced flocculation.¹⁹ The concentrations of PNIPAM-SH and Au are $10 (8.20 \times 10^{-4} \text{ mM})$ based on the NIPAM repeating unit and 19.7 mg/L (0.1 mM), respectively, unless otherwise noted. The concentration of Au was calculated from the amount of used HAuCl₄·4H₂O to prepare gold nanoparticles. For thermogravimetric analysis (TGA), excess PNIPAM-SH was removed from the PNIPAM-coated gold nanoparticles by centrifugation at 6000 rpm for 2 h followed by decantation of supernatants. The precipitate was dissolved in water, and then the PNIPAM-coated gold nanoparticles were recovered by freeze-drying.

Characterization. *Gel-Permeation Chromatography (GPC).* GPC analysis was performed at 40 °C with a Shodex DS-4 pump and an RI-101 refractive index detector using Shodex one KF-805L and three KF803L columns connected in series. THF was used as the eluent at a flow rate of 1.0 mL/min. The number-average molecular weight (M_n), weight-average molecular weight (M_w), and M_w/M_n of the sample polymers were calibrated with standard polystyrene samples.

Transmission Electron Microscopy (TEM). TEM measurements were performed on a Hitachi model H-600 instrument operated at an accelerating voltage of 100 kV. Sample was prepared by placing a drop of a gold nanoparticle solution on a carbon-coated copper grid followed by air-drying.

Nuclear Magnetic Resonance (NMR). ¹H NMR spectra were obtained with a Bruker DRX-500 spectrometer operating at 500 MHz. PNIPAM and PNIPAM-SH samples were analyzed in D₂O at 25 °C. Chemical shifts were determined using 3-(trimethylsilyl)-propionic-2,2,3,3-*d*₄ acid, sodium salt, as an internal reference.

Thermogravimetric Analysis (TGA). TGA was carried out using a Rigaku Thermo plus TG-8120 instrument. Samples were heated from 25 to 800 °C at 5 °C/min. The surface density of PNIPAM (chains/nm²) can be estimated by TGA as follows

$$\text{surface density of PNIPAM (chains/nm}^2\text{)} = \frac{\left(\frac{W_{\text{PNIPAM}}}{100 - W_{\text{PNIPAM}}} \right) \times \rho \times V_{\text{particle}} \times N_A}{M_{\text{PNIPAM}} \times S_{\text{particle}}} \quad (1)$$

Here, W_{PNIPAM} is the percent weight loss corresponding to the decomposition of the PNIPAM chains, ρ is the density of bulk gold (19.32 g/cm³), V_{particle} is the volume of bare gold nanoparticle calculated from the R_h of the particle (9.24 nm), N_A is Avogadro's number, M_{PNIPAM} is the molecular weight of the PNIPAM chain (M_n), and S_{particle} is the specific surface area of bare gold nanoparticle calculated from R_h .

UV-vis absorption spectra. UV-vis absorption spectroscopic measurements were performed with a JASCO V-530 spectrophotometer with a 1.0 cm path length quartz cell at various temperatures. The temperature was changed from 20 to 50 °C with a heating rate of 0.5 °C/min with a JASCO ETC-505T thermostat system.

Quasi-elastic Light Scattering (QELS). QELS data were obtained with an Otsuka Electronics Photol DLS-7000HL light scattering spectrometer equipped with an ALV-5000/EPP multi- τ digital time correlator at various temperatures. Sample solutions were filtrated with a 0.2 μm pore size membrane filter prior to measurements. The temperature of the sample solutions was controlled by a Tokyo Rikakikai NCB-1200 circulating water bath with a thermostat. The solutions were permitted to stand for at least 10 min at a constant

(11) Lowe, A. B.; Sumerlin, B. S.; Donovan, M. S.; McCormick, C. L. *J. Am. Chem. Soc.* **2002**, *124*, 11562–11563.

(12) Zhu, M.-Q.; Wang, L.-Q.; Exarhos, G. J.; Li, A. D. Q. *J. Am. Chem. Soc.* **2004**, *126*, 2656–2657.

(13) Shan, J.; Chen, J.; Nuopponen, M.; Tenhu, H. *Langmuir* **2004**, *20*, 4671–4676.

(14) Shan, J.; Nuopponen, M.; Jiang, H.; Kauppinen, E.; Tenhu, H. *Macromolecule* **2003**, *36*, 4526–4533.

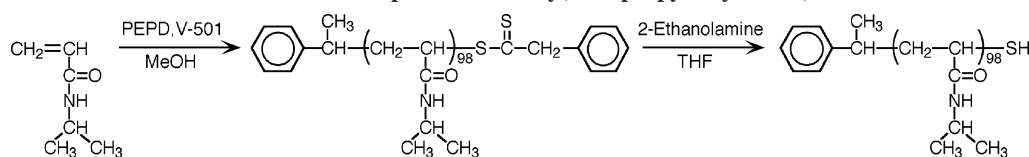
(15) Raula, J.; Shan, J.; Nuopponen, M.; Niskanen, A.; Jiang, H.; Kauppinen, E. I.; Tenhu, H. *Langmuir* **2003**, *19*, 3499–3504.

(16) VanDurme, K.; Rahier, H.; VanMele, B. *Macromolecules* **2005**, *38*, 10155–10163.

(17) Quinn, J. F.; Rizzardo, E.; Davis, T. P. *Chem. Commun.* **2001**, 1044–1045.

(18) Freeman, R. G.; Hommer, M. B.; Grabar, K. C.; Jackson, M. A.; Natan, M. J. *J. Phys. Chem.* **1996**, *100*, 718–724.

(19) Satoh, N.; Hasegawa, H.; Tsujii, K.; Kimura, K. *J. Phys. Chem.* **1994**, *98*, 2143–2147.

Scheme 1. Preparation of Poly(*N*-isopropylacrylamide)

temperature. A He–Ne laser (10 mW at 632.8 nm) was used as a light source. To obtain the relaxation time distribution, $\tau A(\tau)$, the inverse Laplace transform (ILT) analysis was performed using the algorithm REPES^{20,21}

$$g^{(1)}(t) = \int \tau A(\tau) \exp(-t/\tau) d \ln \tau \quad (2)$$

Here, τ is the relaxation time and $g^{(1)}(t)$ is the normalized autocorrelation function. The relaxation time distributions are given as a $\tau A(\tau)$ versus $\log \tau$ profile with an equal area. The translational diffusion coefficient (D) is calculated from $D = \Gamma/q^2$, where Γ is the relaxation rate ($1/\tau$) and $q = (4\pi n/\lambda) \sin(\theta/2)$ with n being the refractive index of solvent, λ being the wavelength (632.8 nm) and θ being the scattering angle. The hydrodynamic radius (R_h) is calculated using the Einstein–Stokes relation $R_h = k_B T / 6\pi\eta D$, where k_B is Boltzmann's constant, T is the absolute temperature, and η is the solvent viscosity.

Results and Discussion

Synthesis of PNIPAM-SH and PNIPAM-Coated Gold Nanoparticles. Ray et al.²² reported that RAFT polymerization of NIPAM proceeds in a controlled fashion using PEPD as a chain transfer agent. The dithioester group at the PNIPAM chain end can easily be cleaved by a primary amine to yield the corresponding thiol group.²³ Cleavage of the terminal phenyldithioacetate group was confirmed by UV–vis absorption and ¹H NMR measurements. PNIPAM prepared by the RAFT process shows UV absorption at 312 nm due to the phenyldithioacetate group at the chain end. The UV absorption of the terminal phenyldithioacetate group disappeared as a result of its cleavage (Supporting Information Figure S2). Furthermore, cleavage was confirmed from a decrease in ¹H NMR peak intensity around 7.2–7.5 ppm associated with the terminal phenyl protons in the terminal 1-phenylethyl and phenyldithioacetate groups (Scheme 1). The resonance peaks associated with the methine proton of the pendent *N*-isopropyl group are observed at 3.9 ppm (Supporting Information Figure S3). After the cleavage reaction, the ratio of the area intensity for the terminal phenyl protons at 7.2–7.5 ppm to that for the pendent methine proton at 3.9 ppm decreased to one-half that for PNIPAM, i.e., from 0.092 for PNIPAM to 0.046 for PNIPAM-SH. This decrease is indicative of the quantitative cleavage of the terminal phenyldithioacetate group of PNIPAM.

The M_n and M_w/M_n values for PNIPAM and PNIPAM-SH determined by GPC analysis using THF as eluent are nearly the same: $M_n = 1.14 \times 10^4$ and $M_w/M_n = 1.07$ for PNIPAM and $M_n = 1.22 \times 10^4$ and $M_w/M_n = 1.08$ for PNIPAM-SH (Supporting Information Figure S4). The GPC data indicated the absence of bimodal coupling products that could be formed from disulfide bond (PNIPAM-S-S-PNIPAM).²⁴

TGA measurements on the purified PNIPAM-coated gold nanoparticles indicated that PNIPAM decomposed in the

temperature range 230–420 °C. From an observed weight loss of 23.7%, the residual mass of gold was calculated to be 76.3%. The TGA curves for bare gold nanoparticles and PNIPAM-coated gold nanoparticles are given in Supporting Information Figure S5. Thus, the surface density of PNIPAM chain can be calculated from eq 1 to be 0.9 chains/nm². Fukuda et al.²⁵ reported a gold nanoparticle coated with poly(methyl methacrylate) (PMMA) prepared by surface-initiated ATRP of methyl methacrylate with the initiator-coated gold nanoparticle. The graft density on the gold surface is nearly constant independent of the molecular weight of PMMA and estimated to be 0.3 chains/nm². Tenhu et al.¹⁴ reported PNIPAM-coated gold nanoparticles prepared by the Schiffrin reaction in the presence of PNIPAM bearing a dithiobenzoate end group or PNIPAM end capped with a thiol group. The graft density of PNIPAM chains ranged from 1.8 to 3.6 chains/nm².

In general, when aqueous solutions of conventional gold nanoparticles are freeze-dried, they tend to aggregate and cannot redissolve in water. However, PNIPAM-coated gold nanoparticles recovered in a powdery form by freeze-drying techniques can be completely redissolved in water.

UV–vis Absorption Spectra. UV–vis absorption spectra associated with the surface plasmon band for gold nanoparticles are known to be sensitive to the shape of the gold nanoparticles²⁶ and their surrounding environments.²⁷ When gold nanoparticles are close to each other, the characteristic plasmon band around 520 nm shifts toward longer wavelengths because of an electronic dipole–dipole interaction between plasmons of neighboring gold nanoparticles.^{28,29} Figure 1 compares typical UV–vis absorption spectra of PNIPAM-coated gold nanoparticles at 25 and 40 °C in pure water and in a 50 mM NaCl aqueous solution. In pure water, there is no or little effect of temperature on the plasmon resonance absorption. As can be seen in Figure 1a, the absorption maximum of the plasmon band of 522 nm at 25 °C only slightly shifts to 524 nm as the temperature is increased to 40 °C. This slight red shift may be attributed to a change in the dielectric constant of the microenvironment around the gold nanoparticles.³⁰ At 25 °C, the PNIPAM-coated gold nanoparticles should be hydrated because PNIPAM chains at this temperature are sufficiently solvated. At 40 °C, however, the coated gold nanoparticles would be dehydrated due to the intrachain coil-to-globule transition of the surrounding PNIPAM chains. On the other hand, in a 50 mM NaCl aqueous solution, a considerable red shift from 523 to 580 nm in the plasmon band was observed when the temperature was increased from 25 to 40 °C (Figure 1b). This red shift should be due to an interparticle aggregation

(25) Ohno, K.; Koh, K.-M.; Tsujii, Y.; Fukuda, T. *Macromolecules* **2002**, *35*, 8989–8993.

(26) Foss, C. A., Jr.; Hornyak, G. L.; Stockert, J. A.; Martin, C. R. *J. Phys. Chem.* **1992**, *96*, 7497–7499.

(27) Hostetler, M. J.; Wingate, J. E.; Zhong, C.-J.; Harris, J. E.; Vachet, R. W.; Clark, M. R.; Londono, J. D.; Green, S. J.; Stokes, J. J.; Wignall, G. D.; Glush, G. L.; Porter, M. D.; Evans, N. D.; Murray, R. W. *Langmuir* **1998**, *14*, 17–30.

(28) Heath, J. R.; Knobler, C. M.; Leff, D. V. *J. Phys. Chem. B* **1997**, *101*, 189–197.

(29) Collier, C. P.; Saykally, R. J.; Shiang, J. J.; Henrichs, S. E.; Heath, J. R. *Science* **1997**, *277*, 1978–1981.

(30) Kreibitz, U.; Vollmer, M. *Optical Properties of Metal Clusters*; Springer Series in Material Science 25; Springer-Verlag: Berlin, 1995.

(20) Jakes, J. *Czech. J. Phys.* **1988**, *B38*, 1305–1316.

(21) Schillén, K.; Brown, W.; Johnsen, R. M. *Macromolecules* **1994**, *27*, 4825–4832.

(22) Ray, B.; Isobe, Y.; Matsumoto, K.; Habaue, S.; Okamoto, Y.; Kamigaito, M.; Sawamoto, M. *Macromolecules* **2004**, *37*, 1702–1710.

(23) Nakayama, M.; Okano, T. *Biomacromolecules* **2005**, *6*, 2320–2327.

(24) Scales, C. W.; Convertine, A. J.; McCormick, C. L. *Polym. Prep.* **2005**, *46*, 393–394.

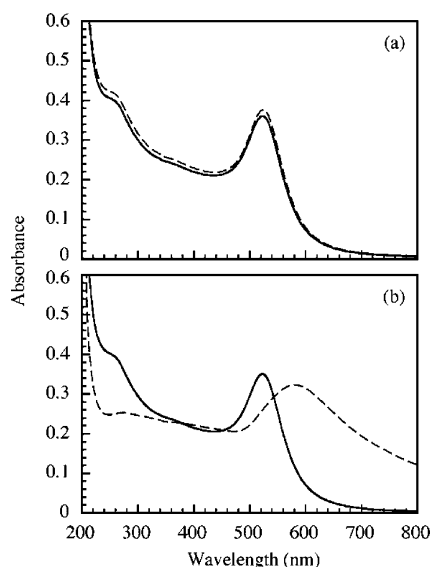


Figure 1. UV-vis absorption spectra for PNIPAM-coated gold nanoparticles in pure water (a) and 50 mM NaCl aqueous solutions (b) at 25 (solid lines) and 40 °C (dotted lines).



Figure 2. Photographs for 50 mM NaCl aqueous solutions of the PNIPAM-coated gold nanoparticles at 25 (a) and 40 °C (b).

of the PNIPAM-coated gold nanoparticles in a 50 mM NaCl aqueous solution at temperatures above LCST for PNIPAM.

For the PNIPAM-coated gold nanoparticles, no macroscopic phase separation was observed at 40 °C, presumably because of the following two reasons. First, the PNIPAM concentration is so low (10 mg/L) that the macroscopic phase separation due to the aggregation of collapsed PNIPAM chains above LCST is invisible. Second, dehydrated PNIPAM chains at 40 °C may be prevented from undergoing aggregation by electrostatic repulsion of negative charges of the gold nanoparticles.

Figure 2 compares the colors of 50 mM NaCl aqueous solutions of the PNIPAM-coated gold nanoparticles at 25 and 40 °C. A pink color at 25 °C turns to a blue-purple color without precipitation when the solution is heated to 40 °C. The color change was completely reversible responding to the temperature change. These observations indicate that the heat-induced interparticle association and dissociation occur reversibly. In the case of the PNIPAM-coated gold nanoparticles in pure water, however, such a color change upon a temperature change was not recognized.

Figure 3 compares the absorbance at 600 nm for the PNIPAM-coated gold nanoparticles in pure water and in a 50 mM NaCl

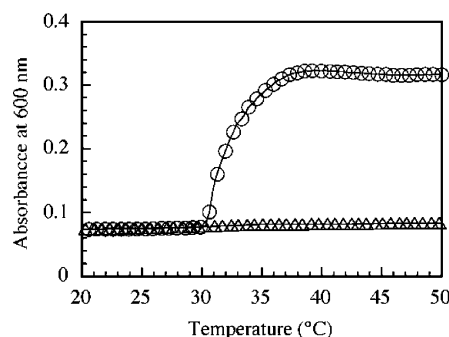


Figure 3. Absorbance at 600 nm for 0 (Δ) and 50 mM NaCl (\circ) aqueous solutions of the PNIPAM-coated gold nanoparticles as a function of temperature: the temperature was changed with a heating rate of 0.5 °C/min.

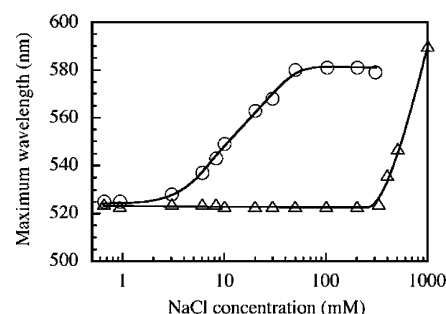


Figure 4. Maximum wavelength of the surface plasmon band of the PNIPAM-coated gold nanoparticles in aqueous solutions as a function of the concentration of NaCl at 25 (Δ) and 40 °C (\circ).

aqueous solution plotted as a function of temperature. The absorbance at 600 nm in pure water is almost constant independent of the temperature over the range of 20–50 °C. On the other hand, the absorbance at 600 nm in the 50 mM NaCl aqueous solution is ca. 0.07 below 30 °C, and the absorbance starts to increase rather abruptly with increasing temperature at a temperature near 30 °C, reaching ca. 0.32 above 38 °C. This observation indicates that interparticle associations of the PNIPAM-coated gold nanoparticles begin to occur near 30 °C in a 50 mM NaCl aqueous solution. This temperature is slightly lower than LCST for PNIPAM homopolymer in water (i.e., 32 °C).^{4,5}

It is known that an electrolyte added, such as NaCl, NaOH, and KCl, would make dispersed solutions of bare gold nanoparticles unstable because the surface charges on the gold nanoparticles are electrostatically screened. When the ionic strength of a dispersed solution of bare gold nanoparticles is increased, a pink color of the solution turns to a bluish-purple color quickly due to the flocculation of the bare gold nanoparticles. After a while, the color fades and a dark precipitate eventually forms and the precipitate cannot redissolve in water.³¹ Such a phenomenon is commonly referred to as a “salting-out effect”.³²

In Figure 4 the maximum wavelength of the surface plasmon band of the PNIPAM-coated gold nanoparticles is plotted against the concentration of NaCl ([NaCl]) on the logarithmic scale. The maximum wavelength at 25 °C is practically constant at ca. 523 nm, independent of the NaCl concentration when [NaCl] \leq 300 mM. However, the maximum wavelength begins to increase rapidly with increasing NaCl concentration in the region where [NaCl] > 300 mM. In this region of high salt concentrations,

(31) Lévy, R.; Thanh, N. T. K.; Doty, R. C.; Hussain, I.; Nichols, R. J.; Schiffrin, D. J.; Brust, M.; Fernig, D. G. *J. Am. Chem. Soc.* **2004**, *126*, 10076–10084.

(32) Evans, D. F.; Wennerstrom, H. *The Colloidal Domain where Physics, Chemistry, Biology, and Technology Meet*, 2nd ed.; Wiley-VCH: New York, 1999.

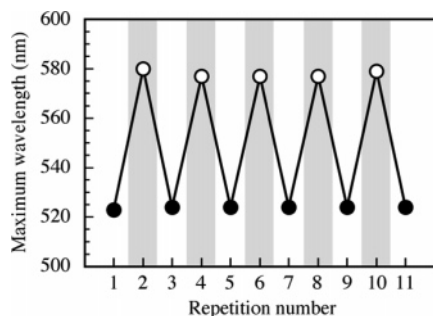


Figure 5. Change in maximum wavelength of surface plasmon absorption band for the PNIPAM-coated gold nanoparticles in 50 mM NaCl aqueous solutions at 25 (●) and 40 °C (○) switched with a 30 min cycle.

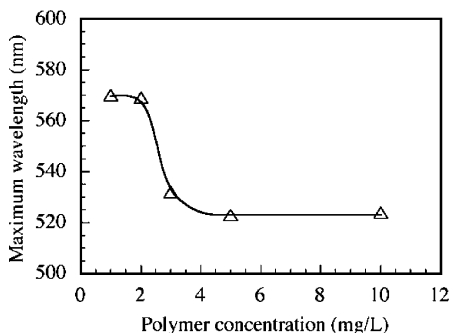


Figure 6. Maximum wavelength of the surface plasmon band of the PNIPAM-coated gold nanoparticles in 150 mM NaCl aqueous solutions as a function of the polymer concentration at 25 °C.

formation of unstable aggregates was observed as a precipitate. Therefore, it can be concluded that the gold nanoparticles are stabilized by coating with PNIPAM when $[\text{NaCl}] \leq 300$ mM at 25 °C. At 40 °C, the maximum wavelengths are practically constant at 523 nm when $[\text{NaCl}] < 1$ mM. However, the maximum wavelength begins to increase at $[\text{NaCl}] > 3$ mM with increasing salt concentration, reaching a value of ca. 580 nm at $[\text{NaCl}] \geq 50$ mM. This red-shifted maximum wavelength is an indication of the interparticle association of the gold nanoparticles. At 40 °C, a temperature higher than LCST of PNIPAM, the interaction between the polymers predominates over the interaction between the polymer and solvent, which leads to the interparticle association. The presence of a certain level of NaCl in water may induce the dehydrated PNIPAM chains to associate, and thereby interparticle aggregation is enhanced.

Figure 5 shows thermoresponsive changes of the absorption maximum for a 50 mM NaCl aqueous solution of PNIPAM-coated gold nanoparticles observed with a temperature cycle between 25 and 40 °C with a 30 min interval. The maximum wavelength changes reversibly between 523 and 580 nm responding to the temperature change, i.e., a pink color at 25 °C changes to a bluish-purple color at 40 °C over many cycles.

All experiments were performed in the presence of excess free polymer chains except for TGA. We studied an effect of polymer concentration (C_p) on the stability of the PNIPAM-coated gold nanoparticles in NaCl aqueous solutions by UV-vis absorption spectroscopy. The maximum wavelength of the surface plasmon band was measured as a function of polymer concentration in the presence of 150 mM NaCl at 25 °C (Figure 6). When $C_p < 5$ mg/L, the maximum wavelengths of the PNIPAM-coated gold nanoparticles were observed at longer wavelengths, suggesting the presence of the association of the PNIPAM-coated gold nanoparticles. When $C_p \geq 5$ mg/L, the red shift was not observed, indicating that the PNIPAM-coated gold nanoparticles are

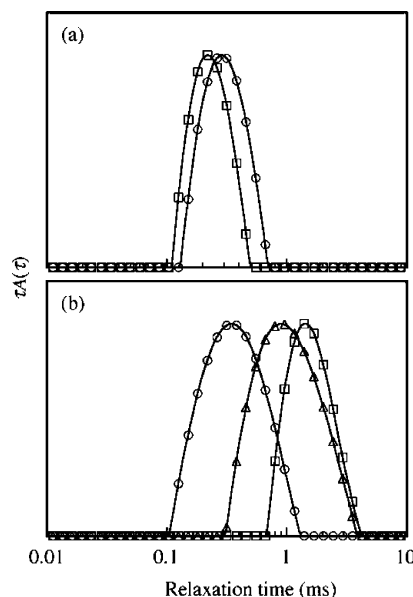


Figure 7. Typical examples of QELS relaxation time distributions for the PNIPAM-coated gold nanoparticles in pure water (a) and 50 mM NaCl aqueous solutions (b) at 25 (○), 32 (△), and 40 °C (□), where the scattering angle is kept at 90°.

dispersed thoroughly at $C_p \geq 5$ mg/L. The maximum wavelength was independent of the polymer concentration in the range 5–10 mg/L at 25 °C. Furthermore, at 40 °C the maximum wavelengths at $C_p = 5$ and 10 mg/L were almost the same, i.e., 581 nm. Therefore, the influence of free PNIPAM chains may be negligible for the stability of the PNIPAM-coated gold nanoparticles in the polymer concentration region from 5 to 10 mg/L.

Quasi-elastic Light Scattering (QELS). The heat-induced interparticle association behavior of the PNIPAM-coated gold nanoparticles was confirmed by QELS measurements at varying temperatures in pure water and in a 50 mM NaCl aqueous solution. It is known that a single molecule of PNIPAM in a dilute solution collapses into a densely packed single-chain globule (i.e., a coil-to-globule transition) upon an increase in temperature above LCST.³³ Figure 7 compares QELS relaxation time distributions for the PNIPAM-coated gold nanoparticles at different temperatures. All relaxation time distributions are unimodal. In pure water (Figure 7a), mean hydrodynamic radii (R_h) at 25 and 40 °C were calculated to be 19.1 and 14.9 nm, respectively. This decrease of R_h at the higher temperature corresponds to a 52.5% decrease of the volume of PNIPAM-coated gold nanoparticles upon a temperature increase from 25 to 40 °C. The contour length of a fully extended PNIPAM chain with a DP_n of 98 is calculated to be ca. 24.5 nm. The R_h value of bare gold nanoparticles was 9.24 nm as determined by QELS. Therefore, the observed R_h value (19.1 nm) at 25 °C was smaller than the R_h value ($24.5 + 9.24 = 33.7$ nm) calculated from the fully extended PNIPAM chain length and radius of bare gold nanoparticles, which implies that interparticle association of PNIPAM-coated gold nanoparticles may not occur in pure water. As the temperature is increased from 25 to 40 °C, the mean R_h value for the PNIPAM-coated gold nanoparticles in pure water decreases, as can be seen from the shift of the relaxation peak toward shorter relaxation times in Figure 8a. This observation suggests that the PNIPAM-coated gold nanoparticles in pure water may not associate to form interparticle aggregates at 40 °C, although the PNIPAM chains surrounding gold nanoparticles collapses into a more compact form due to dehydration at the

(33) Wu, C.; Zhou, S. *Macromolecules* **1995**, *28*, 8381–8387.

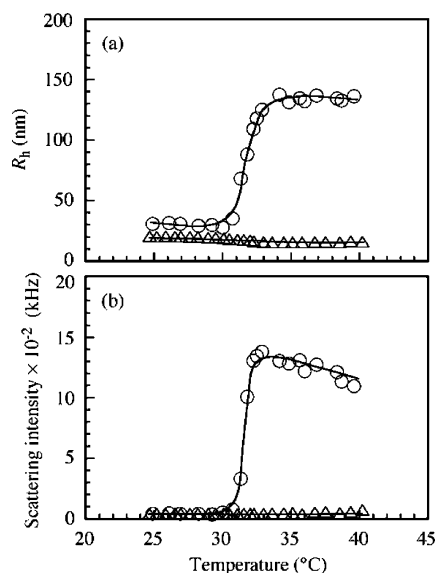


Figure 8. (a) Hydrodynamic radius (R_h) and (b) scattering intensity for the PNIPAM-coated gold nanoparticles in pure water (Δ) and 50 mM NaCl aqueous solutions (\circ) as a function of temperature, where the scattering angle is kept at 90° .

higher temperature. These findings agree well with the observations in UV-vis absorption spectra (Figure 1a). The plasmon band around 522 nm in pure water was scarcely influenced by temperature because PNIPAM-coated gold nanoparticles do not associate to form interparticle aggregates.

In 50 mM NaCl aqueous solutions (Figure 7b), mean R_h values for distributions at 25, 32, and 40 °C were calculated to be 30.9, 88.2, and 137 nm, respectively. The R_h value in the 50 mM NaCl aqueous solution at 25 °C is 30.9 nm, which is larger than that in pure water at 25 °C ($R_h = 19.1$ nm). This finding suggests that several PNIPAM-coated gold nanoparticles associate to form small interparticle aggregates presumably consisting of 2–3 PNIPAM-coated gold nanoparticles in 50 mM NaCl aqueous solutions at 25 °C. It is noteworthy that the mean R_h values in pure water decrease with increasing temperature, whereas the mean R_h values in 50 mM NaCl aqueous solutions increase with increasing temperature. The aggregation number of PNIPAM-coated gold nanoparticles may increase with increasing temperature in the 50 mM NaCl aqueous solution. At high temperatures, the gold nanoparticles come into association due to the enhancement of the polymer–polymer interaction. This observation is in accordance with the observations of UV-vis absorption spectra. The maximum wavelength corresponding to the plasmon band of 523 nm at 25 °C shifts to 580 nm at 40 °C in the 50 mM NaCl aqueous solution, which indicates that the PNIPAM-coated gold nanoparticles associate to form interparticle aggregates at higher temperature.

In Figure 8 are plotted the R_h values for the PNIPAM-coated gold nanoparticles in pure water and in 50 mM NaCl aqueous solutions as a function of the temperature. Below 30 °C the PNIPAM-coated gold nanoparticles in 50 mM NaCl aqueous solutions may form small aggregates with an R_h on the order of 28–31 nm. As the temperature is increased, R_h starts to increase abruptly at a temperature near 30 °C, reaching a maximum value of 131–137 nm at 34 °C. In pure water, the R_h values (19–20 nm) below 30 °C are indicative of a single PNIPAM gold nanoparticle with hydrated PNIPAM chains. When the temperature is increased to 30 °C or higher, the R_h value decreases, reaching a value of 15–16 nm, suggesting that the PNIPAM chains surrounding gold nanoparticles are dehydrated. Tenhu et al.¹³ reported that PNIPAM brushes on gold nanoparticles with a

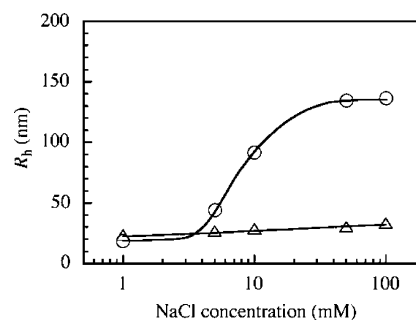


Figure 9. Hydrodynamic radius (R_h) values of the PNIPAM-coated gold nanoparticles in aqueous solutions as a function of the concentration of NaCl at 25 (Δ) and 40 °C (\circ).

high density of ca. 2.4 chains/nm² exhibit two transitions with increasing temperature in aqueous solutions. The PNIPAM chains in the inner zone undergo the first transition at a lower temperature, and the PNIPAM chains in the outer zone show the second transition at a higher temperature. In the case of our PNIPAM-coated gold nanoparticles, a two-step transition was not found in the effect of temperature on the absorbance at 600 nm (Figure 3) and R_h (Figure 8) presumably because the graft density is lower than that reported by Tenhu and co-workers.

Figure 8b plots scattering intensities for the aqueous solutions of the PNIPAM-coated gold nanoparticles against temperature. As the temperature is increased, the scattering intensities in 50 mM NaCl aqueous solutions begin to increase at 30 °C. This temperature agrees well with those at which the R_h values (Figure 8a) and absorbance at 600 nm (Figure 3) start to increase. On the other hand, in pure water, the scattering intensities are almost constant in the whole range of temperatures examined, indicating that no interparticle association occurs.

Figure 9 shows the dependence of R_h of the PNIPAM-coated gold nanoparticles on the concentration of NaCl at 25 and 40 °C. Values of R_h at 25 °C are practically constant at 25–30 nm, indicating that the PNIPAM-coated gold nanoparticles are stable in the whole range of [NaCl] studied. On the other hand, R_h at 40 °C starts to increase abruptly at [NaCl] \geq 5 mM with increasing [NaCl], reaching a maximum value of ca. 137 nm at [NaCl] \geq 50 mM. At [NaCl] > 100 mM, QELS data were unable to be obtained because the sample solution was partially precipitated. The dependence of R_h for PNIPAM-coated gold nanoparticles on the salt concentration agrees well with the relationship between the maximum wavelength and salt concentration (Figure 4). These observations indicate that the presence of NaCl at a certain level in water induces the dehydrated PNIPAM chains to associate between surface-coated gold nanoparticles.

Conclusion

Thermoresponsive PNIPAM with well-controlled structure was prepared by RAFT radical polymerization. The dithioester group at the PNIPAM chain end was cleaved by 2-ethanolamine to give thiol-terminal PNIPAM (PNIPAM-SH). By treating with PNIPAM-SH, gold nanoparticles were coated with PNIPAM. The thermoresponsive changes in color due to the surface plasmon resonance and in the hydrodynamic size of the PNIPAM-coated gold nanoparticles were sensitively influenced by added salt. The color of the coated gold nanoparticles in pure water was pink independent of the temperature. The hydrodynamic size of the PNIPAM-coated gold nanoparticle decreased with increasing temperature because the PNIPAM chain was dehydrated and collapsed on the surface of the gold nanoparticle at temperatures > LCST. Thus, no interparticle association of the PNIPAM-coated gold nanoparticles was observed in pure water. In 50 mM

NaCl aqueous solutions, the pink color of the PNIPAM-coated gold nanoparticle at 25 °C turned to a blue-purple color when heated to 40 °C. This heat-induced color change observed for the salt-containing solutions was completely reversible. In 50 mM NaCl aqueous solutions at 25 °C, the PNIPAM-coated gold nanoparticles were found to form aggregates with $R_h = 30.9$ nm. This size of the PNIPAM-coated gold nanoparticle aggregates started to increase at 30 °C when the temperature was increased, reaching a maximum R_h value of 137 nm at 40 °C. These findings indicate that interparticle association occurs in the salt-containing aqueous solutions and that the size of the aggregate depends on the temperature. The change in the size of the aggregate caused

by changing temperature was completely reversible. Accordingly, the PNIPAM-coated gold nanoparticles in 50 mM NaCl aqueous solutions exhibited a reversible change in color due the surface plasmon resonance responding to the temperature.

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Supporting Information Available: TEM micrograph of bare gold nanoparticles and UV-vis absorption, ^1H NMR, GPC, and TGA traces. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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