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Application of Stimuli-Responsive Nanogels for High-Performance Nanoreactor and Nanoreservoir of Gold Nanoparticles

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Size-controlled synthesis of gold nanoparticles (AuNPs) was successfully achieved through the autoreduction of $HAuCl_4$ using stimuli-responsive PEGylated nanogels, composed of cross-linked polyamine core and tethered PEG chains. Transmission electron microscopy images revealed that the synthesized AuNPs located only in the polyamine gel core of the nanogels. Number of the AuNPs per nanogel decreased with the increase in the N/Au (number of amino groups / number of Au atoms) ratio. Mean diameter of the AuNPs prepared at 60 °C was almost constant (~ 8 nm) regardless of the N/Au ratio, whereas that prepared at 25°C was not controllable. Worth noticing that the mean diameter of the AuNPs prepared at N/Au = 1 decreased with the increase in temperature. We found that the degree of protonation of core-polyamine is far different between 25 °C and 60 °C. This result indicates that shrinking and hydrophobic polyamine gel core at 60 °C may play an important role for the stabilization and size-controlled synthesis of AuNPs. Therefore, stimuli-responsive PEGylated nanogels act as both nanoreactor and nanoreservoir for AuNPs.

Key words: nanogel, gold nanoparticles, size, temperature, stimuli-responsive

1. Introduction

Gold nanoparticles (AuNPs) have received increasing attention in the field of nanomaterials such as optical and electronic devices, catalyst, and biomedical materials due to their unique properties different from those of bulk materials and single atoms. One particular interest in AuNPs is that they show a very intense surface plasmon band (SPB) at around 520 nm, which is useful for biomedical applications such as DNA sensor [1] and immunoassay [2]. Since SPB is known to be extremely sensitive not only to the shape and size, but also to the surroundings of AuNPs [3], coagulation of AuNPs and nonspecific adsorption of biocomponents often decrease in sensitivity and selectivity [4]. The control of the size, shape, organization of AuNPs with non-fouling character is a major challenge in biomedical applications. In this regard, a variety of polymer systems interacting with AuNPs have been studied so far as a means of stabilization, particle size control and organization.

Our group recently reported a simple and effective synthetic approach to the concomitant stabilization and functionalization of AuNPs based on the autoreduction of the chloroaurate ions ($AuCl_4^-$) in the presence of poly(ethylene glycol)-*block*- poly[2-(*N,N*-dimethylamino)ethyl methacrylate] copolymer bearing a reactive acetal group as a bio-tag installation moiety at the PEG end [5]. In the viewpoint of not only excellent stability under physiological conditions and minimal interaction with biological components, but also specific molecular recognition with its acetal end group is superior point of this system compared to the other conventional polymer-stabilized AuNPs systems.

Taking advantage of this system, our group reported a next generation of AuNPs with specified properties and functions using pH-sensitive PEGylated nanogels composed of cross-linked poly[2-(*N,N*-diethylamino) ethyl methacrylate] (PEAMA) core and tethered PEG chains [6]. The PEGylated nanogels showed extremely high dispersion stability as well as reversible volume phase transition (swelling) in response to the pH due to the protonation of the cross-linked PEAMA core. Indeed, the formation of the AuNPs with relatively low size distribution in the PEAMA core of the PEGylated nanogels spontaneously occurred without any additional reducing agents, *viz.* tertiary amino groups in the PEAMA core play a crucial role in the autoreduction of Au(III) ions (nanoreactor) as well as the binding of the AuNPs (nanoreservoir) (Fig.1) [7,8]. The resulting PEGylated nanogels containing AuNPs showed high dispersion stability under physiological conditions as well as the change in SPB in response to pH.

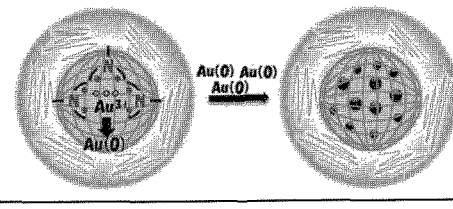

Fig.1 Schematic illustration of nanogel acts as nanoreactor and nanoreservoir.

Worth noticing that the average number of AuNPs in single nanogel ("organization") was controllable by varying the molar ratio of amino groups in the nanogel to Au(III) ions (N/Au ratio). As a specified properties and functions of this system, our group also reported pH-responsive on-off regulation of catalytic activity for reactive oxygen species using pH-responsive PEGylated nanogel containing platinum nanoparticles [9].

Nevertheless, this synthetic method still had a difficulty in the size-control of the AuNPs. A unique finding, which we would like to describe here, is organization- and size-controlled synthesis of AuNPs through an autoreduction of Au(III) ions in the cross-linked PEAMA core of PEGylated nanogels at various temperature. Note that pH-sensitive PEGylated nanogel also shows the volume phase transition of cross-linked PEAMA core in response to temperature [10] *viz,* the synthesis of organization- and size-controlled AuNPs was achieved in the PEAMA core at appropriate temperature. Novel synthetic route of PEGylated AuNPs has a possibility which enables several biomedical applications such as immuno sensor taking advantage of mono-dispersity and high-density of AuNPs within the functionalized nanogel-core.

2. Experimental part

2.1 Materials

Ethylene glycol dimethacrylate (EGDMA; Wako) and 2-(*N,N*-diethylamino)ethyl methacrylate (EAMA; Wako) were distilled over CaH_2 under reduced pressure. Potassium persulfate (KPS; Wako) was purified by recrystallization from water and then dried in vacuo. Tetrachloroauric acid ($HAuCl_4$; Wako) was used without further purification. Water was purified using the Milli-Q system (Millipore).

2.2 Preparation of the stimuli-responsive PEGylated nanogel

The α-acetal-ω-vinylbenzyl-poly(ethylene glycol) macromonomer (Mn = 7,200, Mw/Mn = 1.03) was synthesized as described in our previous report [11]. The vacuum and nitrogen purging cycles of the reactor containing acetal-PEG-vinylbenzyl (0.5 g, 69 μmol) and KPS (29.3 mg, 108 μmol) were repeated three times, followed by the successive addition of deionized-distilled water (33 mL), EGDMA (20.5 μL, 0.11 μmol, 1.0 mol%), and EAMA (2.18 mL, 10.8 mmol). Emulsion copolymerization process was carried out at 4 °C and resulting solution was stirred for 24 h.

2.3 Synthesis of pH-Responsive PEGylated nanogel containing AuNPs

A typical procedure for the nanogel containing AuNPs at N/Au (number of amino groups / number of Au atoms) ratio of 4 at 60 °C is as follows. The $HAuCl_4$ aqueous solution (40 μL, 5 mg/mL, [Au] = 12 mM, pH = 6) was added to the nanogel solution (1.96 mL, 0.23 mg/mL, [N] = 0.98 mM, pH = 6), and the resulting mixture was precisely adjusted to pH = 6 by HClaq or NaOHaq. The reaction mixture was stirred at 60 °C until the intensity of SPB was saturated.

2.4 Characterization of pH-Responsive PEGylated nanogel containing AuNPs

To measure the size of pH-responsive nanogel, dynamic light scattering (DLS) measurements were carried out at each temperature using Zetasizer Nano Series (Nano-ZS, Malvern Instruments Ltd, UK). To determine the *pKa* of the pH-responsive nanogel, the pH-responsive nanogel (6 mg) was dissolved in 40 mL water and adjusted to pH = 3 by HCl, and titrated with 0.01 M NaOH. An automatic titrator (DL-21, METTLER) was used for titration. In this case, the titrant was added in quantities of 0.05 mL after the pH values were stabilized (minimal interval: 30 s). The α/pH curves were determined from the obtained titration curves. To characterize the optical property of the pH-responsive nanogel containing AuNPs, UV-vis spectra were recorded using a Shimadzu UV-2400PC spectrometer with a 1 cm quartz cell. Transmission electron microscope (TEM) samples were prepared by mounting a drop of the solution on carbon-coated Cu grids and allowing them to dry in the air. TEM analysis was carried out using JEOL JEM-100 operating at 100 kV to measure the size of AuNPs. The average diameter and number of AuNPs from TEM images were calculated by ImageJ software. The diameters of approximately 100 particles were used for calculations.

3. Results and discussion

3.1 Synthesis of stimuli-responsive PEGylated nanogel

The stimuli-responsive PEGylated nanogel was prepared at room temperature by emulsion copolymerization of EAMA with acetal-PEG-vinylbenzyl (M_n = 7,200, M_w/M_n = 1.03) in the presence of KPS and EGDMA (1 mol.%) as a cross-linker. The diameter of the obtained nanogel increased proportionally with decreasing in the pH from 7 to 5, reaching to a 8.9-fold larger hydrodynamic volume at pH = 5 (diameter = 180 nm) compared to that at pH = 7 (diameter = 87 nm) (Fig.2a), due to an increase in the ion osmotic pressure and solvation of the PEAMA core caused by the protonation of the tertiary amino groups in the core of the nanogel. Note that volume phase transition point was shifted to lower pH region with increase in the temperature from 25 °C to 60 °C. This tendency is also observed in the α/pH curves as measured in different temperatures (Fig.2b), *viz. pKa* value of the PEGylated nanogel at 60 °C (*pKa* = 4.6) was lower than that at 25 °C (*pKa* = 6.0). This is most likely due to an increase in the mobility of the water molecules. It can be concluded that synthesized PEGylated nanogel is both pH- and temperature-responsive smart nanomaterial.

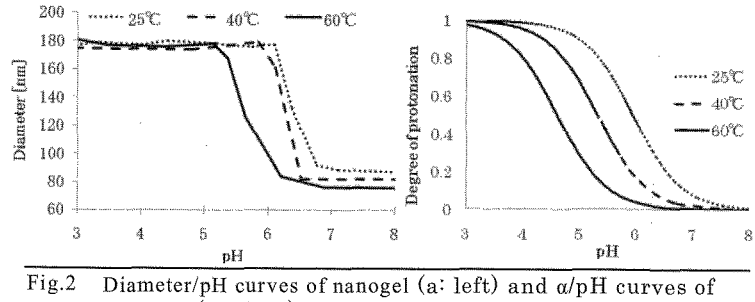

Fig.2 Diameter/pH curves of nanogel (a: left) and α/pH curves of nanogel (b: right) measured under different temperature.

3.2 Temperature-dependent formation of AuNPs within PEGylated nanogel

In our previous work, it was revealed that the PEGylated nanogel composed of PEAMA core and the tethered PEG chains acts as both nanoreactor and nanoreservoir for AuNPs formed from autoreduction of tetrachloroauric acid ($HAuCl_4$) at around pH = 6 [6]. However, aggregation of the AuNPs was observed under low pH reaction conditions (pH = 4) due to the weak coordination of protonated PEAMA core to AuNPs. On the other hand, no formation of the AuNP was observed under high pH reaction conditions (pH = 10), because of the formation of unreducible $Au(OH)_4^-$ species [14] and/or deprotonated, hydrophobic PEAMA core. Thus, temperature-dependent formation of AuNPs in the PEGylated nanogel was studied here at pH = 6.

Figure 3a shows TEM images of PEGylated nanogels containing AuNPs prepared under different temperature (25 °C, 40 °C, and 60 °C) and N/Au ratio (1, 2, 4, 8, and 16). Spherical AuNPs (high contrast) were dispersed in the PEAMA core of the nanogels (~100 nm, low contrast) for all images. The average number of AuNPs in single nanogel decreased with increase in N/Au ratio at each temperature as shown in Fig.3b. For instance, average numbers of AuNPs in single nanogel for N/Au = 1, 4, and 16 at 60 °C were found to be 27, 2, and 1, respectively. However, aggregation of the AuNPs was observed at N/Au = 0.5 (data was not shown), indicating that the N/Au ratio of 1 (stoichiometric ratio) is a limited value to synthesize AuNPs with well-defined structure. It should be noticed that effect of temperature on both the average number and the size of AuNPs was obviously observed at boundary N/Au ratio (= 1), *viz.* the average numbers of AuNPs in nanogel synthesized at 60 °C, 40 °C, and 25 °C were 27, 23, and 15 particles /nanogel. Furthermore, size of the AuNPs synthesized at 60 °C, 40 °C, and 25 °C were 8.0, 8.6, and 11.0 nm. This tendency is most likely due to difference in the size, environment, and/or protonation of PEAMA core (Figs 2a and 2b). Worth noticing is that the mean diameter of AuNPs synthesized at 60 °C was found to be almost constant (~ 8 nm) with narrow size distribution regardless of the N/Au ratio, whereas AuNPs synthesized at 25 °C were not controllable. Indeed, there is another report on the relationships between polymer hydrophobisity and the size of the AuNPs [12]. Sakai et al. suggested that the high hydrophobicity of the poly(propylene oxide) segment is one of the important factor to form the smaller size of stable AuNPs due to its adsorption on the surface on AuNPs [13]. In the case of reaction at 25 °C, the PEAMA core was 50 % protonated and hydrophilic (diameter of the nanogel = 180 nm). On the other hand, PEAMA core was completely deprotonated and hydrophobic (diameter of the nanogels = 100nm) at 60 °C. Thus, such differences in the environment of the PEAMA core may play an important role for the formation of size-controlled AuNPs.

The most interesting point of this autoreduction system based on temperature-induced phase transition of the PEAMA core compared to pH-induced previous autoreduction system is that change in temperature only affects the environment of PEAMA core without change in Au(III) ion species. It is known that $HAuCl_4$ has many Au(III) ion species such as $AuCl_4^-$, $[AuCl_3(OH)]^-$, $[AuCl_2(OH)_2]^-$, $[AuCl(OH)_3]^-$, and $Au(OH)_4^-$, because the ligand exchange process between Cl^- and OH^- occurred as a function of the pH (concentration of OH^- ions) [14]. For instance, although the PEAMA core of the nanogel is hydrophobic at pH > 7 at 25 °C, $HAuCl_4$ preferentially forms $Au(OH)_4^-$ whose redox potential is lower than that of $AuCl_4^-$ [14, 15, 16], leading to the no reduction of Au(III) to Au(0).

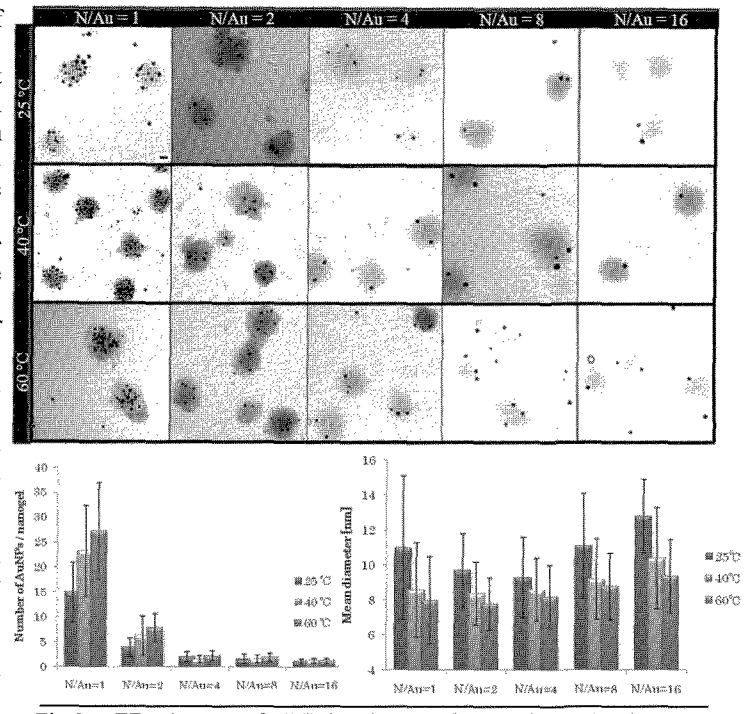

Fig.3 TEM images of PEGylated nanogels containing AuNPs prepared under different temperature (bar: 20nm) (a: top). Analytical results of the average number of AuNPs / nanogel (b: left) and mean diameter of AuNPs (c: left).

3.3 Temperature-dependent time-conversion of Au(III) ions

It is also reported that the rate of conversion of Au(III) ions to Au(0) is one of the important factors on the formation of AuNPs [16]. Toshima et al. reported that clear distinguish between the rapid nucleation process of Au clusters and succeeding growth process of AuNPs is required to obtain the AuNPs with narrow size distribution [17]. As shown in Figure 3, intensity of the SPB of the reaction solution in the presence of nanogel rapidly increased at 60 °C within 1 h even though the hydrophobic core, whereas the SPB was observed after 12 h at 25 °C. This indicates that both nucleation and growth processes in the presence of nanogel at 60 °C are faster than those at 25 °C. To confirm the incorporation of Au(III) ions into the hydrophobic PEAMA core, time-conversion of the Au(III) ions within the PEAMA core of the PEGylated nanogel was also examined as a function of the temperature. The incorporation rate of Au(III) ions into PEAMA core was measured as follows.

The reaction solution was prepared as described in the experimental section (2.3). Then, at a proper reaction time, one portion of the reaction solution was treated with centrifugal ultrafiltration to separate free Au(III) ions and Au-incorporated nanogels. Filtrate was adjusted to pH = 2 using HClaq to form LMCT (Ligand (π)-to-Metal (σ^*) Charge Transfer) complex which has strong absorbance at 313 nm, *viz.* amount of the free Au(III) ions can quantify by the UV spectrum of LMCT band [14].

The incorporation rate of Au(III) ions into hydrophobic PEAMA core of the nanogel at 60 °C (30 min) was faster than that of hydrophilic PEAMA core of the nanogel at 25 °C, indicating that interaction between Au(III) ion and amino group is not only electrostatic interaction but also the coordination of loan pair of amino group to the empty d-orbital of Au. Thus, the driving force for the incorporation of Au(III) ions into hydrophobic and deprotonated PEAMA core may be mainly coordination. Note that overlap of the incorporation process (decrease of LMCT) and growth process (increase of SPB) was observed at 60 °C (Fig.4b), whereas both processes (Fig.4c) were clearly separated at 25 °C. This indicates that the formation of AuNPs is different by reaction temperatures in this system. Considering the general theory that separation between nucleation step and growth step of Au species is lead to monodispersed AuNPs [17], synthesized AuNPs within nanogels under 60 °C unlikely to be monodisperse. However, in this case, obtained AuNPs at 60 °C is comparatively monodisperse (SD = 1.8) than that of 25 °C (SD = 2.3).

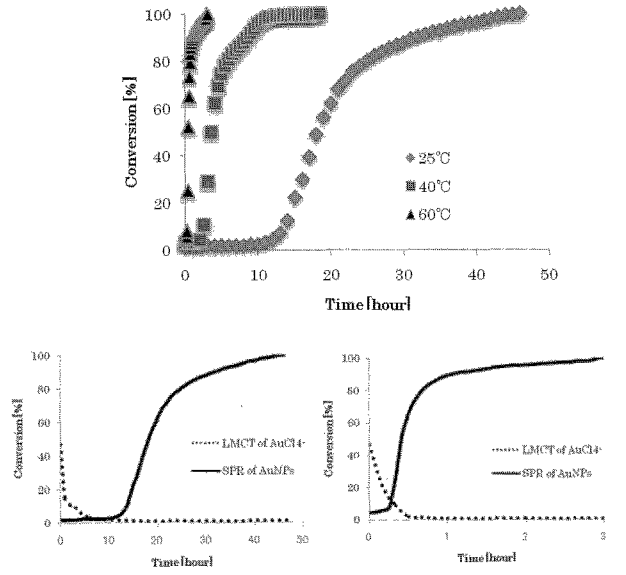

Fig.4 Time course measurement of SPB at 25 °C, 40°C, 60°C (a: top) and LMCT & SPB at 25 °C (b: left), 60°C (c: right).

4. Conclusions

In conclusion, size-controlled synthesis of AuNPs was successfully achieved through the autoreduction of $HAuCl_4$ in PEGylated nanogels at 60 °C, pH = 6. TEM images revealed that synthesized AuNPs located only in the core of the nanogels. The mean diameter of the AuNPs prepared at 60 °C was almost constant (~ 8 nm) regardless of the N/Au ratio, whereas that prepared at 25°C was not controllable. At the most densely N/Au ratio (N/Au = 1), the AuNPs prepared at 60 °C were more smaller-sized (8 nm) and more densely-packed (26 particles / nanogel) than that prepared at 25 °C (11nm, 14 particles / nanogel). Trace of the each reaction stages while Au(III) ions converted to AuNPs in the nanogel core was performed by ultrafiltration and UV-vis spectroscopy. This result indicates that rapid nucleation of Au clusters was occurred in the nanogels which treated with $HAuCl_4$ at 60 °C, and the formed Au clusters were effectively stabilized with highly deprotonated hydrophobic nanogel core. Since this hydrophobicity was realized by temperature-responsive of nanogels, there is no need to consider about the formation of less-reactive $Au(OH)_4^-$ species, in case adjusted its hydrophobisity by NaOHaq. These results indicate that PEGylated nanogels act as both stimuli-responsive nanoreactor and nanoreservoir for AuNPs.

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