PEGylated Polyamine Nanogel as a Nanoreactor of Silica/Gold Hybrid Nanoparticle Preparation

Md. Amran Hossain1, Yutaka Ikeda1,2,3 and Yukio Nagasaki1,2,3,4,5*

1 Graduate School of Pure and Applied Sciences, University of Tsukuba,
2 Tsukuba Research Institute for Interdisciplinary Materials Science, University of Tsukuba,
3 Center for Tsukuba Advanced Research Alliance, University of Tsukuba 305-8573, Japan
4 Master’s School of Medical Sciences, University of Tsukuba
5 Satellite Laboratory of International Center for Materials Nanoarchitectonics, National Institute of Materials Science (NIMS), Ten-noudai 1-1-1, Tsukuba, Ibaraki 305-8573, Japan

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1. Introduction

Nano-sized gold particles attracted considerable attention, because of their novel optical properties from surface plasmon resonance and their various biological and medical applications such as colorimetric biosensors [1-2], drug delivery [3-4], cancer imaging [5-6] and cancer therapies [7-8]. However, the instability in high ionic strength environment and nonspecific adsorption of biomolecules is the main barrier for applying the metallic nanoparticles in biological systems. Various types of low molecular weight stabilizers as well as of water-soluble polymers are often used to improve in their dispersibility and to reduce the nonspecific adsorption under the physiological conditions [9].

We have recently reported one-pot synthesis of pH-responsive PEGylated nanogel containing gold nanoparticles (AuNPs) through an autoreduction of chloroaurate ions within the cross-linked poly[2-(N,N-diethylamino)ethyl methacrylate] (PEAMA) core [10]. The PEGylated nanogel composed of PEAMA core and the tethered PEG chains [11] acts as a nanoreactor as well as stabilizer of the AuNPs formed from autoreduction of tetrachloroauric acid (HAuCl4). The surface plasmon band (SPB) of PEGylated nanogel containing AuNPs was observed at 523 nm and the gold particles were 6 nm in size [10]. If this nanogel template preparation of metal nanoparticle can be extended to other nanoparticle preparations, their applications will be further expanded. To investigate this opportunity, we have tried preparation of silica nanoparticle in the presence of nanogel. As a result, well-size-controlled silica was obtained in the core of nanogel (SiNG). It is interesting to note that silica/gold hybridized nanoparticle could be prepared by the self-reduction of aurate cation in the presence of SiNG. Due to the protonation ability of the cross-linked PEAMA core and the steric stabilization of tethered PEG chains surrounding of the Si/Au nanoparticles, the PEGylated nanogel containing Si/Au nanoparticles showed significant volume phase transition (swelling) in response to acidic pH and excellent stability under physiological conditions.

Figure 1: Schematic representation of the synthesis of PEGylated nanogel containing Si/Au hybrid nanoparticles.
obtained Si/Au nanogel is promising as new nano-based materials.

2. Experimental

2.1. Materials:

Ethylene glycol dimethacrylate (EGDMA; Wako) and 2-(N,N-diethylamino)ethyl methacrylate (EAMA; Wako) were distilled over CaH₂ under reduced pressure. Potassium persulfate (KPS; Wako) was purified by recrystallization from water and then dried in vacuo. Tetraethyl orthosilicate (TEOS; Aldrich) and tetrachloroauric acid (HAuCl₄, Aldrich) were used without further purification. Water was purified using the Milli-Q system (Millipore).

2.2. Synthesis of PEGylated nanogel containing silica nanoparticles (SiNG):

The nanogel was prepared as described [11]; briefly, EAMA was polymerized with EGDMA (3 mol %) by emulsion polymerization in the presence of acetal-PEG-Ph-CH=CH₂ (Mₙ=6400) as emulsifier in addition to co-monomer. KPS was used as initiator. The copolymerization was carried out at room temperature for 24 h. As a typical procedure for the pH-responsive PEGylated nanogel containing SiNPs at N/Si ratio of 4.0, 3.0, 2.0 and 1.5 is described, 42 mg of nanogel in 1 ml H₂O (N = 162.0 µmol) was kept in a glass vial and placed it on a magnetic stirrer at 350 rpm. Added desired amount of TEOS (Si = 40.6, 54.1, 81.2 and 108.3 µmol) and the reaction mixture was stirred for 24 h at room temperature.

2.3. Synthesis of PEGylated nanogel containing Si/Au nanoparticles:

500 µl of prepared SiNG was taken in a glass vial and adjusted at desired pH conditions (pH = 6.0, 7.0 and 9.0). Added 500 µl of HAuCl₄ (6 mg/ml in H₂O) and the resulting was stirred for 24 h at room temperature.

2.4. Characterization:

The size of SiNG and PEGylated nanogel containing Si/Au NPs was evaluated by dynamic light scattering (DLS) on a Zetasizer Nano ZS (Malvern Instruments, Ltd., U.K.) equipped with a 4 mW He-Ne ion laser (λ = 633 nm). The DLS measurements were carried out at 25.0°C at a detection angle of 173°. To determine the amount of the Si immobilized into pH-responsive PEGylated nanogels, inductively coupled plasma atomic emission spectroscopy (ICP-AES) analysis of SiNG was carried out using Nippon Jarrell Ash ICP-P-575 emission spectrometer. To characterize the optical property of the pH-responsive nanogel containing Si/Au nanoparticles, UV-vis spectra were recorded using a Thermo Scientific SkanIt® Software for Varioskan® Flash version 2.4.3 spectrometer. 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 air. TEM analysis was carried out using JEOL JEM-1400 operating at 80 kV.

3. Results and discussion

3.1. Synthesis of PEGylated nanogel containing silica nanoparticles (SiNG):

The PEGylated SiNG was prepared through hydrolysis of TEOS by the polyamine core of the PEGylated nanogel. When TEOS was added to the nanogel, it was dissolved in the polyamine core and hydrolysis reaction occurred by the lone pair electron of tertiary amine group. As a result, the SiNPs formed in the core. Figure 2 shows the pH dependency of the diameter of nanogel and SiNG measured by DLS in water containing 0.15 M NaCl at 25°C; direction angle, 173°.

Figure 2: pH dependency of the diameters of the pH-responsive PEGylated nanogel containing SiNPs (SiNG) and nanogel measured by DLS in water containing 0.15 M NaCl at 25°C; direction angle, 173°. DLS. Both the nanogel and SiNG show the size distribution under swelling at pH < 7.5 and shrinking at pH > 7.5 conditions. The degree of phase transition, V swelling/V shrinking (SiNG-3, 1.9; SiNG-1.5, 1.5) for SiNG is smaller than that of
PEGylated nanogel (V swelling/V shrinking = 2.5). The amount of immobilized Si in SiNG after purification by centrifugation was determined by ICP-AES analysis (Table 1). Note that the amount of immobilized Si increased with the decrease in N/Si ratio (increase of the amount of TEOS) and it seems constant after N/Si ratio 2. This observation suggested the formation of SiNPs in the PEGylated nanogel.

### 3.2. Synthesis of PEGylated nanogel containing Si/Au nanoparticles:

The PEGylated nanogel containing Si/Au nanoparticles were prepared by a simply addition of HAuCl₄ into previously prepared SiNG at various pH conditions. Because of silica nanoparticle should be negatively charged in positively charged nanogel core, the Au (III) ions might access to negatively charged SiNPs under suitable pH conditions. Our idea was that Si/Au hybrid particles should be obtained, if the coordinated Au ion on the silica particle was reduced by surrounding amines. Figure 3 shows the UV-vis spectra of PEGylated nanogel containing Si/Au NPs prepared at various pH conditions. The absorption at 618 nm was observed for the sample prepared at pH 9.0 that might indicate the formation of Si/Au hybrid particles. In contrast, the absorption around 520 nm was observed for the samples prepared at neutral or acidic pH, suggesting the formation of AuNPs by the ion exchange reaction between protonated amino group and the AuCl₄⁻ [10].

Transmission electron microscopy (TEM) images of SiNG and PEGylated nanogel containing Si/Au nanoparticles were prepared by a simply addition of HAuCl₄ into previously prepared SiNG at various pH conditions. Because of silica nanoparticle should be negatively charged in positively charged nanogel core, the Au (III) ions might access to negatively charged SiNPs under suitable pH conditions. Our idea was that Si/Au hybrid particles should be obtained, if the coordinated Au ion on the silica particle was reduced by surrounding amines. Figure 3 shows the UV-vis spectra of PEGylated nanogel containing Si/Au NPs prepared at various pH conditions. The absorption at 618 nm was observed for the sample prepared at pH 9.0 that might indicate the formation of Si/Au hybrid particles. In contrast, the absorption around 520 nm was observed for the samples prepared at neutral or acidic pH, suggesting the formation of AuNPs by the ion exchange reaction between protonated amino group and the AuCl₄⁻ [10].

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size of the smaller and high contrast particles in Figure 4.a and Figure 4.b are about 18 nm and 36 nm respectively. Larger size for Si/Au in nanogel than SiNG might be proof of the preparation of gold surface on Silica particles in nanogel. Figure 5 shows the pH dependency of the diameter of the PEGylated nanogel containing Si/Au NPs measured by DLS. The diameter of the PEGylated nanogel containing Si/Au NPs increased proportionally with a unimodal size distribution (PDI < 0.15) with decreasing pH, reaching to a 1.4-fold larger hydrodynamic volume at pH < 7.5 (diameter = 137.2 nm) compared to that at pH > 7.5 (diameter = 122.4 nm).

4. Conclusion:
The synthesis of pH-responsive PEGylated nanogel containing Si/Au NPs was carried out through a matrix catalyzed hydrolysis of TEOS, followed by addition of HAuCl4 at pH 9.0. The SPB of PEGylated nanogel containing Si/Au NPs was observed in longer wavelength as compared to the SBP observed for PEGylated nanogel containing AuNPs [10]. The prepared PEGylated nanogel containing Si/Au NPs show reversible volume phase transition (swelling) in response to the pH. Therefore, the pH-responsive PEGylated nanogel containing Si/Au NPs may have important applications in biomedical fields.

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6. References: