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One-pot green synthesis of luminescent gold nanoparticles using imidazole derivative of chitosan

Alexander Nazirov[a], Alexander Pestov[a,b], Yuliya Privar[a], Alexander Ustinov[a], Evgeny Modin[a,c], Svetlana Bratskaya[a,*]

[a] Institute of Chemistry, Far East Branch of Russian Academy of Sciences, 159, Prosp. 100-letiya Vladivostoka, Vladivostok 690022, Russia
[b] I. Ya. Postovsky Institute of Organic Synthesis, Ural Branch of Russian Academy of Sciences, 20, S. Kovalevskoy Str., Yekaterinburg 620990, Russia
[c] Far Eastern Federal University, 8, Sukhanova Str., Vladivostok 690950, Russia

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ABSTRACT

Water soluble luminescent gold nanoparticles with average size 2.3 nm were for the first time synthesized by completely green method of Au(III) reduction using chitosan derivative—biocompatible nontoxic *N*-(4-imidazolyl)methylchitosan (IMC) as both reducing and stabilizing agent. Reduction of Au(III) to gold nanoparticles in IMC solution is a slow process, in which coordination power of biopolymer controls both reducing species concentration and gold crystal growth rate. Gold nanoparticles formed in IMC solution do not manifest surface plasmon resonance, but exhibit luminescence at 375 nm under UV light excitation at 230 nm. Due to biological activity of imidazolyl-containing polymers and their ability to bind proteins and drugs, the obtained ultra-small gold nanoparticles can find an application for biomolecules detection, bio-imaging, drug delivery, and catalysis. Very high catalytic activity (as compared to gold nanoparticles obtained by other green methods) was found for Au/IMC nanoparticles in the model reaction of *p*-nitrophenol reduction providing complete conversion of *p*-nitrophenol to *p*-aminophenol within 180–190 s under mild conditions.

1. Introduction

In the recent years, luminescent gold nanoclusters and nanoparticles have attracted substantial researchers attention due to their potential applications in bio-imaging and biomedicine, sensing, and catalysis (Biondi, Laurenczy, & Dyson, 2011; Yarramala, Doshi, & Rao, 2015; Zheng, Zhou, Yu, & Liu, 2012). Size, nature of surface ligands, valence states of gold at the surface and other parameters have a significant influence on the luminescence properties of such particles, which are much less understood in comparison with gold plasmons (Zheng et al., 2012).

Many approaches to fabrication of luminescent gold particles using synthetic (Bao et al., 2007; Biondi et al., 2011) and natural (Xu et al., 2014; Yarramala et al., 2015) polymers as stabilizers and sometimes as reductants (Bao et al., 2007) have been recently reported. Such particles are typically protected with thiolates and other sulfur-containing ligands (Bigioni, Whetten, & Dag, 2000; Liu et al., 2006Liu, Peng, & Yao, 2006; Negishi et al., 2004) providing stability due to very high affinity of sulfur to gold. Another approach to stabilization is an entrapment of small nanoparticles inside box-like cavities of cyclodextrines (Shibu & Pradeep, 2011) or dendrimers (Bao et al., 2007; Zheng, Petty, & Dickson, 2003).

However, there still exist challenging issues in the synthesis of gold nanoclusters and nanopartilces of a size below 3 nm, which do not manifest plasmon resonance (Alvarez et al., 1997). In most cases, ultrasmall gold nanoparticles are obtained using toxic reducing agents, such as $NaBH_4$ (Link et al., 2002; Zheng et al., 2003; Zheng, Zhang, & Dickson, 2004), or organic solvents (Selvam & Chi, 2010) that limits their biomedical applications. Another problem is low yield of gold clusters and nanoparticles with ultrasmall size. Size-tunable Au nanodots represent only one of the fractions of gold nanoparticles obtained in solutions of second and fourth generation OH-terminated poly(amidoamine) via $NaBH_4$ reduction and, therefore, must be separated from larger gold nanoparticles through centrifugation (Zheng et al., 2003, 2004). It is worth mentioning that reduction with ascorbic acid yielded a mixture of nanoclusters of various sizes with green and red emissions, while smaller and narrow sized blue emitting nanoclusters were obtained without any external reductant due to reduction of gold ions, coordinated by tertiary amino groups of dendrimer, with surface hydroxyl groups (Bao et al., 2007). In both cases, much higher yields of gold nanoclus-

* Corresponding author.
E-mail address: sbratska@ich.dvo.ru (S. Bratskaya).

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ters (as compared to $NaBH_4$ reduction (Zheng et al., 2003, 2004) were observed.

It is well known that the nature of reducing agents and, hence, the reduction rate to a great extent determines the size and shape of the metal nanoparticles formed (Hussain, Iqbal, & Mazhar, 2008; Murugadoss & Sakurai, 2011; Shervani & Yamamoto, 2011). Strong reductants such as hydrazine and $NaBH_4$ added in excess often lead to formation of large aggregates due to very high reduction rate and inability of stabilizers to accommodate at the particle surface and form a protective layer (Daniel & Astruc, 2004; Shervani & Yamamoto, 2011). At the same time, due to immediate formation of a large number of nucleation centers in solutions with low concentrations of precursor metal ions and high concentration of stabilizers, very small nanoparticles can be obtained (Murugadoss & Sakurai, 2011; Hussain et al., 2008). So the final size of metal nanoparticles synthesized in polymer solutions is determined by the balance of nucleation and crystal growth rates, which can be controlled via variation of reductant strength and concentration and coordination power of the polymer (Rozenberg & Tenne, 2008; Sarkar, Guibal, Quignard, & SenGupta, 2012; Warshawsky & Upson, 1989).

In green synthesis of gold plasmon nanoparticles and nanodots, the efficient coordination of precursor ion Au(III) at the first stage of the process is extremely important (Bao et al., 2007; Bao, Shen, Liu, & Li, 2013; Pestov, Nazirov, Modin, Mironenko, & Bratskaya, 2015; Vo, Guillon, Dupont, Kowandy, & Coqueret, 2014; Xu et al., 2014; Zheng et al., 2004). Moreover, the balance of coordinating and reducing moieties is a crucial factor that determines the size of gold nanoparticles formed in biopolymer solutions without external reductants (Xu et al., 2014). When reducing moieties are in excess, rapid reduction of Au(III) ions in protein solutions leads to formation of gold plasmons, whereas smaller fluorescent gold nanoparticles can be obtained, if coordinating amino groups are present in protein in sufficient amounts.

Recently, we have shown that reducing species responsible for formation of gold nanoparticles in chitosan solution are generated "in situ" due to hydrolysis of glycosidic bond catalyzed by Au(III) ion (Pestov et al., 2015). Thus, in this system the coordination power of biopolymer controls both reducing species concentration and crystal growth rate, which depends on the presence of free Au(III) ions. Motivated by the fact that efficient complexation and slow reduction rate are possible clues to fabrication of ultrasmall luminescent gold nanoclusters, we modified chitosan with a stronger complexing moiety (methylimidazole) and used the obtained low molecular weight water-soluble polymer for Au(III) reduction. Aside from being strong complexing agents, imidazole-containing polymers coupled with nanoparticles and nanoclusters have very high and still unexplored potential for biomedical and biophysical applications, e.g., detection of biomolecules, bioimaging, and drug delivery due to their biocompatibility (Jana, Patra, Saha, Basiruddin, & Pradhan, 2009) and ability to bind drugs and proteins (Anderson & Long, 2010).

2. Experimental

2.1. Gold nanoparticles preparation

Medium molecular weight chitosan with a deacetylation degree of 84% (^{1}H NMR data) and a molecular weight of $\sim 2.5 \times 10^5$ Da was purchased from JSC "Sonat" (Moscow, Russia). *N*-(4-Imidazolyl)methylchitosan (IMC) was synthesized as described in Pestov, Ezhikova, Kodess, Azarova, and Bratskaya (2014). DS was determined by ^{1}H NMR spectroscopy and was equal to 0.2 (see Supporting information for details).

IMC stock solutions of a concentration of 0.1% were prepared by dissolution of the required amount of polymer in 0.1% acetic acid solution. $H[AuCl_4]$ solution of a concentration of 0.01 mol/l was prepared by dissolution of an appropriate amount of gold foil in aqua regia followed by three cycles of evaporation/addition of concentrated HCl. Stock solution of $H[AuCl_4]$ was added into freshly prepared IMC solutions to obtain Au(III)/IMC(monomer) molar ratios 1:10, 1:20, and 1:40. The mixtures, which are further referred to as Au/IMC with corresponding molar ratio, were permanently stirred and kept at 25 °C for at least 7 days.

2.2. UV–vis and photoluminescence spectroscopy

UV–vis absorption spectra of Au/IMC solutions were recorded using a UV-1650PC spectrophotometer (Shimadzu, Japan). The emission spectra of Au/IMC solutions were recorded using an RF-5301PC spectrofluorophotometer (Shimadzu, Japan) at excitation wavelength of 230 nm. The slit width for the excitation and emission was set to 5 nm.

2.3. ^{1}H NMR spectroscopy

^{1}H NMR spectra of Au/IMC composite (precipitated at pH 10 7 days after mixing IMC and $H[AuCl_4]$ solutions) and of supernatant solution dried at 35 °C under vacuum were recorded on a Bruker AVANCE-500 spectrometer. Samples were dissolved in D_2O/DCl (concentration 10 mg/ml); sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS) was used as an internal standard.

2.4. X-ray photoelectron spectroscopy (XPS)

Investigations of the gold oxidation state in IMC solution were carried out 30 min, 1 day, 3 days, 5 days, and 7 days after $H[AuCl_4]$ addition. Samples for measurements were obtained by dropping Au/IMC solution on quartz wafer and drying at 35 °C under vacuum. XPS spectra were recorded by means of high vacuum photoelectron spectrometer (Specs, Germany) equipped with MgKα X-ray radiation source. All recorded peaks were shifted by the same value to set the C1s peak to 285.0 eV. The high resolution spectra were deconvoluted by means of a computer routine using binding energy, height, full width at half maximum as free parameters of the component peaks.

2.5. Transmission electron microscopy (TEM)

Samples were prepared by dropping Au/IMC solutions after 7 days of reaction with $H[AuCl_4]$ onto the copper grid coated with a Formvar film. TEM images were obtained using a Carl Zeiss Libra 200 FE transmission electron microscope at an accelerating voltage of 200 kV in the bright field mode. To gain the required statistical data, the mode of image matching was used: panoramic images of 4×4 frames of total area of 3.3×3.5 μm were obtained. TEM images processing for calculating the particles number and sizes was carried out using the ImageJ software (Schneider, Rasband, & Eliceiri, 2012). The images contrast was increased by histogram normalization or adjustment. The area of interest was segmented through the image binarisation: overlapping particles were marked out using the watershed segmentation algorithm.

2.6. Catalytic reduction of p-nitrophenol

Catalytic activity of ultra-small gold nanoparticles was investigated as follows: 0.250 ml of Au/IMC 1:20 solution (7 days after mixing) or Au(0)/carboxyethylchitosan (CEC) 1:20 solution containing 60 μg/ml of gold and 1 ml of 1 mM *p*-nitrophenol solution were mixed with 8.75 ml of deionized water, after that 3.8 mg