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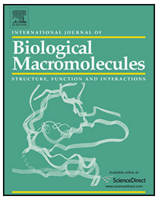

Original Research

pH dependent green synthesis of gold nanoparticles by completely C6-carboxylated curdlan under high temperature and various pH conditions

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ABSTRACT

A C6-carboxylated curdlan (C6-Cc) obtained from 4-acetamido-TEMPO-mediated oxidation of curdlan was used both as a reducing and stabilizing agent for green synthesis of pH-responsive AuNPs, which was carried out by controlling the pH of the C6-Cc solution at a high temperature (100 °C). C6-Cc presented a semi-flexible random coil chain in the aqueous medium at pH 5.5 and became more expanded and rigid in alkaline conditions (pH 7.1–12.0), though the primary chemical structure of C6-Cc was virtually unchanged with the pH variation. The AuNPs prepared with C6-Cc at various pHs were characterized by various instrumental measurements. The shapes and sizes of AuNPs were found to be strongly dependent on the pH of the C6-Cc solution. The C6-Cc-decorated AuNPs exhibited a more well-dispersed spherical morphology with smaller particle sizes under alkaline conditions (pH 7.1–12.0). Through this study, a facile, simple, and green method has been demonstrated for preparation of stimuli-sensitive AuNPs using biocompatible polyanionic polysaccharides.

1. Introduction

During the last two decades, gold nanoparticles (AuNPs), one of the noble metal NPs, have attracted considerable attention worldwide owing to their unique optical, electrical, and photothermal properties, exhibiting diverse applications in catalysis, chemical/biochemical sensing, photonics, biomedicine, and biotechnology [1–5]. Size, shape, and surface morphology play important roles in controlling the optical, physicochemical, and electronic properties of AuNPs [6–9]. Thus, the preparation of AuNPs with controllable size and shape potentially utilized in biomedicine and cosmetics has become the focus of several studies. Currently, a significant number of techniques have been developed for the feasible synthesis of AuNPs, including chemical [4,10], photochemical [3,11], sonochemical [12], and radiolytic [13,14] reduction. Among these, the chemical reduction in solution ("wet chemistry") by using citrate or sodium borohydride as a reducing agent was considered as the most popular method for the preparation of AuNPs. In recent years, to avoid potential environmental and biological risks, considerable effort has been extensively exerted into the development of "green" strategies for the synthesis of size- and shape-controlled AuNPs [15,16].

Polysaccharides (PS) and their derivatives derived from natural resources like plants, animals, and microorganisms possess a broad spectrum of biological activities and multiple functionalities, as well as superior biodegradability, biocompatibility, and nontoxicity [17]. PS, which possess abundant functional groups such as hydroxyl, amino, and carboxyl groups, have been potential candidates as both mildly reducing and stabilizing agents in aqueous medium for the green synthesis of AuNPs. For example, Huang et al. successfully used a polysaccharide of chitosan as the reducing agent and stabilizer to synthesize and stabilize AuNPs [18]. Since then, a number of PS and their derivatives, such as alginate [13], chitosan [14], carboxymethyl cellulose [19], guar gum [20], glucoxylan [21], lentinan [22], and xanthan gum [23], have been potentially devoted for use as mild reducing agents and stabilizers for green synthesis of AuNPs in aqueous medium. Some researchers have especially prepared pH-responsive AuNPs in aqueous solutions by using carboxymethylated (CM) chitosan or CM-cellulose as both reducing and stabilizing agents, and the results indicated that the size and shape of the as-prepared AuNPs are closely dependent on the pH values [19,24]. However, the carboxymethylation of PS usu-

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ally depends on the use of the esterification reagent chloroacetic acid, which is potentially toxic to the environment. Consequently, the search for a novel and greener reducing and stabilizing agent derived from PS and its derivatives to prepare pH-responsive AuNPs constitutes an urgent priority.

Recent studies have reported the regioselective oxidation of the C6 primary hydroxyl groups of curdlan by using 2,2,6,6-tetramethypiperidine-1-oxyl radical (TEMPO) or its analog as a catalyst under mild conditions [25–27]. For example, Watanable et al. has successfully prepared pure polyglucuronic acids containing carboxylate groups exclusively at the C6-positions from curdlan using the 4-acetamido-TEMPO-mediated oxidation system for their accurate characterization and further applications [28]. Meanwhile, Watanable et al. also found that the water-soluble polyglucuronic acid derived from curdlan was shown to undergo obvious depolymerization by β-alkoxy elimination when it was treated in water at 105 °C and at alkaline conditions (pH 7–13) [29]. More interestingly, these curdlan derivatives bearing the β-1,3-polyglucuronic acid structure have the potential to produce gene carriers, bio-nanomaterials, and other chiral nanowires through hydrogen bonding interactions, hydrophobic interactions, and electrostatic attraction [30]. As expected, TEMPO- or 4-acetamido-TEMPO-oxidized curdlan contains numerous hydroxyl and titratable carboxylic groups, facilitating the formation of AuNPs. In this regard, our research group has successfully developed a facile, simple, and green method of preparing AuNPs by using aqueous solution of carboxylic curdlan (Cc), which was prepared by the 4-acetamido-TEMPO-mediated oxidation curdlan, as both reducing and stabilizing agents under different conditions [31]. The results indicated that the size and shape of the as-synthesized AuNPs can be easily tuned by controlling the reaction time and concentrations of Cc and $HAuCl_4$. The spherical AuNPs were well dispersed and showed high stability because the carboxylic groups (COO^-) in the Cc molecules tend to adsorb and stabilize the surface of the AuNPs. However, to the best of our knowledge, no or few studies have been reported on the green synthesis of pH-responsive AuNPs by controlling the pH of Cc solution without any additional reducing or stabilizing agents in the aqueous medium.

In order to better investigate the titratable carboxylic group-induced synthesis of the AuNPs, in the present study, we firstly prepared the completely C6-carboxylated curdlan (C6-Cc) by 4-acetamido-TEMPO-mediated oxidation curdlan at pH 4.8 and 40 °C for 48 h. The effects of pH on the chemical structure and chain conformation of the C6-Cc were determined and evaluated. Subsequently, the C6-Cc was used as the reducing agent and the stabilizer for the green synthesis of pH-dependent AuNPs in an aqueous medium. The particle size, surface morphology, and structure of the as-obtained AuNPs by C6-Cc were characterized by using UV–visible (UV–vis) spectroscopy, transmission electron microscopy (TEM), X-ray diffraction (XRD), and energy-dispersive X-ray spectroscopy (EDX) measurements.

2. Materials and methods

2.1. Materials and chemicals

Commercial curdlan (M_w: 1.1×10^6 g/mol) and 4-acetamido-TEMPO were purchased from Wako Pure Chemical Corporation (Osaka, Japan). Hydrogen tetrachloroaurate (III) tetrahydrate ($HAuCl_4{\cdot}3H_2O$, 99%) was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals and solvents were of laboratory grade and used without further purification. Ultrapure Milli-Q water (18.2 MΩ cm at 25 °C) was used in all experiments.

2.2. Preparation of completely C6-carboxylated curdlan

C6-Cc was prepared according to the reported method with minor modifications [28]. Briefly, 1.0 g of curdlan, 0.096 g of 4-acetamido-TEMPO, and 0.68 g of $NaClO_2$ were added into 100 mL of 0.2 M acetate buffer at pH 4.8 and at 40 °C for 30 min under continuous stirring. Then, 0.62 mL of 12% NaClO solution was added immediately to the resultant mixture to initiate oxidation for 48 h. The oxidation was terminated by adding excess ethanol, centrifuged, dialyzed, and lyophilized to yield the freeze-dried C6-Cc. The carboxyl content of the C6-Cc was determined by electric conductivity titration based on the previous study [32].

2.3. Treatment of C6-Cc under high temperature and different pH values

A C6-Cc aqueous solution (0.2%, w/v) was previously prepared by dissolving 0.2 g of C6-Cc into 100 mL of deionized water and stored at 4 °C overnight for complete hydration. Then, each 20 mL of the C6-Cc solution was filled in a 50-mL centrifuge tube, and the desired pH was adjusted by adding 0.2 M HCl or 0.2 M NaOH solution dropwise. These tubes were capped and immersed in a water bath at 100 °C for 60 min. After treatment, the sample solution was cooled down immediately in an ice-water bath, neutralized, dialyzed, and then lyophilized. These freeze-dried samples were placed in a seal bag and stored in a desiccator before use. The pH value of the C6-Cc solution was measured by a Shanghai Leici digital PHS-2F acidic meter.

2.4. Size-exclusion chromatography with multi-angle laser-light scattering (SEC-MALLS) analysis

The weight-average molecular weight (M_w), number-average molecular weight (M_n), molecular weight distribution (M_w/M_n), z-average radius of gyration (R_g), and chain conformation of the C6-Cc before and after treatment as described above were investigated by SEC–MALLS (DAWN HELEOS II, $\lambda = 658$ nm: Wyatt Technologies Corporation, USA) on an Agilent 1100 system equipped with two SEC columns (OHpak SB–806 M HQ and SB-805 HQ, 8 mm $\Phi \times 30$ cm, Shodex, Japan) in 0.1 M NaCl at 25 °C and a refractive index detector. The online Astra software (Wyatt Technologies, USA) was used for data collection and analysis. The detailed experiment conditions were reported in our previous study [27].

2.5. FT-IR and ^{13}C NMR spectroscopic analyses

The FTIR spectra of C6-Cc samples before and after treatment was performed with a Nexus 670 FTIR spectrometer (Thermo Nicolet Co., USA) in the wavenumber range of 500 cm^{-1} to 4000 cm^{-1} with KBr pellets and referenced against air. For ^{13}C NMR analysis, C6-Cc and its products were dissolved in D_2O. The ^{13}C NMR spectra were obtained with a Bruker AVANCEIII 400 MHz spectrometer (Bruker, Rheinstetten, Germany) at 25 °C. All chemical shifts were expressed in reference to Me_4Si.

2.6. Green synthesis of C6-Cc-capped gold nanoparticles

C6-Cc aqueous solutions (0.2%, w/v) under different pH values (4.5–12.6) were previously prepared by adding 0.2 M HCl or 0.2 M NaOH solution. For the synthesis of AuNPs, 2.0 mL of 2.0 mM $HAuCl_4$ solution was mixed with an equal volume of C6-Cc (0.2%, w/v) solution before and after treatment. Afterward, the resultant mixture was kept at 100 °C under continuous stirring in a water bath for 60 min to obtain C6-Cc-capped gold nanoparticles (C6-Cc-AuNPs).

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