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One-step green synthesis of gold nanoparticles using casein hydrolytic peptides and their anti-cancer assessment using the DU145 cell line

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ABSTRACT

This study demonstrates casein hydrolytic peptide (CHP)-mediated synthesis of crystalline gold nanoparticles (AuNPs). The AuNPs formation was triggered by the addition of an aqueous NaOH solution due to the catalytic properties of OH^- and/or hydration of the functional groups of CHPs. The CHPs were capable of forming a monolayer on the AuNP surface *via* electrostatic interactions, thus playing an important role in long-term stability (12 months). The AuNPs resulted in lower viability in the human prostate cancer cell line as compared to the normal mouse hepatocyte cell line; this property exhibits the potential of AuNPs for applications in cancer therapy.

Introduction

Engineered nanomaterials are at the leading edge of the rapidly developing field of nanotechnology. They are increasingly being used in a variety of commercial applications such as electronics, pharmaceuticals, environmental science, and materials science due to their unique surface, optical, and electronic properties [1,2]. However, the major hindrance in the commercialization of nanoproducts is their toxicity to environmental systems and human health, as reported previously [3,4]. Researchers have demonstrated that different toxicity patterns of nanomaterials could be observed from different synthesis methods, chemical composition, and surface capping [5]. Therefore, the improvement of nanocomposites through their therapeutic preparations using safe, aqueous, and non-expensive manufacturing methods is essential in decreasing their toxicity. In particular, gold nanoparticles (AuNPs) can be synthesized with very precise size, shape, and surface chemistry at the nanoscale level, promising improved safety in therapeutic applications [6]. The synthesis of AuNPs from biological scaffolds, such as amino acids, proteins, and DNA, is being explored to control their formation and surface functionalization on the molecular level [7–9]. Different types of microbial species or dissimilar plant resources are also being employed for the synthesis of metal nanoparticles [10–12]. However, the use of conventional and synthetic peptides is not yet well-established for the synthesis of various types of NPs due to problems pertaining to their precipitation and aggregation [13,14]. Therefore, continual development of an alternative green technique is crucial for obtaining higher AuNP yield and for solving problems related to the aggregation and stability of AuNPs in aqueous media.

All principles of green chemistry can be employed for the improved transformation of Au ions into AuNPs using casein hydrolytic peptides (CHPs) as a renewable bioresource without producing any toxic waste. The CHPs can reduce Au(III) into Au(0) effectively and deliver the long-term stabilization of AuNPs intended for biomedical applications, as reported earlier [15,16]. Previous studies indicated that nanomaterial composition, size, and surface chemistry greatly influence cellular uptake and toxicity in biomedical applications [17]. However, as we noticed in literature, present knowledge about the toxicity induced by AuNPs is still largely incomplete, and thus is of particular interest.

This study investigates the green synthesis of AuNPs using CHPs for the production of high-concentration aqueous dispersion of AuNPs. The proposed methods used water as an environmentally benign solvent and CHPs, a renewable bioresource, as the reducing and capping agents. The alkaline conditions in the reaction system had a significant effect on the kinetics of AuNP production by using CHPs as a strong reducing

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agent *via* their oxidation. Characterization of the obtained AuNPs, including the average core size, morphology, purity, surface capping, crystal structure, and optical properties, was carried out using UV–vis spectroscopy, transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS), and Fourier transform infrared spectroscopy (FT-IR) spectral analysis. Highly dispersed hexagonal-shaped AuNPs were used in *in-vitro* toxicity experiments with both normal and cancer cell lines at different concentration ranges in aqueous media.

1. Experimental

1.1. Materials

Gold chloride (≥99.5%) and casein hydrolytic peptides (CHPs) (≥98.5%) were purchased from Sigma Chemical Co., USA. Dialysis membrane (25-kDa cutoff) was obtained from Spectrum and used after the recommended treatment. Distilled ultrapure water was used as the environmentally benign solvent throughout the preparation and storage of AuNPs. CHPs are prepared in water and used freshly for the formation of AuNPs.

1.2. Casein hydrolytic peptide (CHP) mediated synthesis of AuNPs

The present green synthetic method, based on CHPs to transform Au ions into AuNPs using substantially low concentration of CHPs in aqueous media, is highly reproducible. The CHP-mediated preparation of AuNPs is quite straightforward and useful in green technological methodology. A stock solution of readily soluble CHPs (0.6%) was prepared by gentle dissolving in water and used freshly for the synthesis of AuNPs. In a typical preparation of AuNPs, 3.0 mL of a 0.6% (wt/v) CHP solution and 0.1 mL of NaOH (1 M) were combined in a 15.4 mL aqueous solution (H_2O) and incubated at 60 °C for an hour. Finally, a 1.5 mL aliquot of a 20 mM solution of Au salt was mixed properly, resulting in a total reaction mixture of 20 mL. The final mixture was maintained at 60 °C for 24 to 48 h at a static condition.

1.3. Characterization of AuNPs

To remove the excess free amino acids and unreduced Au ions from the reaction mixture, the final colloidal AuNP solution was dialyzed against deionized ultrapure water for 12 h using the dialysis membrane (25-kDa cutoff). The purified AuNPs were collected, stored in a refrigerator, and used for characterization and cytotoxicity assessment. UV–vis absorption spectroscopy was used to monitor surface plasmon resonance (SPR) and spectral analysis of AuNPs using an Agilent UV-8453 spectrophotometer at 1 nm resolution. The AuNPs obtained by the CHPs were imaged by TEM (Hitachi H-7600 AMT V600). All microscopic samples were prepared by distributing 50 μL of colloidal solution onto a carbon-coated copper grid, followed by drying at room temperature. XPS was carried out to investigate the surface atomic compositions and chemical states of the AuNP thin film. The purified AuNPs were used for FT-IR (Perkin Elmer, Shelton, Connecticut) analysis using a KBR pellet.

1.4. In-vitro *stability of AuNPs*

The *in-vitro* stability of AuNPs up to 12 months was determined by observing the constancy of the SPR band, while AuNP solutions were combined with water and NaCl. In brief, 2 mL of AuNPs was added to a 0.8 mL solution of water and NaCl (10%). Stability with respect to the SPR band was determined by recording the UV–vis absorbance at different intervals for up to 12 months.

1.5. In-vitro *cytotoxicity assay*

An *in-vitro* cytotoxicity test of AuNPs was performed to investigate cellular fate and feasibility with two different cell lines at identical culture conditions. Toxicity and cell count of both normal and cancer cell lines were determined after treatment with different concentration of AuNPs. The AuNPs were tested with increasing concentrations (0.05, 0.1, and 0.5 mM) for both normal mouse hepatocyte (NCTC1469) and human prostate cancer (DU145) cell lines. Cell viability was measured at 48 h post-incubation and calculated with an automated cell counter. Control experiment growth was considered to be 100% viable.

2. Results and discussion

2.1. Green synthesis of AuNPs by CHPs

In the present study, the potential application of CHPs for the production of stable AuNPs in aqueous media was investigated and the AuNPs were employed for cytotoxicity and anti-cancer applications. All principles of green chemistry were employed to exploit safer nanosynthesis of AuNPs using CHPs as a renewable bioresource. In addition, CHPs were modified into alkaline–CHPs, which were responsible for the formation of stable AuNPs. The proposed CHP-based nanosynthesis was one-step and facile; mixing CHPs (0.6% (wt/v)) with $HAuCl_4$ (1.5 mM) and NaOH (0.1 mM) at 60 °C quickly initiated the appearance of a red wine color, indicating the formation of AuNPs. This color change was observed due to excitation of surface plasmon vibrations with AuNPs [18]. UV–vis spectral analysis of the red-colored AuNP solution at different time intervals (6, 12, and 24 h) confirmed the presence of a single, sharp, and characteristic peak at 535 nm, as shown in Fig. 1. The CHPs that were used to facilitate the functional reduction of Au ions under aqueous conditions completely satisfied the fundamental principles of green chemistry as discussed previously [19–21].

Herein, the formation of AuNPs based on CHPs was triggered by the addition of an aqueous NaOH solution and the reaction was completed within 12 to 24 h. The reducing capability of CHPs was likely controlled by carboxylic and amine groups in the presence of an alkaline additive. The NaOH ensures that the amino acids of CHPs are deprotonated, providing the mono-anion form in an aqueous solution, making it a stronger complexing agent to direct crystal growth and increase the productivity as reported previously [22]. In contrast, the reduction reaction progressed very slowly

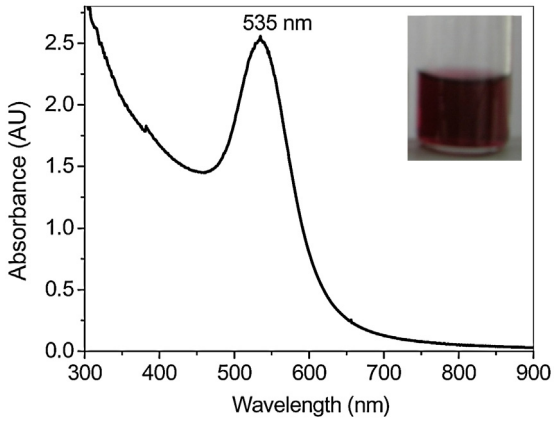

Fig. 1. Absorption spectrum of AuNPs prepared with CHPs. Inset: representative color of a colloidal solution indicating the formation of AuNPs. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)