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Construction, stability, and enhanced antioxidant activity of pectin-decorated selenium nanoparticles

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ABSTRACT

Selenium nanoparticles (SeNPs) as a new replacement source of other Se forms applied in nutritional supplements have been associated with health-related issues. Pectin (PEC) as a well-known food-grade polysaccharide has been considered as a potential soft template for the preparation and stabilization of SeNPs in aqueous medium. In this study, therefore, PEC was used as a stabilizer and dispersing agent to form well-dispersed and stable SeNPs under a simple redox system of selenite and ascorbic acid. Se/PEC ratios significantly affected the color of the suspension, particle size, and surface morphology of the as-prepared SeNPs in the presence of PEC. PEC-SeNPs with a Se/PEC ratio of 1:2 appeared amorphous and exhibited a well-dispersed and stable spherical structure with an average size of ∼41 nm, which corresponds to the strong hydrogen bonding between the hydroxyl groups of PEC and SeNPs. The PEC-SeNPs (Se/PEC = 1:2) remained highly stable in different acidic solutions for at least 1 month. Small and highly stable PEC-SeNPs (Se/PEC = 1:2) possessed the strongest DPPH radical scavenging ability and antioxidant capacity among the evaluated PEC-SeNPs. They also possessed a low cytotoxic activity against cancer cells (SPCA-1 and HeLa) and normal cells (RWPE-1) *in vitro*. These findings suggested that pectin as a surface decorator could be effectively used to improve the stability and antioxidant capacity of SeNPs remarkably.

1. Introduction

Selenium (Se) is an essential trace element in organisms and involved in maintenance of health and growth [1]. The most important function of Se is its antioxidant activity through glutathione peroxidase (GSH-Px) [2,3]. In the presence of a reduced glutathione, GSH-Px can effectively remove harmful peroxides from the body and protect the membrane structure of organisms from damage [4,5]. Se can also stimulate the formation of antibodies for humoral immunity, enhance the number and activity of immune-active cells, and activate immune cytokines, thereby enhancing the body's immunity and exhibiting a remarkable immunomodulatory function [3]. A broad spectrum of biological activities of Se mainly depends on its chemical form and absorbed dose. Inorganic Se and most organic Se compounds are relatively toxic [6,7]. Se has a very narrow margin between the thresholds of functionality and toxicity [8]. Therefore, considerable efforts have been devoted to developing a new Se source exhibiting low toxicity and high efficiency and showing potential for applications in dietary supplements.

Selenium nanoparticles (SeNPs) have been extensively investigated as an optimum replacement source of other Se forms in clinical practices because of their excellent bioavailability, high biological activity, and low cytotoxicity [9,10]. Similar to other NPs, SeNPs possess important chemical, physical, and biological properties and exhibit unique functionalities because of their nanoscale size [11]. Chemical reduction is usually applied to form SeNPs. In the presence of dispersants, Se compounds, including selenate, selenite, and selenium dioxide, are reduced by ascorbic acid to obtain red nano-Se. Particle size and stability play an important role in the functionalities and biological activities of the as-obtained SeNPs [12]. As an antioxidant, SeNPs with sizes ranging from 5 nm to 100 nm elicit scavenging effects on free radicals, especially reactive oxygen species (ROS), and can be used as protective agents against DNA oxidation [13]. During preparation, soft template regulators are often utilized to control NP agglomerations or aggregations, resulting in ideal particle size and morphological characteristics of SeNPs. Many template regulators, such as proteins [14], polyphenols [15], polysaccharides [16,17], monosaccharides [18], and amino acids [19], have been used as a stabilizer and dispersing agent to prepare stable and uniform SeNPs in aqueous media. For example, Zhang et al. prepared water-dispersible SeNPs by using natural hyperbranched polysaccharides (HBP) as a stabilizer and capping agent under safe conditions and found that spherical SeNPs are ligated by HBP to

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form nanocomposites in an aqueous solution. These ligated SeNPs remain stable for over 1 month [17]. In this regard, considerable effort has been devoted to developing SeNPs with new physiological functions and improved bioactivities by selecting biomolecules, especially polysaccharides, as morphological and functional regulators in aqueous solutions.

Pectin (PEC) is a well-known gelling and thickening agent or source of stabilizers for acidic milk drinks, antioxidant fortified foods, and nutraceutical and functional ingredients in processed foods [20]. PEC is mainly composed of an anionic heteropolysaccharide complex derived from chains of linear regions of (1→4)-α-D-galacturonosyl units and their methyl esters but is interrupted by (1→2)-α-L-rhamanopyranosyl units. Given the excellent biocompatibility, abundant availability, and non-toxicity of PEC, it is widely utilized in food, pharmaceutical, and biomedical applications, particularly engineering drug vehicles for oral drug delivery [21]. Coupling PEC with nanomaterials has been explored to create bio-nanocomposites, which are essential for food applications. PEC has been found to be favorably utilized as templates for the green synthesis of NPs such as AgNPs, AuNPs, and ZnONPs, which have a small particle size, good monodispersity, and stability [22–24]. In the presence of abundant hydroxyl, carboxyl, and ester groups along the backbone of a PEC chain, we supposed that stable and well-dispersible SeNPs could be prepared by using PEC as a stabilizer and dispersing agent. However, the formation and stability of SeNPs have yet to be fully explored by using PEC as a stabilizer and dispersing agent in an aqueous medium.

In the present study, PEC was used as a stabilizer and capping agent to fabricate stable and homogeneous SeNPs in aqueous solutions. The particle size, morphological characteristics, structure, and stability in different acidic solutions (pH 3–5) of the as-prepared SeNPs (PEC-SeNPs) were investigated. The *in vitro* antioxidant activities and cytotoxicity of the as-prepared PEC-SeNPs were also evaluated.

2. Materials and methods

2.1. Materials and chemicals

Commercial pectin (PEC) from citrus peel with galacturonic acid (≥74%, dried basis) and methoxyl groups (≥6.7%, dried basis), Sodium selenite (Na_2SeO_3), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), and 2,4,6-tris (2-pyridyl)-s-triazine were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Alamar Blue (AB) was purchased from Bio-Rad Laboratories Inc. (Hercules, CA, USA). Fetal bovine serum (FBS), Dulbecco's modified Eagle medium (DMEM), minimum essential medium (MEM), Defined Keratinocyte-SFM and antibiotics (penicillin 100 units/mL, streptomycin sulfate 100 μg/mL) were supplied by Gibco (Life Sciences Inc., USA). All other chemicals and solvents were of laboratory grade and used without further purification.

2.2. Synthesis of PEC-SeNPs

PEC (1.2 g) was dissolved in a 1.2 L of deionized water under continuous stirring at 25 °C overnight until completely hydration. Different volumes (0.3–15.4 mL) of Na_2SeO_3 solutions (0.1 M) were added to 200 mL of the PEC aqueous solution (1 mg/mL) and stirred at 25 °C for 1 min. Thereafter, 1.0 mL of ascorbic acid solution (0.2 M) was added dropwise into the resulting mixture and stirred at 25 °C for 24 h. The resulting mixtures were dialyzed against deionized water in dialysis bags for 48 h (MWCO: 3500 Da). The dialyzates were lyophilized to obtain red pectin-selenium nanoparticle composites labeled as PEC-SeNPs. The SeNPs without PEC were prepared with the same procedure and used as the control.

2.3. Characterization of PEC-SeNPs

The UV–vis spectroscopy (UV–vis) absorption spectrum of the sample (1.0 mg/mL) was measured by a Varian Cary 100 spectrophotometer (Varian Co., USA) in the wavelength range of 190–600 nm at 25 °C. The morphology and particle size of the PEC-SeNPs at different ratios were observed by using high-resolution transmission electron microscopy (HRTEM, Tecnai 12, Philips, 120 kV). For TEM analysis, a drop of properly diluted sample was placed on a 300 mesh carbon-coated copper grid and air dried at room temperature before examination. The Z-average diameter (particle size) and zeta potential of PEC-SeNPs at different Se/PEC ratios were analyzed by a combined dynamic light scattering (DLS) and particle electrophoresis instrument (Zetasizer Nano ZS, Malvern Instruments, UK). All measurements were performed at least three times with 10 measurements each at 25 °C, and the results given were averaged. Energy-dispersive X-ray spectroscopy (EDX, Noran, Thermo Fisher Scientific, USA) attached to the SEM (S4800, Hitachi Ltd., Japan) was used to obtain the elemental composition and distribution of the PEC-SeNPs. X-ray diffraction (XRD) measurements were performed on an X-ray diffractometer (D8-Advance, Bruker, Germany). The XRD patterns were recorded with Cu Kα radiation at 40 kV and 40 mA in the 2θ ranging from 20° to 90° with a step speed of 4°/min. Fourier-transform infrared (FT-IR) spectra were recorded on a Nexus 670 FT-IR instrument (Thermo Nicolet Co., USA) from 500 to 4000 cm^{-1} with KBr pellets and referenced against air using. Raman spectra were recorded from 50 to 3400 cm^{-1} using a DXR Raman microscope (Thermoelectron Co., USA) equipped with He-Ne laser (532 nm, 10 mW).

2.4. Stability measurement of PEC-SeNPs

The PEC-SeNPs (Se/PEC = 1:2) suspensions (1.0 mg/mL) were prepared by dissolving the freeze-dried PEC-SeNPs products in 0.1 M citrate–sodium citrate buffer solutions (pH 3, 4, and 5) with continuous stirring at room temperature for more than 2 h. Afterward, all of the samples were centrifuged at 8000 rpm for 20 min. The obtained supernatants were subjected to a storage stability experiment. The appearance, particle size, zeta potential and surface morphology of the PEC-SeNPs in different acidic solutions and storage durations were determined through imaging, DLS analysis, and TEM observation.

2.5. In vitro antioxidant activity

In this work, three different assays, namely, the DPPH radical scavenging activity, Trolox equivalent antioxidant capacity (TEAC) assay, and the ferric reducing ability of plasma (FRAP) assay, were employed to evaluate the *in vitro* antioxidant activities of PEC and PEC-SeNPs at different Se/PEC ratios (1:40, 1:10, 1:5, 1:2, 1:1, 4:3). Vitamin C (Vc) was used as the positive control. Details of the operation conditions and methods have been reported previously [25].

2.6. Cell culture and cytotoxicity assay

Human lung adenocarcinoma cancer cell lines (SPCA-1), human cervical cancer cell lines (HeLa), and human prostate normal cells (RWPE-1) were obtained from Cell Resource Center of Shanghai Institute of Life Sciences, Chinese Academy of Sciences (Shanghai, China). SPCA-1 cells were cultured in DMEM supplemented with FBS (100 μg/mL), penicillin (100 units/mL), and streptomycin sulfate (100 μg/mL) at 37 °C and 5% CO_2 in an incubator with a humidified atmosphere. HeLa cells were cultured in MEM supplemented with FBS (100 μg/mL), sodium pyruvate (0.11 g/L), penicillin (100 units/mL), and streptomycin sulfate (100 μg/mL) at 37 °C and 5% CO_2 in an incubator with a humidified atmosphere. RWPE-1 cells were cultured in Defined Keratinocyte-SFM supplemented with FBS (100 μL/mL), penicillin (100 units/mL) and streptomycin sulfate (100 μL/mL) at 37 ℃ and

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