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Electron beam induced water-soluble silk fibroin nanoparticles as a natural antioxidant and reducing agent for a green synthesis of gold nanocolloid

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HIGHLIGHTS

- WSSF-NPs were successfully prepared using electron beam irradiation.
- Electron beam irradiation reduces MW of SF from 250 to 37 kDa.
- WSSF-NPs are spherical with nanoscaled size as small as 40 nm.
- WSSF-NPs show effective antioxidant and reducing power than non-irradiated SF.
- WSSF-NPs display powerful reducing agent for green synthesis of gold nanocolloid.

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ABSTRACT

The research proposes a novel water-soluble silk fibroin nanoparticles (WSSF-NPs) created by electron beam irradiation. In this report, we demonstrate the effects of electron beam irradiation doses ranging from 1 to 30 kGy on the molecular weight (MW), nanostructure formation, antioxidant activity and reducing power of the WSSF-NPs. Electron beam-induced degradation of SF causing MW reduction from 250 to 37 kDa. Chemical characteristic functions of SF still remained after exposing to electron beam. The WSSF-NPs with the MW of 37 kDa exhibited spherical morphology with a nanoscaled size of 40 nm. Antioxidant activities and reducing powers were investigated using 2,2-diphenyl-1-picrylhryl free radical ($DPPH^{\bullet}$) scavenging activity and ferric reducing antioxidant power (FRAP) assays, respectively. The WSSF-NPs showed greater antioxidant activity and reducing power than non-irradiated SF. By increasing their antioxidant and reducing power efficiencies, WSSF-NPs potentially created gold nanocolloid. WSSF-NPs produced by electron beam irradiation would be a great merit for the uses as a natural antioxidant additive and a green reducing agent in biomedical, cosmetic and food applications.

1. Introduction

Silk waste is a part of the cocoons of silkworms (*Bombyx mori*) (Altman et al., 2003) and this by-product is produced in a large amount in Thailand. Although it is unsuitable for silk textile production, the composition of such silk waste is similar to that of good silk (Prommuak et al., 2008). It composes of fibroin core polymer (75–83%) and sericin glue-like protein as a coating (17–25%). Primary structure of silk fibroin (SF) polymers mainly consists of amino acid sequence of $[Gly\text{-}Ala\text{-}Gly\text{-}Ala\text{-}Gly\text{-}Ser]_n$, existing in glycine (Gly, 43%), alanine (Ala, 30%) and serine (Ser, 12%) units (Kojthung et al., 2008; Vepari and Kaplan, 2007). SF forms an arrangement of repetitive protein layers of antiparallel β-sheet microcrystalline. It is a natural protein with a semi crystalline structure providing stiffness and strength. Because of its impressive biocompatibility with human tissue, biodegradability, drug permeability, flexible morphology, good mechanical property and non-toxicity (Vepari and Kaplan, 2007), SF is of great interest to be applied in biomedicine and healthcare materials, such as

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food and cosmetic additive, tissue engineering (Sofia et al., 2001) and drug delivery system (Altman et al., 2003; Xing et al., 2012). SF protein from the *B. mori* silkworm is FDA approved and it has been used in biomedical applications. Particularly, SF has been the most widely developed as scaffold materials and successfully used in wound healing and in tissue engineering of bone, cartilage, tendon and ligament tissues (Vepari and Kaplan., 2007; Ude et al., 2014). Although the chemical structure of SF is similar to collagen, SF exhibits a less inflammatory response than the collagen and poly (lactic acid) (She et al., 2008).

More recently, it has been reported that SF produced from *B. mori* harbor antioxidant and hypolipidemic properties (Ali and Arumugam, 2011; Zhang et al., 2012). Most of the antioxidant molecules commonly contain hydroxyl, –OH (e.g. phenolics), sulfhydryl, –SH (e.g. cysteine and glutathione) or amino, $–NH_2$ groups (e.g. uric acid, spermine and proteins) (Decker, 1998). Due to plenty of hydroxyl, amine and carboxyl groups (Engel, et al., 1987), SF protein is of great interest as a natural antioxidant for applying in food, cosmetic, pharmaceutical and biomedicine (Moure et al., 2001). Although the SF protein provides some antioxidant functions, its inherent drawbacks are non-reactivity and water insolubility because of strong inter- and intra-molecular hydrogen bonding and Vander Waals force as a result of self-assembled anti-parallel β-sheet structure (Murphy and Kaplan, 2009). Molecular weights of SF are various with a light-chain of 25 kDa and a heavy-chain of 325 kDa (Horan et al., 2005). Unlike sericin, SF polymer with higher MW is difficult to dissolve in water and able to dissolve in hot acid and alkaline solution (Vaithanomsat and Punyasawon, 2006). Although some reports have mentioned about the antioxidant properties of SF, to the best our observation, the strategy to study and improve the antioxidant activities of SF polymer has not yet been reported. A few studies have proposed the extraction of antioxidant pigments, i.e., carotenoids (0.7 mg) and flavonoids (5.1 mg) from a gram of yellow Thai silk waste using ethanol extraction (Prommuak et al., 2008).

Up to date, SF has been developed in micro- and nanoparticles for drug delivery applications. The SF nanoparticles have been produced using emulsion-solvent evaporation/extraction, phase separation or coacervation, self-assembly, solvent displacement, rapid expansion of supercritical solution, and spray-drying methods (Wang et al., 2010); however, less known aspects of radiation-induced degradation and nanostructure formation of the SF. Recently, we published a creation of water-soluble chitosan nanoparticles using gamma-irradiation. We proposed its possibility to be a natural antioxidant and reducing agent for a green synthesis of gold nanoplatforms (Pasanphan et al., 2015). Herein, it is a challenge to produce water-soluble SF in nanoscaled particle and enhance its antioxidant activity using irradiation technique. Gamma-irradiation method has been proposed to promote degradation of SF by creating the weakness of peptide bonding in polypeptides and reducing the β-sheet structure in the SF (Kojthung et al., 2008).

Given such fact, there is a strong possibility that the water-soluble SF nanoparticles (WSSF-NPs) are able to be produced using radiation-induced degradation in order to improve the antioxidant activity similar strategy, which has been observed in a polysaccharide, that is chitosan (Pasanphan et al., 2015). Aside from antioxidant improvement, the possibility of the obtained WSSF-NPs to reduce gold ion (Au^{3+}) ion to gold atom (Ag^0) leading to production of gold nanocolloid is also a novel challenge. Since green synthesis of gold nanocolloid is one of the important issues in biomedical applications (Barhatea et al., 2014), the discovery in this work would be a good candidate for such valuable purpose. Therefore, in this report, we study the effect of electron beam irradiation on the MW reduction, antioxidant activity and reducing power of SF. The chemical structure and nanostructural morphology of the irradiated SF were also observed. Furthermore, the ability of the irradiated WSSF-NPs to promote green synthesis of AuNPs was also included in this paper.

2. Experimental methods

2.1. Materials

Silk fibroin (SF) from cocoons of Thai silkworm *B. mori* (variant Nangnoi) was obtained from the Queen Sirikit Department of Sericulture (Saraburi, Thailand). Silver nitrate ($AgNO_3$), Sodium chloride (Na_2CO_3) and Calcium chloride ($CaCl_2$) were bought from Merck (Darmstadt, Germany). Dialysis membrane from Membrane Filtration Products (Texas, USA). Protein molecular weight standards Dual Color, were purchased from Biorad (California, USA). Methanol (CH_3OH), ethanol (C_2H_5OH) and potassium ferricyanide ($K_3Fe(CN)_6$) were bought from Lab Scan, Co., Ltd. (Thailand). 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) was purchased from Tokyo Chemical Industry (TCI) Co., Ltd. (Japan). Trichloroacetic acid (CCl_3COOH) and iron (III) chloride ($FeCl_3$) were obtained from Carlo ErbaReagents (Italy). Gold chloride hydrate ($HAuCl_4$) 99.999% was purchased from Sigma-Aldrich (USA).

2.2. Instruments and equipment

Electron beam irradiation was carried out using an electron beam accelerator (MB20-16 model MEVEX, Canada) with a voltage of 10 MeV and a current of 10 mA. It was supported by Gems Irradiation Center, Thailand Institute of Nuclear Technology (Public Organization). All samples were irradiated with the doses of 1–30 kGy in air under ambient temperature and pressure. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used for molecular weight (MW) determination. Samples were resolved on a 5% stacking gel and 8% resolving gel in comparison to Dual Color (10–250 kDa). Protein bands were visualized by staining with $AgNO_3$ (0.1%w/v) solution and developed in Na_2CO_3 (3% w/v) solution. Fourier transform infrared (FTIR) spectra were recordedina Bruker (USA) Tensor27. FTIR spectrometer, with 32 scans at a resolution of 2 cm^{-1} in a frequency range of 4000–650 cm^{-1}. The morphologies of the dried powder sample on a conductive carbon tape were taken by scanning electron micrographs (SEM) using the QUANTA 450, FET (Netherlands). The nano dimensions of the synthesized WSSF-NPs were investigated by a transmission electron microscope using a Hitachi H-7650 Zero (Japan) TEM at 100 kV. Aqueous sample was diluted to suitable concentrations (5 μg/mL). The samples were vigorous stirred and sonicated before dropping onto a carbon coated copper grid. The samples were dried in a desiccator for 24 h before TEM measurement. UV–vis spectrophotometer was operated from 200 to 900 nm using a 1-cm path length quartz cuvette with a Libra S32 spectrophotometer, Biochrome (UK).

2.3. Preparation of water-soluble silk fibroin nanoparticles (WSSF-NPs)

Cocoons (30 g) were incubated in an autoclave at 121 °C for 30 min. The cocoons were boiled in an aqueous solution of Na_2CO_3 (0.02 M, 1000 mL) at 100 °C for 30 min and boiled in deionized water at 100 °C for 15 min. The sample was dried in an air oven at 60 °C for overnight to obtain degummed cocoons. The degummed cocoons were dissolved in a ternary solvent system of $CaCl_2$:C_2H_5OH:H_2O with a mole ratio of 1:2:8 and heat at 80 °C for 2 h. The fibroin solution was filtrated using a filter paper (11 μm) to obtain a clear solution of fibroin. The solution was poured into cellulose dialysis tubes (MWCO = 12,000–14,000 Da) in deionized