Carbohydrate Polymers 150 (2016) 400–407

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Antioxidant activity of levan coated cerium oxide nanoparticles

Sun-Jung Kim[a,*,2], Bong Hyun Chung[a,b,*,1]

[a] Hazards Monitoring Bionano Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), 125 Gwahak-ro, Yuseong-gu, Daejeon 305-806, Republic of Korea
[b] BioNano Health Guard Research Center, 125 Gwahak-ro, Yuseong-gu, Daejeon 305-806, Republic of Korea

ARTICLE INFO

Article history:
Received 8 April 2016
Accepted 8 May 2016
Available online 17 May 2016

Keywords:
Levan
Cerium oxide
Nanoparticles
Antioxidant
ROS

ABSTRACT

Levan coated cerium oxide nanoparticles (LCNPs) with the enhanced antioxidant activity were successfully synthesized and characterized. Levan and their derivatives are attractive for biomedical applications attributable to their antioxidant, anti-inflammation and anti-tumor properties. LCNPs were synthesized using the one-pot and green synthesis system with levan. For production of nanoparticles, levan plays a role as a stabilizing and reducing agent. Fourier transform infrared spectroscopy (FT-IR) analysis showed that LCNPs successfully synthesized. The morphology and size of nanoparticles were confirmed by transmission electron microscopy (TEM) and dynamic light scattering (DLS). LCNPs have good water solubility and stability. The conjugation of levan with cerium oxide nanoparticles improved antioxidant activity. Moreover the level of ROS was reduced after treatment of LCNPs to H_2O_2 stimulated NIH3T3 cells. These results demonstrate that the LCNPs are useful for applying of treatment of ROS induced diseases.

1. Introduction

Cerium oxide is a lanthanide metal oxide and rare earth element, and it is widely used as a catalyst (Trovarelli, 1996; Wang & Lin, 2004), ultraviolet absorber (Dao, Luu, Nguyen, & Kim, 2011; Zholobak et al., 2011) and biomedical materials because of the redox activities (Hayat, Andreescu, Bulbul, & Andreescu, 2014; Lee et al., 2013; Xu & Qu, 2014). Cerium oxide nanoparticles (nanoceria) can reversibly change from Ce^{3+} to Ce^{4+} ions and form oxygen vacancies, which exhibit redox potentials.

However, naked cerium oxide nanoparticles have poor water solubility, causing various constraints for biological applications. Therefore, many researchers reported that cerium oxide nanoparticles could be well-dispersed in aqueous solution using dextran (Perez, Asati, Nath, & Kaittanis, 2008; Wang, Perez, & Webster, 2013), polyethylenglycol (PEG) (Karakoti et al., 2009; Qi et al., 2012) polyacrylic acid (PAA) (Asati, Santra, Kaittanis, Nath, & Perez, 2009), and similar compounds. The polymer coating of nanomaterials improves the water solubility, stability and biocompatibility. Dextran-coated cerium oxide nanoparticles have a pH-dependent antioxidant property (Perez et al., 2008), PEGylated nanoceria was reported as a radical scavenger (Karakoti et al., 2009), and folic acid-conjugated PAA-coated cerium oxide nanoparticles have oxidase-like activity, which can be used in the modified enzyme-linked immunosorbent assay (ELISA) (Asati et al., 2009). Reactive oxygen species (ROS) have an important role in biological systems, such as its role in cellular proliferation. However, a high ROS concentration in the body causes organ aging, cell destruction and diseases, such as cancer, cardiovascular diseases and immune-related diseases. Therefore, the elimination and reduction of ROS are important, and antioxidant therapies have attracted attention. Several nanoparticles demonstrate antioxidant activity, which reduces and removes ROS *in vivo* and *in vitro* system (Giri et al., 2013; Huang et al., 2010; Stevanović et al., 2014).

Fructans (polyfructose) are fructose-conjugated polymers that contain inulin and levan. Inulin is a beta-2, 1-linked polyfructose, and levan is a beta-2, 6-linked fructose polymer. Specifically, levan possesses amphiphilicity, high biodegradability and biocompatibility (Han, 1990; Kang et al., 2009, ch. 6). Levan can be used in the food, cosmetic and pharmaceutical industries. Moreover, several studies reported the anti-tumor (Yoo, Yoon, Cha, & Lee, 2004) anti-inflammation (Srikanth et al., 2015) and antioxidant activities (Dahech et al., 2013) of levan. Recently, we reported self-assembled levan nanoparticles for breast cancer imaging (Kim, Bae, & Chung, 2015). Levan is expected to be widely used in biomedical applications (Ahmed, Kalla, Uppuluri, & Anbazhagan, 2014; Sezer, Kazak, Öner, & Akbuğa, 2011; Vereyken, Chupin, Demel, Smeekens, & Kruijff, 2001). In this study, levan-coated cerium oxide nanopar-

* Corresponding authors.
E-mail addresses: kimsj@kribb.re.kr (S.-J. Kim), chungbh@kribb.re.kr (B.H. Chung).
[1] Deceased, February 24, 2016.
[2] Current affiliation: BioNano Health Guard Research Center.

http://dx.doi.org/10.1016/j.carbpol.2016.05.021
0144-8617/

ticles (LCNPs) were synthesized using the one-pot system with the coprecipitation method. Levan acted as a reducing and stabilizing agent to produce cerium oxide nanoparticles. The size and distribution of the nanoparticles were measured by transmission electron microscopy (TEM) and dynamic light scattering (DLS). The formation of levan coated cerium oxide nanoparticles confirmed by FT-IR. The lattice and crystalline patterns were also measured using X-ray diffraction (XRD). The antioxidant activity was measured using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and *in vitro* ROS scavenging was observed by 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) fluorescence assay.

2. Materials and methods

2.1. Materials

Levan (Bacterial levan produced by levansucrase, estimated MW < 2000 kDa) was purchased from Realbiotech (Korea). Cerium (III) nitrate, cerium oxide nanoparticles (CNPs), inulin (I2255, from chicory), dextran (D1037, MW 425–575 kDa), ammonium hydroxide solution, methanol, dimethyl sulfoxide (DMSO) and hydrogen peroxide were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Starch (424495000) was obtained from ACROS ORGANICS, and hyaluronic acid (MW 1000 kDa) was purchased from Humedix (Korea). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Alfa Aesar (Heysham, United Kingdom), 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) and 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) were purchased from Sigma-Aldrich. Dulbecco's Modified Eagle Medium (DMEM) was purchased from Gibco BRL (Grand Island, NY, USA).

2.2. Synthesis of levan-coated cerium oxide nanoparticles

Levan-coated cerium oxide nanoparticles (LCNPs) were synthesized using coprecipitation methods. In brief, cerium (III) nitrate (0.25 M, 0.5425 g) was dissolved in distilled water (5 ml); 10% levan was also dissolved in water (10 ml). After these two materials were mixed, the mixture was added to a 30 ml ammonium hydroxide solution. The solution was stirred for 24 h at room temperature and dialyzed for 24 h using a dialysis membrane in distilled water (MWCO 12,000). The levan-coated nanoparticles were centrifuged at 2000 rpm to remove large aggregates for purification. The supernatant solution was lyophilized using a freeze dryer.

2.3. Characterization of nanoparticles

To confirm the levan-coated nanoparticles, a fourier transform infrared analysis (FT-IR, Bruker Optics IF66, Billerica, MA, USA) was performed. The absorbance of nanoparticles was determined using UV–vis spectroscopy (Beckman Coulter, DU-800, Brea, CA, USA). The morphology and size of the nanoparticles were confirmed using transmission electron microscopy (TEM, JEM-2100F (HR), Japan) and dynamic light scattering (DLS, Otsuka Electronics, Osaka, Japan). The crystallinity and diffraction pattern of the nanoparticles were determined by X-ray diffraction (XRD, RIGAKU, D/MAX-2500, Japan).

2.4. H_2O_2 response activity

Levan-coated cerium oxide nanoparticles (1 mg/ml in distilled water) were prepared. Hydrogen peroxide (H_2O_2, 30%) was added to the cerium oxide nanoparticle solution. After 5 min, the transmission of nanoparticles was measured using UV–vis spectroscopy. The autocatalytic activity was evaluated using the transmission of nanoparticles after incubation for 10 days at 37 °C. After 10 days treatment of H_2O_2, transmission of nanoparticles was measured using UV–vis spectroscopy. These steps were repeated 2times (Perez et al., 2008).

2.5. Antioxidant activity

The antioxidant activity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. DPPH was dissolved in methanol (0.1 mM). Naked cerium oxide nanoparticles were purchased from Sigma Aldrich. Other polysaccharide coated cerium oxide nanoparticles were synthesized by same method. The polysaccharide (levan, inulin, dextran, starch and hyaluronic acid) coated cerium oxide nanoparticles and naked cerium oxide nanoparticles were dissolved in distilled water (1 mg/ml). The two solutions were mixed for 30 min at room temperature, and the absorbance (O. D.) was measured using UV–vis spectroscopy at 520 nm. The inhibition rate of the DPPH free radical was calculated as follows (Brand-Williams, Cuvelier, & Berset, 1995).

$$\text{Scavenging effect(\%Inhibition)} = [1 - \{(\text{sample absorbance (in DPPH)}/\text{control absorbance (only DPPH)}\}] \times 100$$

2.6. Cellular cytotoxicity

The cellular cytotoxicity was evaluated using an MTT assay. NIH3T3 cells (1×10^4 cells/well) and HEK293T cells (1×10^4 cells/well) were incubated in 96-well cell culture plates using DMEM medium, which contained 10% FBS and penicillin streptomycin, for 24 h. Then, nanoparticles at various concentrations were added to the medium and incubated with the cells for 24 h. After 24 h, the cells were washed with phosphate-buffered saline (PBS) and given fresh culture medium. Then, 10 μl of MTT solution (5 mg/ml in PBS) was added for 4 h. The cells were washed, and the produced formazan salt was dissolved in dimethyl sulfoxide (DMSO; 100 μl). The absorption was measured at 570 nm using a microplate reader (SpectraMax M2, Molecular Devices). And the cytotoxicity of nanoparticles was caluculated by percentage.

2.7. In vitro *ROS scavenging assay*

The intracellular ROS was detected using the DCFH-DA (2, 7-dichlorodihydrofluorescein diacetate) fluorescence compound. In brief, the NIH3T3 cells (3×10^5 cells/well) were incubated in 6-well plates for 24 h. The cells were incubated with DCFH-DA (40 μM) for 45 min. After being washed, the cells were incubated with levan-coated cerium oxide nanoparticles at various concentrations for 4 h. Then, the cells were detected and lysed on the plates. The lysed cells were centrifuged for 10 min at 13,500 rpm. The fluorescence intensity of the supernatant was measured using excitation at 488 nm and emission at 520 nm using a microplate reader (SpectraMax M2, Molecular Devices). The intracellular ROS was also observed using fluorescence microscopy. The NIH3T3 cells (1×10^4 cells/well) were cultured on the 8-well chamber slides. The cells were incubated with DCFH-DA (40 μM) for 45 min. After being washed, the cells were incubated with various concentrations of levan-coated cerium oxide nanoparticles for 4 h. The cells were mounted and observed using fluorescence microscopy (DAPI and FITC filter).

2.8. Statistical analysis

Several independent experiments were carried out and the data were expressed as the means and standard deviation (SD). Statistical significance was determined by Student's *t*-test.