International Journal of Pharmaceutics 515 (2016) 300–306

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Zein-alginate based oral drug delivery systems: Protection and release of therapeutic proteins

Sungmun Lee, Ph.D.[a,*], Yeu-Chun Kim[b], Ji-Ho Park[c]

[a] Department of Biomedical Engineering, Khalifa University of Science, Technology, and Research, PO Box 127788, Abu Dhabi, United Arab Emirates
[b] Department of Chemical and Biomolecular Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 34141, Republic of Korea
[c] Department of Bio and Brain Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 34141, Republic of Korea

ARTICLE INFO

Article history:
Received 6 June 2016
Received in revised form 22 September 2016
Accepted 9 October 2016
Available online 15 October 2016

Keywords:
Zein
Alginate
Oral drug delivery
Superoxide dismutase

ABSTRACT

Reactive oxygen species (ROS) play an important role in the development of inflammatory bowel diseases. Superoxide dismutase (SOD) has a great therapeutic potential by scavenging superoxide that is one of ROS; however, *in vivo* application is limited especially when it is orally administered. SOD is easily degraded *in vivo* by the harsh conditions of gastrointestinal tract. Here, we design a zein-alginate based oral drug delivery system that protects SOD from the harsh conditions of gastrointestinal tract and releases it in the environment of the small intestine. SOD is encapsulated in zein-alginate nanoparticles (ZAN) *via* a phase separation method. We demonstrate that ZAN protect SOD from the harsh conditions of the stomach or small intestine condition. ZAN (200:40) at the weight ratio of 200 mg zein to 40 mg of alginate releases SOD in a pH dependent manner, and it releases 90.8 ± 1.2% of encapsulated SOD at pH 7.4 in 2 h, while only 11.4 ± 0.4% of SOD was released at pH 1.3. The encapsulation efficiency of SOD in ZAN (200:40) was 62.1 ± 2.0%. SOD in ZAN (200:40) reduced the intracellular ROS level and it saved 88.9 ± 7.5% of Caco-2 cells from the toxic superoxide in 4 hours. Based on the results, zein-alginate based oral drug delivery systems will have numerous applications to drugs that are easily degradable in the harsh conditions of gastrointestinal tract.

1. Introduction

Inflammatory bowel diseases (IBD), such as ulcerative colitis (UC) and Crohn's disease (CD), are chronic, immune-mediated disorders of the gastrointestinal tract (Sartor, 2006; Xavier and Podolsky, 2007). The prevalence of IBD is the highest in industrialized world, including northern Europe (2.2 million people) and North America (1.5 million people) (Ananthakrishnan, 2015). One of key components in risk factors of IBD is oxidative stress caused by reactive oxygen species (ROS) and reactive nitrogen species (Itzkowitz and Yio, 2004; Zhu and Li, 2012). ROS are chemically reactive oxygen compounds such as superoxide, singlet oxygen, hydroxyl radicals, and hydrogen peroxide including free radicals (Apel and Hirt, 2004; Bhattacharyya et al., 2014). ROS can be generated as by-products of intracellular metabolism or extracellularly by cigarette smoking and radiation (Bhattacharyya et al., 2014). High level of superoxide, one of ROS, can damage cells, intestinal mucosal barrier and tissues leading to the symptoms of IBD (Bhattacharyya et al., 2014).

Antioxidant proteins such as superoxide dismutase (SOD) can scavenge superoxide and alleviate the symptoms of IBD (Li and Zhou, 2011; Moura et al., 2015). However, clinical trials with therapeutic proteins have provided little benefits when they are delivered orally. Although the oral route is the most convenient way for the therapeutic administration, proteins are easily degraded in vivo under the harsh conditions of gastrointestinal (GI) tract (Woodley, 1994). In order to enhance the efficacy of therapeutic drugs, it is imperative to develop and design an effective oral drug delivery system.

Current technologies to deliver SOD include liposome-based delivery (Corvo et al., 1999), direct conjugation of folate to SOD (Lee and Murthy, 2007), nanoparticle-mediated delivery (Reddy and Labhasetwar, 2009), and biodegradable microspheres (Lee et al., 2007; Seshadri et al., 2010). These drug delivery systems have shown promise for enhancing the delivery of SOD; however, they need some modification to improve the efficacy of SOD on oral administration. For example, folate-conjugated SOD is a well-

* Corresponding author.
E-mail address: sung.lee@kustar.ac.ae (S. Lee).

http://dx.doi.org/10.1016/j.ijpharm.2016.10.023
0378-5173/

designed drug delivery system targeting activated macrophages; however, the folate conjugation cannot protect SOD from the harsh conditions of GI tract. One of effective oral drug delivery systems is based on prolamin proteins. Prolamin proteins are a group of plant storage proteins that have a high proline content (Shewry and Halford, 2002). Prolamins include gliadin from wheat, hodein from barley, and zein from corn (Shewry and Halford, 2002; Shewry and Tatham, 1990). The first orally effective delivery system of superoxide dismutase is Glisodin® that protects SOD from the harsh conditions (Cloarec et al., 2007). However, Glisodin® is composed of SOD mixed with gliadin that may cause an autoimmune disorder, termed Celiac disease (Briani et al., 2008; Fasano and Catassi, 2001). The disease can damage the inner surface of the small intestine and interfere with nutrient absorption (Chaptal et al., 1957). In order to avoid the autoimmune disorder, a zein-based oral drug delivery system has been developed (Lee et al., 2013). Zein, one of prolamins, can also protect therapeutic proteins, catalase and SOD, from the harsh conditions of GI tract. Although zein can protect therapeutic drugs from GI tract, controlled release of SOD can be enhanced by alginate. Alginate is a water-soluble linear polysaccharide separated from brown seaweed (Leal et al., 2008). Alginates are composed of two alternating monomers, α-L guluronic acid and β-D-mannuronic acid (Leal et al., 2008). Alginates can be cross-linked by divalent cations such as calcium ion, which produces hydrogel beads. Due to the pH sensitivity and low cost, alginate has significant potential for drug delivery applications.

Here, we develop a zein-alginate based oral drug delivery system for SOD. We demonstrate that zein protects SOD from the harsh conditions of GI tract and alginate enhances their enzymatic activities by releasing SOD into the small intestine. Based on their high delivery efficacy, we anticipate that a zein-alginate based drug delivery system will have numerous applications for the treatment of IBD and other inflammatory diseases in the small intestine.

2. Materials and methods

2.1. Preparation of zein-alginate nanoparticles (ZAN) encapsulating superoxide dismutase

The nanoparticles were prepared using a phase separation method. Briefly, zein (200 mg) (Sigma-Aldrich, St. Louis, MO) was dissolved in 1.0 mL of 70% ethanol. Alginate (20, 40, 60, or 80 mg) (Sigma-Aldrich, St. Louis, MO) was dissolved in 2 mL of pH 9.0 bicarbonate buffer solution and the solution was added to zein in ethanol. For encapsulating antioxidant proteins in ZAN, 100 μL of superoxide dismutase (SOD) (2000 U), therapeutic proteins, was added into the above solution. Deionized water (7.0 mL) was added to the zein-alginate solution with SOD while the solution was dispersed by sonication (10 W/cm^2) for 1 min. The resulting particles were isolated by centrifugation (10,000 rpm) for 3 min generating a yellowish solid powder. Particle size and shape were determined by a scanning electron microscopy (SEM) using JEOL JSM-7500 SEM (JEOL, Peabody, MA, USA).

2.2. Fluorescein isothiocyanate (FITC) labeling of SOD

SOD was labeled with fluorecein isothiocyanate (FITC) for the encapsulation of SOD in ZAN and the release of SOD from ZAN *in vitro*. Briefly, SOD was dissolved in pH 9.0 buffer solution (100 mM, carbonate buffer) and 50 μL of 10 mg/mL FITC in DMSO was mixed with SOD solutions for 2 h at room temperature. The FITC labeled SOD were separated from unreacted FITC using a PD-10 desalting column equilibrated in PBS (pH 7.4).

2.3. Encapsulation of SOD in ZAN

FITC-labeled SOD (FITC-SOD) was encapsulated in ZAN with 200 mg zein and various amounts of alginate (0 mg, 10 mg, 20 mg, 40 mg, 60 mg, and 80 mg). FITC-SOD in ZAN were separated from the aqueous solution by ultracentrifugation at 10,000 rpm for 3 min. The amount of SOD encapsulated in the nanoparticles was determined by the amount of SOD in supernatant, which is the difference between the total amount of SOD used to prepare the nanoparticles and the amount of SOD present in the aqueous solution.

2.4. The release of SOD from ZAN in vitro

The release of SOD from ZAN was evaluated using FITC-labeled SOD. FITC-SOD-loaded ZAN (2.0 mg) were suspended in either pH 1.3 or pH 7.4 buffer solutions (1.0 mL). For statistical analysis, 3 independent samples per group were prepared. The suspensions were kept at 37 °C under gentle shaking. At specific time points, the suspensions were centrifuged at 10,000 rpm for 2 min and the fluorescence of supernatant was then analyzed with an Infinite® 200 Pro microplate reader (Tecan Trading AG, Switzerland) ($\lambda_{ex}/\lambda_{em}$ = 488/520 nm). The pellets were re-suspended with fresh buffer solutions (1.0 mL) and the procedure was repeated for each time point.

2.5. Activities of SOD encapsulated in ZAN

The activity of SOD encapsulated in ZAN was determined by scavenging superoxide from potassium superoxide (KO_2). KO_2 (1.0 mg) was dissolved in 1 mL of anhydrous dimethyl sulfoxide (DMSO) by sonication (10 W/cm^2) for 1 min. As shown in Fig. S1, sonication enhances the superoxide concentration from potassium superoxide (KO_2). The insoluble KO_2 was removed by centrifugation at 10,000 rpm for 3 min. Either free SOD (20 U) or equivalent SOD amount in ZAN was dispersed in 160 μL of deionized water. SOD was mixed with 20 μL of KO_2 solution and 20 μL of 50 μm dihydroethidium (DHE) (Invitrogen, Carlsbad, CA). The activities of SOD were determined by the fluorescence of DHE ($\lambda_{ex}/\lambda_{em}$ = 518/605 nm) using an Infinite® 200 Pro microplate reader. Empty nanoparticles of each ZAN were used as control groups.

2.6. pH stability of SOD in ZAN

The ability of ZAN to protect and release SOD in GI conditions was investigated. Either SOD (20 U) in ZAN or free SOD (20 U) was placed in hydrochloric acid (pH 1.3 with 349.1 μg/mL pepsin) for 20 min to mimic the stomach condition or pH 7.4 with 143.0 μg/mL trypsin for 30 min to mimic the intestine condition. SOD activities of both SOD in the nanoparticles and SOD released from the nanoparticles were measured together by scavenging superoxide from KO_2 in anhydrous DMSO using DHE.

2.7. Cell culture and cytotoxicity using MTT reduction assay

Caco-2 cells (ATCC, Manassas, VA), human epithelial colorectal adenocarcinoma cells, were grown at 37 °C under a humidified atmosphere of 5% CO_2 in Eagle's Minimum Essential Medium (Hyclone, Logan, UT) containing 20% (v/v) FBS (Hyclone, Logan, UT). MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction assay was performed to measure the cytotoxicity of superoxide. The Caco-2 cells (2×10^4 cells/well, 96 well plate) in 190 μL medium were treated with 10 μL of KO_2 in DMSO in the presence or absence of either free SOD (20 U) or equivalent SOD amount in ZAN. At specific time points, cell toxicity or cell viability was measured by MTT test. MTT solution (20 μL)