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Entrapment and delivery of α-tocopherol by a self-assembled, alginate-conjugated prodrug nanostructure

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ABSTRACT

The aim of the study was to deliver α-tocopherol by a combination of prodrug and entrapment techniques, and to evaluate the ability of entrapped α-tocopherol to inhibit lipid oxidation in an oil-in-water emulsion. The amphiphilic alginate-α-tocopherol conjugate was synthesized via esterification reaction and confirmed by IR, ^{1}H NMR, and fluorescence spectroscopy as well as esterase hydrolysis. The bound α-tocopherol in the conjugate was 20.6 μmol/g. The conjugate self-assembled into nanostructures in water, with a critical association concentration of 0.076 g/L. The percent entrapment and loading efficiency of α-tocopherol in the self-assembled nanostructures of the conjugate were, respectively, 4.92–16.82% and 78.83–92.32%, depending on the initial loading of α-tocopherol. Both blank and α-tocopherol-loaded nanostructures presented a negative zeta potential ranging from −50 to −65 mV and exhibited spherical shape with particle sizes in the range of 190–253 nm. More than 40% of α-tocopherol was retained after 216 h (i.e. 9 days) in phosphate buffer solutions (pH 7.4). The release of α-tocopherol was faster for nanostructures with lower α-tocopherol theoretical loading. Based on the thiobarbituric acid reactive substances assay for lipid oxidation, the blank nanostructures of the conjugate did not show antioxidant activity while the α-tocopherol-loaded nanostructures protected lipids against oxidation better than free α-tocopherol entrapped in the oil phase or in Tween 20 micelles. The results indicated that the newly prepared α-tocopherol-loaded nanostructure is a promising platform for solubilizing and delivering antioxidant activity of α-tocopherol in aqueous dispersions.

1. Introduction

Antioxidants and free radical scavengers have been proposed as protective or therapeutic agents against reactive oxygen species-mediated injuries (Rayner, Bottle, Gole, Ward, & Barnett, 2016). However, the application of antioxidants is limited by factors including low solubility for hydrophobic antioxidants, poor stability, bioavailability and targeted specificity (Liu et al., 2016), together with the side effects when used at high levels (Carocho & Ferreira, 2013). In recent decades, novel delivery systems have been developed to overcome these limitations, in an effort to achieve specific or targeted functionality (Hood, Simone, Wattamwar, Dziubla, & Muzykantov, 2011; Sandhir, Yadav, Sunkaria, & Singhal, 2015). Since most of the biological processes occur at the nanoscale, nanoparticulate technology has been considered as a promising approach to improve the bioavailability and *bioefficacy* of antioxidants. Results showed that nanodelivery systems including nanoemulsions (Li, Hwang, Chen, & Park, 2016), liposomes, solid lipid nanoparticles (Pandita, Kumar, Poonia, & Lather, 2014), nanostructured lipid carriers, micelles (Ye, Lei, Wang, & Zhao, 2017) and polymeric nanoparticles (Alqahtani et al., 2015; Astete, Sabliov, Watanabe, & Biris, 2009; Huang et al., 2016; Kamil et al., 2016; Murugeshu, Astete, Leonardi, Morgan, & Sabliov, 2011) can increase water solubility, thermal stability, and oral bioavailability of antioxidants, protect them from degradation in the body and prolong their circulation time. Moreover, some antioxidant nanoparticulates exhibit higher differential uptake efficiency in the targeted cells over normal cells, enhanced permeation and retention effect in diseased tissues (Kamil et al., 2016; Sandhir et al., 2015).

The loading of antioxidants onto nanoparticulates can be

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achieved by physical entrapment (Astete et al., 2009; Huang et al., 2016), chemical conjugation (Lee et al., 2009; Nie et al., 2007; Sanna et al., 2014; Williams, Lepene, Thatcher, & Long, 2008) or combining both methods (Astete, Dolliver, Whaley, Khachatryan, & Sabliov, 2011). Antioxidants can be located in the inner core (Huang et al., 2016), or just attached on the surface (Astete et al., 2011; Nie et al., 2007), depending on the methods used and exhibit enhanced functionality. For example, trolox-functionalized gold nanoparticles showed enhanced antioxidant activity, which was eight times greater toward 2,2-diphenyl-1-picrylhydrazyl radicals than that of Trolox (a water soluble vitamin E analogue) in solution (Nie et al., 2007). Enhanced antioxidant properties were detected for nanogold based conjugates of polyphenols (epigallocatechin-3-gallate, resveratrol, and fisetin), which was correlated well with the polyphenols content in nanogold (Sanna et al., 2014). Similarly, for organic nanoparticles, poly(ethylene glycol)-glutathione conjugate self-assembled into uniform nanoparticles with sizes of 283 nm suitable for efficient antioxidant delivery (Williams et al., 2008). Ascorbic acid conjugated α-tocopherol reported by Astete et al. (2011) showed both surfactant and antioxidant activities, which was employed to stabilize poly(lactic-*co*-glycolic) acid nanoparticles and confer antioxidant properties to the nanoparticles as well.

Alpha-tocopherol, one of the eight isoforms of vitamin E, has been considered the most active isoform with regard to antioxidant as well as biological activities (Tucker & Townsend, 2005). However, like other lipophilic nutraceuticals, the use of α-tocopherol for supplementation of food and beverages is mainly hindered because of its insolubility in water, biological instability and variable bioavailability (Cheong, Tan, Man, & Misran, 2008; Sabliov et al., 2009). To address these challenges, the development of delivery systems for α-tocopherol has gained attention.

Nanoparticles made from synthetic polymers such as poly(D, L-lactide-*co*-glycolide) (Zigoneanu, Astete, & Sabliov, 2008) and poly ε-caprolactone (Byun et al., 2011) or biopolymers such as gliadin (Duclairoir, Orecchioni, Depraetere, & Nakache, 2002) and zein (Luo, Zhang, Whent, Yu, & Wang, 2011) have been considered as promising nanocarriers for delivery and controlled release of entrapped α-tocopherol. The next generation, pro-drug delivery systems involved α-tocopherol conjugation to polymers followed by self-assembly of the conjugated polymer into nanodelivery systems. Quiñones et al. (2013) reported that α-tocopheryl monoesters chemically conjugated to soluble chitosan derivatives (O6-succinylated chitosan and glycol chitosan) self-assembled into nanoparticles to sustain the release of α-tocopherol. It is worthy to note that α-tocopherol conjugated polysaccharides are able to self-assemble into nanoparticulate systems, which are considered as effective nanocarriers for delivery of hydrophobic bioactives (Jena & Sangamwar, 2016; Liang et al., 2012; Mandracchia, Tripodo, Latrofa, & Dorati, 2014). To the best of our knowledge, α-tocopherol derivatives (α-tocopheryl polyethylene glycol 1000 succinate or α-tocopheryl succinate) rather than α-tocopherol had been mainly employed to synthesize α-tocopherol-conjugated amphiphilic polymers with spacer arm lengths. Herein, an amphiphilic alginate-α-tocopherol conjugate prodrug was designed as a nanocarrier for delivery of α-tocopherol as shown in Fig. 1. The conjugate prodrug was constructed by attaching hydrophobic α-tocopherol to the alginic acid chains (Fig. 1A). The conjugate prodrug readily formed stable nanostructures in aqueous phase and was used to entrap free α-tocopherol (Fig. 1B). The release profiles and the antioxidant activity of the prepared α-tocopherol-loaded nanostructures were examined to assess the ability of the novel pro-drug, nanodelivery system to improve functionality of α-tocopherol.

2. Materials and methods

2.1. Materials

Alginic acid from brown algae (average molecular weight of 240 kDa, approximately 61% mannuronic acid and 39% guluronic acid), (±)-α-tocopherol (≥96% by HPLC), 3-dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), dimethyl sulfoxide (DMSO), dimethylformamide (DMF), and 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) and pyrene were purchased from Sigma–Aldrich Corporation (St Louis, MO, USA). All other reagents and solvents were of analytical grade and used as received.

2.2. Conjugation of α-tocopherol with alginic acid

Alpha-tocopherol was conjugated to alginic acid through the DCC/DMAP coupling reaction as illustrated in Fig. 1. Alginic acid (500 mg) was suspended in anhydrous DMSO (30 mL). DCC (170 mg) and DMAP (100 mg) were dissolved in 10 mL of anhydrous DMSO separately prior to introducing into the suspension. Under nitrogen atmosphere, α-tocopherol (430 mg diluted by 10 mL DMF) was dripped into the reaction flask after the 2 h activation of the carboxyl group of alginic acid. The reaction was carried out at room temperature (21 °C) for 36 h under gentle magnetic stirring and then 0.200 mL of triethylamine was added to neutralize the carboxyl group of alginic acid. The reaction suspension was then dialyzed for one day against anhydrous ethanol followed by three days dialysis against deionized water using a dialysis membrane of molecular weight cut-off of 12–14 kDa to remove the unreacted molecules. Finally, alginate-α-tocopherol conjugate was lyophilized (Labconco, MO, USA) and stored in refrigerator for further studies.

2.3. Characterization of alginate-α-tocopherol conjugate

2.3.1. Determination of bound α-tocopherol on alginate-α-tocopherol conjugate

In this study, the amount of bound α-tocopherol on alginate-α-tocopherol conjugate refers to the degree of substitution (DS), which is an important parameter reflecting the reaction efficiency. In order to determine the bound α-tocopherol, the sample (5 mg) was dissolved in 0.250 mL of PBS buffer (10 mmol/L, pH 7.0). 0.020 mL of the aqueous solution of esterase from porcine liver (6 mg/mL) was added into the alginate-α-tocopherol conjugate solution (20 mg/mL). The released α-tocopherol was extracted by chloroform and then subjected to HPLC analysis. The amount of bound α-tocopherol on alginate-α-tocopherol conjugate was calculated in accordance with the standard curve. DS was defined as the number of tocopheryl groups per one thousand repeating monosaccharide units of alginic acid.

2.3.2. ^1H NMR analysis of alginate-α-tocopherol conjugate

^1H NMR spectra of alginic acid and alginate-α-tocopherol conjugate were measured in D_2O at room temperature (21 °C) by a Bruker AV 600 MHz NMR spectrometer (Bruker, Karlsruhe, Germany).

2.3.3. Determination of critical association concentration

The critical association concentration (CAC) is the concentration at which the amphiphilic polymer chains self-assemble to form nanostructures. The CAC of prepared alginate-α-tocopherol conjugate nanostructure was estimated by fluorescence spectroscopy using pyrene as a fluorescent probe (Ray, Chakraborty, & Moulik, 2006). Briefly, a stock solution (1×10^{-3} mol/L) of pyrene was prepared in ethanol. The alginate-α-tocopherol conjugate aqueous solutions with different final concentrations (5×10^{-4} to 1 g/L)