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Controlled release of alpha-lipoic acid through incorporation into natural polysaccharide-based gel beads

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ABSTRACT

Alpha-lipoic acid (ALA) is an essential cofactor for mitochondrial multi-enzyme complexes related to energy production. However, it is unstable under light and heat and its biological half-life ($t_{1/2}$) is short. In this study, ALA was incorporated into alginic acid (Alg) gel beads to achieve stabilisation and sustained release. The cationic polymer chitosan (CS) was also added to the Alg gel beads to better control ALA release. In artificial gastric juice (pH 1.2), ALA release from the Alg gel beads was controlled through simple diffusion from the gel matrix. However, in artificial intestinal juice (pH 6.8), the Alg gel beads were immediately disintegrated. On the other hand, Alg gel beads with CS were not disintegrated in artificial intestinal juice (pH 6.8), and achieved pH-independent sustained release of ALA through an electrostatic interaction between the carboxyl groups of Alg and the amino groups of CS. ALA contained within the Alg gel beads was protected from light (UV) and heat (65 °C) compared with ALA alone. Addition of CS to the Alg gel beads further increased the stability of ALA. Thus, stabilisation and controlled release of ALA was achieved through incorporation of ALA in Alg gel beads containing CS.

1. Introduction

Oxidative stress is considered to be one of the primary causal factors for ageing and various diseases, such as arteriosclerosis, cardiovascular disease, cerebral diseases, diabetes, inflammatory diseases, and cancer (Kunitomo, 1997; Ochi & Sakai, 2003). It is thought that scavenging of reactive oxygen species (ROS) is important for prevention of these diseases.

Alpha-lipoic acid (ALA) or 1,2-dithiolane-3-pentanoic acid, also referred to as 6,8-thioctic acid, is an essential cofactor for mitochondrial multi-enzyme complexes related to energy production. ALA has been recognised as a powerful antioxidant (Biewenga, Haenen, & Bast, 1997; Li, Zhao, Yu, & Jiang, 2004; Packer, Witt, & Tritschler, 1995). Unlike other antioxidants, ALA is both fat- and water-soluble and is easily absorbed and transported across cell membranes. Therefore, it is widely used as a drug for prevention of various chronic diseases associated with oxidative stress, and is administered as a daily supplement for dietary purposes, anti-ageing, diabetes, and cardiovascular disease (Hagen et al., 1999; Jacob et al., 1999; Maritim, Sanders, & Watkins, 2003; Smith, Shenvi, Widlansky, Suh, & Hagen, 2004; Wollin & Jones, 2003). However, ALA is unstable under light and heat and gradually decomposes at room temperature. Temperatures greater than its melting point (59–62 °C) cause immediate polymerisation and render it unusable, as polymerised ALA is insoluble in almost all solvents (Wagner et al., 1956). Furthermore, the decomposition of ALA is accompanied by an unpleasant odour due the sulphur it contains. The short biological half-life ($t_{1/2}$) of ALA (about 30 min) may also inhibit effective medical use (Teichert, Kern, Tritschler, Ulrich, & Preiβ, 1998).

For controlled delivery and stabilisation of a drug, incorporation of a drug into polymer matrix is one of the useful devices. The drug release from a typical polymer matrix is controlled by three primary mechanisms; diffusion, disintegration, and swelling followed by diffusion (Brannon-Peppas, 1997).

Alginic acid (Alg) is a copolymer of D-mannuronic acid and L-guluronic acid. Chitosan (CS), which obtained by deacetylation of chitin, is a copolymer of D-glucosamine and N-acetyl-D-glucosamine. They are abundant natural mucopolysaccharides that are nontoxic and biocompatible (Tønnesen & Karlsen, 2002; Paul & Sharma, 2000; Mi, Tan, Liang, & Sung, 2002), and have therefore been used as materials of a vehicle for drug delivery. In particular, CS is a cationic polymer; it interacts with an anionic material. The formation of CS–Alg complexes is a useful technique for preparing a vehicle for controlling drug release (Murata, Maeda, Miyamoto, & Kawashima, 1993). The amino groups in the CS and the carboxyl groups in the ALA also form an electrostatic complex, which stabilizes the ALA (Kofuji, Nakamura, Isobe, Murata, & Kawashima, 2008).

In this study, ALA was incorporated into a vehicle consisting of Alg and CS, and controlled release of ALA from the vehicle was

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investigated. The stability of ALA within the vehicle was also examined. Controlled release and stabilisation of ALA will provide a more effective, continuous supply of ALA within the body.

2. Experimental

2.1. Materials

(±)-Alpha-lipoic acid (ALA), *p*-nitrophenol, and ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid tetrasodium salt (EDTA) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Sodium alginate (300 cps) was purchased from Nacalai Tesque Inc. (Kyoto, Japan). CS (molecular weight [MW]: 129.8×10^4 Da, degree of deacetylation [DA]: 88%) was received as a generous gift from Nippon Kayaku Food Techno Co., Ltd. (Tokyo, Japan). Chitin (Chitin 1000) was purchased from Seikagaku Corporation (Tokyo, Japan). All other reagents were of reagent grade.

2.2. Determination of ALA

p-Nitrophenol (100 μL, 6.0–100.0 μg/mL) was dissolved in demineralised water as an internal standard and added to 500 μL of an ALA solution. The mixture was filtered (Cosmonice Filter W, 0.45 μm; Millipore, Billerica, MA, USA) and subjected to high performance liquid chromatography (HPLC). A 20-μL aliquot of the sample was loaded onto a column (COSMOSIL $5C_{18}$-MS-II Waters, 150 mm × 4.6 mm; Nacalai Tesque) with a precolumn (CAPCELL PAK NH_2 UG80; Shiseido, Tokyo, Japan) and eluted with 40% acetonitrile/demineralised water containing 0.1% phosphate as the mobile phase at a flow rate of 0.3 mL/min (LC-10AS; Shimadzu, Kyoto, Japan). The ALA in the effluent from the column was detected at 333 nm using a UV spectrophotometer (SPD-10AVVP; Shimadzu).

2.3. Preparation of Alg gel beads

Sodium alginate (1% w/w) was dissolved in demineralised water and 10% w/w ALA was then added and suspended in the Alg solution. Ten drops (about 0.5 g) of this suspension was slowly added dropwise to 20 mL of 0.1 M calcium chloride aqueous solution using a pipette and left to stand at room temperature for 30 min. Alg hydrogel beads formed spontaneously. The amount of ALA in the hydrogel beads was calculated by subtracting the amount of ALA detected in the preparation solution after bead formation from the theoretical total amount of ALA added to the initial Alg solution. Alg hydrogel beads were then dried at 37 °C for 24 h in a dish before being desiccated under a vacuum in the presence of P_2O_5. Alg gel beads with CS were prepared following the same procedure described above, with CS suspended in the sodium alginate solution (1% w/w) containing 10% ALA.

2.4. Stability of ALA under light

ALA powder (10 mg) was added to a measuring flask and made up to 100 mL with various solutions (ion-exchanged water, 0.1 M acetate buffer [pH 4.5], 0.1 M phosphate buffer [pH 7.4], 0.1 M borate buffer [pH 9.2], 0.1 N NaOH). Insoluble ALA residue was removed using a filter (DISMIC-25, 0.2 μm; Advantec, Dublin, CA, USA). The filtered ALA solution (15 mL) was added to a glass test tube. The solutions were irradiated using a UV lamp (16 W at a 15-cm distance). Over a period of 2 h, 500-μL aliquots of the solution were removed for analysis at regular intervals. The test solution was subjected to HPLC as described above. The ALA remaining in the solution was calculated as a percentage of the amount of ALA in a control sample that did not undergo UV irradiation but was left to stand for the same amount of time.

In addition, the stability of ALA in the gel beads was also determined. Alg hydrogel beads with or without CS were each added to 5 mL of ion-exchanged water in a glass test tube. Each bead was irradiated using a UV lamp for 2 h, and replicate beads were periodically removed for analysis. The bead was then degraded and dissolved in 0.1 N NaOH with 100 mg of EDTA, and the solution was made up to 5 mL. The test solution was subjected to HPLC as described above. The residual ALA remaining in the gel beads and the amount released into the test solution were calculated as percentages of the amount of ALA in a control sample that did not undergo UV irradiation but was left to stand for the same amount of time.

2.5. Stability of ALA under heat

ALA powder (20 mg) was added to a 1.5-mL sample tube. The tube was floated in a water bath at 65 °C for 2 h using a floating tube rack. The tube was periodically taken up and 1 mL of 0.1 N NaOH was added to the tube to dissolve the ALA. The tube was then centrifuged at 3500 rpm for 10 min, and the supernatant was filtered (Cosmonice Filter W, 0.45 μm) and subjected to HPLC to determine the amount of residual ALA. Similarly, a single Alg dried gel bead with or without CS was added to a 1.5-mL sample tube and incubated as described above. After incubation at 65 °C for 2 h, the bead was then dissolved in 0.1 N NaOH with 100 mg of EDTA, and the solution was made up to 5 mL. The sample was then centrifuged at 3500 rpm for 10 min, and the supernatant was filtered (Cosmonice Filter W, 0.45 μm) and subjected to HPLC determination of the amount of residual ALA.

2.6. Dissolution test

The rates of ALA release from Alg gel beads with and without CS were determined in artificial gastric juice (pH 1.2) for 2 h and then in artificial intestinal juice (pH 6.8) for 4 h. Ten Alg gel beads were added to 500 mL of dissolution medium in a Japanese Pharmacopoeia dissolution test apparatus (paddle method, 100 rpm, 37 °C). A 500-μL aliquot of the solution was periodically removed for analysis and replaced with 500-μL of the dissolution medium (prewarmed to 37 °C) to maintain a constant volume. The test solution was subjected to HPLC and the ALA released from the Alg gel beads with and without CS was calculated as a percentage of the initial amount of ALA incorporated into the Alg gel beads with and without CS prior to dissolution test.

3. Results and discussion

3.1. 1. Preparation of ALA gel beads

When sodium alginate solution containing ALA was dropped into 0.1 M calcium chloride aqueous solution, Alg hydrogel beads (diameter: 3.9 ± 0.2 mm) formed spontaneously and retained 96.1 ± 0.4% of the theoretical total amount of ALA. Alg dried gel beads were obtained by drying the Alg hydrogel beads at 37 °C for 24 h, and had a diameter of 2.7 ± 0.2 mm. Similarly, Alg hydrogel beads with CS (diameter: 3.7 ± 0.2 mm) retained 93.2 ± 0.2% of the theoretical total amount of ALA. The Alg dried gel beads with CS had a diameter of 2.3 ± 0.1 mm. All gel beads maintained a spherical shape.

3.2. Stability of ALA

ALA is known to be decomposed by light and heat. The rupture of the S–S bond of the 1,2-dithiolane ring in an ALA molecule results in the disappearance of the 333-nm absorption band and

the formation of dihydrolipoic acid (DHLA), and is accompanied by an unpleasant odour. The antioxidant activity of DHLA is almost same as ALA itself; however, strongly viscous polymeric products of ALA are also formed (Matsugo, Han, Tritschler, & Packer, 1996).

As shown in Fig. 1, UV irradiation gradually reduced the amount of ALA in the solutions. The reduction was similar regardless of the pH of the solution; about 40% of the ALA disappeared after UV irradiation for 2 h. Incorporation of ALA into the Alg gel beads largely prevented degradation of ALA through UV irradiation; the percentage of ALA released from the Alg hydrogel beads with and without CS after irradiation for 2 h was 7.8 ± 1.3% and 12.7 ± 1.3%, respectively. When ALA in the Alg hydrogel beads was irradiated in ion-exchanged water, 93% of the ALA remained after UV irradiation for 2 h, and in Alg gel beads with CS, ALA did not decrease at all under these conditions (Fig. 2).

In addition, when 20 mg of ALA in a 1.5-mL sample tube was incubated at 65 °C, polymerisation of ALA was immediately observed; about 30% of the initial ALA remained after incubation for 2 h. In contrast, incorporation of ALA into Alg dried gel beads with or without CS protected the ALA from heat effects; all of the ALA remained after incubation at 65 °C for 2 h (data not shown). In a previous study, ALA was shown to form an electrostatic complex with CS, and ALA in the ALA–CS complex did not decompose after incubation at 65 °C (Kofuji et al., 2008). It was assumed that complex formation with CS might increase the melting point of ALA, stabilizing the ALA against heat effects. However, incorporation into the Alg gel beads stabilized ALA against UV irradiation and heat regardless of whether CS was added. These results indicate that stabilisation of the ALA is conferred not only through interaction with CS but also through physical incorporation into the Alg gel matrix.

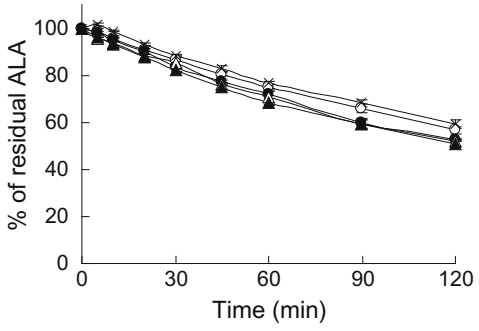

Fig. 1. Stability of ALA in various solutions after UV irradiation. ×, ion-exchanged water; ○, 0.1 M acetate buffer (pH 4.5); ●, 0.1 M phosphate buffer (pH 7.4); △, 0.1 M borate buffer (pH 9.2); ▲, 0.1 N NaOH. Data represent the mean ± SD (n = 3).

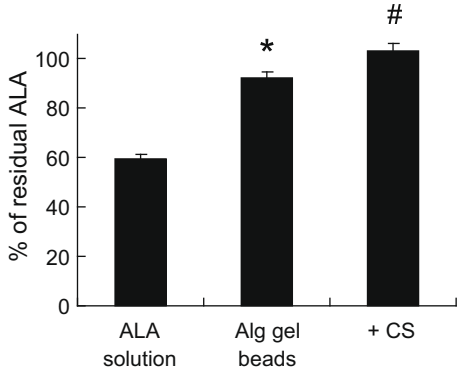

Fig. 2. Effect of incorporation of ALA into Alg gel beads on the stability of ALA after UV irradiation for 2 h. ALA solution, ALA dissolved in ion-exchanged water; Alg gel beads, ALA incorporated into Alg hydrogel beads was added to 5 mL of ion-exchanged water in a glass test tube; +CS, ALA incorporated into Alg hydrogel beads with CS was added to 5 mL of ion-exchanged water in a glass test tube. Data represent the mean ± SD (n = 3). Data were analysed statistically using Student's t-test after F-test. $^{*}P < 0.01$: significantly different from ALA solution, $^{\#}P < 0.01$: significantly different from Alg gel beads.

3.3. Release of ALA from the Alg gel beads

When the Alg hydrogel beads were added to artificial gastric juice (pH 1.2), ALA release from the Alg hydrogel beads was controlled. After a 2-h dissolution test at pH 1.2 (Fig. 3), 43% of the ALA incorporated into the Alg hydrogel beads was released. The release pattern of ALA from the Alg hydrogel beads conformed to Higuchi's square root of time equation (correlation coefficient (r^2) = 0.999), which indicates that ALA was released by simple diffusion from the Alg gel matrix. However, when the same beads used in the pH 1.2 dissolution test were continuously added to artificial intestinal juice (pH 6.8), the beads were immediately disintegrated and the ALA was completely released within 1 h.

A similar result was obtained for the Alg dried gel beads; 26% of the ALA was released after a 2-h dissolution test at pH 1.2. ALA release from the Alg dried gel beads was inhibited compared with the Alg hydrogel beads, likely because the dried gel beads were less permeable to the dissolution medium. However, immediate disintegration was observed after changing the dissolution medium from pH 1.2 to 6.8, and all of the residual ALA in the Alg dried gel beads was also subsequently released within 1 h.

When Alg hydrogel beads with CS were added to the artificial gastric juice (pH 1.2), ALA release tended to be slightly inhibited compared to the Alg hydrogel beads without CS, but a significant difference was not observed. After a 2-h dissolution test at pH 1.2, 39% of the ALA was released. Furthermore, changing the dissolution medium from pH 1.2 to 6.8 did not result in disintegration of the Alg hydrogel beads with CS, and pH-independent release of ALA was shown. After a dissolution test at pH 1.2 for 2 h and at pH 6.8 for 4 h, 86% of the ALA in the Alg hydrogel beads with CS was released.

Release of ALA from the Alg dried gel beads with CS was inhibited compared with the Alg hydrogel beads with CS. After a 2-h dissolution test at pH 1.2, 29% of the ALA was released, and 66% was released after a further dissolution test at pH 6.8 for 4 h.

In contrast to CS, chitin has a low deacetylation degree; addition of chitin in place of CS did not change the release of ALA compared to the Alg hydrogel beads alone (Fig. 4). This indicates that the amino groups in the CS molecule are important for pH-independent sustained release of ALA. In addition, when Alg hydrogel beads with CS were added to artificial intestinal juice (pH 6.8) without

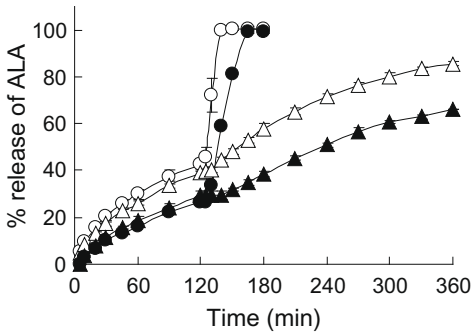

Fig. 3. Effect of CS addition to Alg gel beads on release of ALA into the dissolution medium at pH 1.2 for 2 h and pH 6.8 for 4 h. ○, Alg hydrogel beads; △, Alg hydrogel beads with CS; ●, Alg dried gel beads; ▲, Alg dried gel beads with CS. Data represent the mean ± SD (n = 3).

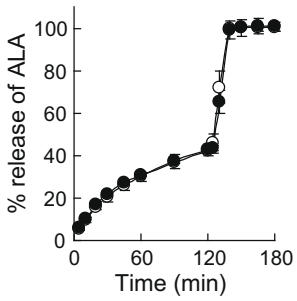

Fig. 4. Effect of chitin addition to Alg hydrogel beads on release of ALA into the dissolution medium at pH 1.2 for 2 h and pH 6.8 for 1 h. ○, Alg hydrogel beads; ●, Alg hydrogel beads with chitin. Data represent the mean ± SD (n = 3).

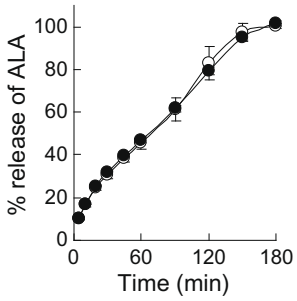

Fig. 5. Effect of CS addition to Alg hydrogel beads on release of ALA into the dissolution medium at pH 6.8. ○, Alg hydrogel beads; ●, Alg hydrogel beads with CS. Data represent the mean ± SD (n = 3).

prior immersion in a dissolution medium at pH 1.2, ALA release was controlled both by diffusion from and disintegration of the Alg gel matrix. Release of ALA from the Alg hydrogel beads was the same with and without addition of CS (Fig. 5), which demonstrates that ionisation of CS under a low-pH environment is required for pH-independent sustained release of ALA.

In a previous study, the authors found that ALA was immediately adsorbed onto CS in natural-pH water (Kofuji et al., 2008). Bernkop-Schnürch, Schuhbauer, Clausen, and Hanel (2004) also prepared a tablet containing ALA and CS, and sustained release of ALA from the tablet was achieved. In that study, sustained release was attributed to ionic interactions between the amino groups of the cationic polymer CS and the carboxyl groups of the anionic drug ALA, consistent with the hypothesis of the present study. In addition, ALA interactions with CS were able to be manipulated by altering the properties of CS. This was likely due to differences in inter- or intra-molecular attractive and repulsive forces in the CS polymers associated with differences in molecular weight, degree of deacetylation, and distribution of acetamide groups. When CS powder (10 mg) was added to 5 mL of ALA-saturated aqueous solution and incubated for 24 h at 25 °C, CS similar to that used in this study (MW: 129.8×10^4 Da, DA: 88%) adsorbed 47% of the ALA. A different CS (MW: 150.6×10^4 Da, DA: 86%) adsorbed only 7% of the ALA in the saturated solution (Kofuji et al., 2008). However, the CS species, and associated differences in interactive ability between CS and ALA, did not affect ALA release from the Alg gel beads with CS (data not shown). Furthermore, ALA adsorbed onto CS was immediately released into solutions whose pH was other than 5.9 (Kofuji et al., 2008). CS is a positively charged polymer with a pK_a of approximately 6.2–6.5 (Domard, 1987; Park, Choi, & Park, 1983), and ALA is a negatively charged drug with a pK_a of approximately 5.2–5.4 (Walton et al., 1955; Ruixia et al., 2004). Thus, the interaction between ALA and CS is maintained at a pH of approximately 5.9 because the degrees of ionisation of ALA and CS are simultaneously high at this pH. A decrease in the degree of ionisation of either CS or ALA due to alterations in the pH causes the release of ALA from the CS. Therefore, controlled release of ALA from the Alg gel beads with CS is not likely due to an interaction between ALA and CS.

pH-Independent sustained release of ALA from the Alg gel beads with CS was observed when the gel beads were immersed beforehand in a dissolution medium at pH 1.2. In this pH environment, the amino groups of CS are ionised and interact with the carboxyl groups of Alg. In addition, chitin, which has few amino groups, did not interact with Alg despite immersion in a dissolution medium at pH 1.2. In previous studies, it has been demonstrated that gel matrix disintegration is able to inhibit through complex formation by electrostatic interactions between an anionic polymer and a cationic polymer (Murata, Miyamoto, & Kawashima, 1996; Murata et al., 1993). Therefore, it would be concluded that pH-independent sustained release of ALA is due to complex formation by electrostatic interactions between Alg and CS rather than between ALA and CS.

4. Conclusion

ALA was stabilised against light (UV) and heat (65 °C) effects through incorporation into Alg gel beads. The use of Alg gel beads also enabled controlled ALA release. Furthermore, the addition of the cationic polymer CS to the Alg gel beads achieved pH-independent sustained release of ALA, resulting from electrostatic interactions between the carboxyl groups of Alg and the amino groups of CS. Thus, the stabilization and controlled release of ALA was achieved through incorporation of ALA into Alg gel beads with CS. These natural polysaccharide-based gel beads are promising vehicles for improving the curative effect of ALA by providing a continuous supply of ALA to the body.

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