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Combined cross-linking treatments of bovine serum albumin gel beadlets for controlled-delivery of caffeine

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ABSTRACT

Combined cross-linking agents (CCLA) of microbial transglutaminase (MTgase) and ribose were applied during production of bovine serum albumin gels via incubation and heating treatment, respectively. CCLA produced stronger gels with lower protein solubility in disruptive solvents (1% sodium dodecyl sulphate plus 1% β-mercaptoethanol) as compared to BSA gels (BSA/Control) or gels produced using single cross-linking agents (SCLA) of MTGase or ribose. The gels were then converted into dried beadlets containing caffeine following a freeze-drying process. *In-vitro* controlled-release of caffeine and swelling ratio studies of the beadlets in artificial saliva or simulated gastric fluid indicated that CCLA beadlets had the slowest release of caffeine and the lowest swelling ratio as compared to other beadlets. Scanning electron microscopy (SEM) data suggested that the improved release and the lower swelling ratio were mainly due to the denser network formed within the CCLA beadlets that had restricted the diffusion of caffeine and hampered the enzymatic breakdown of the matrix. The additional protein cross-linkings formed as a result of MTgase incubation and ribose-induced Maillard reaction could provide a delay action in releasing caffeine that potentially extend the duration of the action of the drug during ingestion.

1. Introduction

Caffeine is a well-known and widely used psychoactive substance for central nervous system from the group of xanthine derivatives and can be used to impart a desired level of increased alertness (McLellan et al., 2005). Most of the caffeine consumed comes from dietary sources such as coffee, tea, cola drinks and chocolate. Thus, it is safe as component of food at doses required to overcoming sleep deprivation and to enhance sport performance. The most notable behavioral effects of caffeine occur after low to moderate doses (50–300 mg), and these are increase in alertness, energy and ability to concentrate (Benowitz, 1990). Moderate caffeine consumption leads very rarely to health risk, whereas higher doses rather induce negative effects such as anxiety, restless, insomnia and tachychardia. These effects have been seen primarily in a small portion of caffeine-sensitive individuals (Benowitz, 1990; James & Stirling, 1983; Nehlig, 1999). As caffeine absorption from the gastrointestinal tract is rapid and reaches 99% in humans about 45 min after ingestion (Blanchard & Sawers, 1983), efforts to control the release of caffeine after ingestion may be beneficial for food and nutraceutical applications.

Typically, synthetic materials such as polyethylene oxide (PEO), polyvinyl alcohol (PVA), ethyl cellulose (EC), polyvinyl pyrrolidone (PVP), poly DL-lactide-co-glycolide (PLGA) and polylactic acid (PLA) are used as carriers for drug-release. Food-based materials such as whey protein, gum arabic, gelatin, alginate and pectin have also been studied for similar application (Gunasekaran, Ko, & Xiao, 2007; Joseph & Venkataram, 1995; Ramakrishnan, Pandit, Badgujar, Bhaskar, & Rao, 2007). The advantages of using protein-based drug/nutraceutical-carrier include non-toxicity and safe for consumption. Approaches to control the release of materials from protein systems include the use of chemical cross-linking such as glutaraldehyde (Fuguet, van Platerink, & Janssen, 2007). The usage of glutaraldehyde compromises the biocompatibility of the cross-linked structures because the toxic residues and degradation products induce cytotoxicity and calcification (Simmons & Kearney, 1993). In this respect, the development of a process that employs food-based materials such as microbial transglutaminase and ribose to modify protein via cross-linking treatments that render the protein more resistant to ingestion may be beneficial.

In this study, bovine serum albumin (BSA) is used as the model protein for drug delivery because of its medically important, abundance, low cost, ease of purification, unusual ligand-binding properties and it is widely accepted in the pharmaceutical industry (Hu, Liu, Sun, Bai, Lü, & Pi, 2006). BSA has been used before as

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a carrier/control release agent for phosphodiester oligonucleotide (Arnedo, Espuelas, & Irache, 2002), anti-inflammatory drug cromolyn sodium (Hu et al., 2006) and β-propranolol (Iemma et al., 2006). Native BSA molecules do not form gels when they are dispersed in water at neutral pH because the intermolecular repulsive interactions (mainly electrostatic, hydration and configurational entropy) dominate over the attractive interactions (mainly van der Waals and hydrophobic). The solution must be heated to a temperature where the globular proteins undergo conformational changes that lead to the exposure of reactive sites, such as hydrophobic and sulfhydryl-containing groups. Under appropriate solution conditions, the resultant increase in protein surface activity is sufficient to promote aggregation and gel formation. The nature of the interactions between the proteins determines the structural organization of the protein molecules within the gel network, which in turn determines the overall physicochemical characteristics of the gel (Baier, Dekker, & McClements, 2004).

MTGase (protein-glutamine: amine γ-glutamyltransferase, E.C. 2.3.2.13) forms cross-links by catalyzing an acyl-transfer reaction between the γ-carboxyamide group of peptide-bound glutamine residues (acyl donors) and variety of primary amines (acyl acceptors), including the ϵ-amino group of lysine residues to form an ϵ-(γ-glutamyl)lysine isopeptide bond (Motoki & Seguro, 1998). This treatment has been used in dairy products (Lorenzen, 2007), legume products (Tang, Li, Wang, & Yang, 2007) and wheat products (Caballero, Gómez, & Rosell, 2007) in enhancing the texture and functional properties. On the other hand, the Maillard or non-enzymatic browning reaction comprises the reaction between reducing sugars and amino groups of amino acids and proteins during heating. "Maillard cross-linking" has been shown to produce cross-linked protein and improved protein gel strength and texture of protein-based products (Hill & Easa, 1998; Md Yasir, Sutton, Newberry, Andrews, & Gerrard, 2007). It is thought that this cross-linking of protein chains occurs during the advanced and the final stages of the Maillard reaction (Gerrard, Brown, & Fayle, 2003). Several investigators have indicated that the sugar-derived dicarbonyl compounds, such as methylglyoxal, 3-deoxyosones or glyoxal (Biemel, Friedl, & Lederer, 2002) attach to lysine, arginine, and tryptophan residues of the protein via one of their carbonyl group (Glomb & Tschirnich, 2001). The proteins, with the bifunctional agents attached, are then thought to polymerize by binding of another second functional group with the remaining lysine and arginine residues of the protein. The cross-links including those of pentosidine, glucosepane, GOLD (glyoxal lysine dimer), MOLD (methylglyoxal lysine dimer), DOGDIC (a lysine arginine dimer), MODIC (methylglyoxal lysine arginine dimer), and GODIC (glyoxal lysine arginine dimer) have been characterized (Biemel, Bühler, Reihl, & Lederer, 2001). Combining the cross-linking agents (CCLA) of microbial transglutaminase (MTGase) with Maillard cross-linking has been attempted in soy protein isolate gels (Gan, Cheng, & Easa, 2008a). The CCLA gels had denser protein network as compared to those of conventional or gels produced with single cross-linking agent (SCLA). The application of the combined cross-linking agents and its methodology has also been tested in microcapsules containing fish oil as well as in yellow noodles (Gan, Cheng, & Easa, in 2008b; Gan, Ong, Wong, & Easa, 2009).

The objective of this study was to investigate the release characteristic and usability of the BSA beadlets produced using combined cross-linking agents (CCLA). BSA beadlets containing caffeine were prepared using CCLA of microbial transglutaminase and ribose and subjected to *in-vitro* controlled-release experiments simulating the mouth and stomach to evaluate the release rates and mechanisms. Beadlets produced using CCLA are expected to enable longer drug-release, thus prolonged effects.

2. Material and methods

2.1. Materials

Bovine Serum Albumin (BSA) was purchased from Merck, Germany. A Ca^{2+} independent microbial transglutaminase (ACTIVA TG-K) was a gift from Ajinomoto Co., Inc. (Tokyo, Japan). D-(−)-Ribose was purchased from Sigma-Aldrich company. Other chemicals (analytical grade) used in this study were obtained from Sigma-Aldrich company and Fluka company.

2.2. Gels and beadlet preparation

Gel-forming BSA solutions were prepared according to the formulation shown in Table 1. The solutions were incubated at 40 °C with constant shaking at 50 rpm for 5 h in LM-570R Orbital Shaker (Yih Der, Taiwan). The solutions were then injected into glass bottles with caps before being subjected to a heating treatment at 90 °C for 3 h in a water bath to produce BSA gels. The dimensions of the gels produced were 2 cm in diameter and 2.5 cm in height (Fig. 1a). The gel samples were subjected to color, pH, gel strength and protein solubility assessments.

For beadlet preparation, a two-piece ball ice cube freezing tray was used as molds for beadlet formation. The tray (24 cm × 9.5 cm × 2.5 cm) that is normally used to produce 1.5 cm diameter of ball-shaped ice cubes, is made from low density polyethylene (LDPE) plastic. The gel-forming solutions were injected into the molds, before incubation and heating, performed as described above. The molds with gels formed inside were left to cool to room temperature (24 ± 0.5 °C) and transferred to a blast freezer (Irinox, Italy) for 1 h. Once frozen, the gels were forced out of the molds and transferred into a freeze-dryer (Labconco, Kansas City, USA) and lyophilized overnight. Dry beadlets with average diameter of ~1.6 cm were produced and these were kept in airtight containers for further analysis.

2.3. BSA gel analysis

Color analysis of the gels was carried out using colorimeter (Minolta, Spectrophotometer CM-3500d, Japan). Gel samples were placed in specimen cell and Minolta color scale was used to measure the lightness as indicated by the L^* value ($L^* = 0$ (black) to $L^* = 100$ (white)). L^* value was used to characterize color changes of the gels as a result of the Maillard reaction.

The pH values of the gels were measured using Inlab 421 electrode attached to a Delta 320 pH meter (Mettler-Toledo, Switzerland).

The rupture force of the gel samples (i.e. gel strength) was determined using Texture Analyzer, TA-TX2 (Stable Micro Systems, Surrey, England) attached with 2-mm diameter cylinder probe. The settings for the analysis were: test mode: compression; pre-test speed: 1.00 mm/s; test speed: 2.00 mm/s; strain: 95%; trigger force: 0.05 N (Note: trigger force is defined as the force threshold above which the measurement commences). The maximum peak force (N) obtained for each sample was recorded as rupture force.

Table 1
Formulations of BSA gel-forming solutions.

Samples	BSA (g)	Caffeine (g)	Distilled water (ml)	2% (w/v) Ribose solution (ml)	MTGase, 40 U/g, (g)
BSA/Control	10	0.5	100	–	–
SCLA-ribose	10	0.5	–	100	–
SCLA-MTGase	10	0.5	100	–	0.5
CCLA	10	0.5	–	100	0.5