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Lactase (β-galactosidase) encapsulation in hydrogel beads with controlled internal pH microenvironments: Impact of bead characteristics on enzyme activity

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ABSTRACT

Individuals who suffer from lactose intolerance can alleviate many of their symptoms by ingestion of lactase (β-galactosidase) with lactose-containing foods. However, this enzyme is deactivated when exposed to highly acidic gastric fluids, which reduces the amount reaching the small intestine. Oral delivery systems are therefore needed to protect lactase in the stomach, and then release it in an active form in the small intestine. In this study, carrageenan-based hydrogel beads were fabricated that contained lactase and a buffer ($Mg(OH)_2$). This buffer was selected because it is insoluble under alkaline conditions, but soluble under acidic conditions, and can therefore maintain a neutral pH inside the beads when they are dispersed in a low pH solution (such as acidic gastric fluids). Small (D = 255 μm) and large (D = 2610 μm) hydrogel beads were prepared using two different injection methods to compare the impact of bead dimensions on the retention of lactase activity. A ratiometric fluorescence method based on confocal laser scanning microscopy was developed to measure changes in the internal pH of the hydrogel beads before and after exposure to simulated gastric conditions. After exposure to stomach conditions, the internal pH of buffer-free beads dropped steeply (from around pH 7.0 to below pH 4.0), but that of buffer-loaded beads remained fairly constant (from around pH 7.2 to pH 6.6). When exposed to small intestine conditions, lactase encapsulated in buffer-loaded beads maintained its enzyme activity, whereas lactase encapsulated in buffer-free beads did not. Smaller beads needed a higher amount of $Mg(OH)_2$ to maintain a neutral internal pH throughout the gastric phase, which was attributed to faster diffusion of hydrogen ions (H^+) into smaller beads. These results suggest that buffer-loaded hydrogel beads may be useful for encapsulation, protection, and delivery of acid-labile enzymes and other bioactive substances.

1. Introduction

Lactose intolerance is a common disease in humans that is associated with the deficiency of a specific enzyme (β-galactosidase) produced within the brush border of the small intestine (Heyman, 2006; Vesa, Marteau, & Korpela, 2000). Individuals with lactose intolerance have a limited ability to digest and absorb lactose in the small intestine. As a result, this disaccharide enters the colon in an undigested form where it can promote health problems, such as gut distension, stomach pain, flatulence, diarrhea, and nausea (Lomer, Parkes, & Sanderson, 2008). Individuals who suffer from lactose intolerance often avoid consuming milk and other dairy products so as to avoid exhibiting these undesirable symptoms. Several other approaches have also been developed to aid individuals with lactose intolerance, including creating lactose-free foods and the co-ingestion of lactase supplements with lactose-containing foods (Montalto et al., 2006). Enzyme supplement treatments are particularly promising because they do not cause undesirable changes in food quality or nutritional profile. Typically, a tablet or capsule containing lactase is taken at the same time as a lactose-containing food, which promotes lactose hydrolysis within the gastrointestinal tract (GIT). β-galactosidase (β-gal) is the most common form of lactase used in enzyme supplements, but it cannot simply be delivered in its free form because it is highly susceptible to denaturation and deactivation under GIT conditions (Nichele, Signoretto, & Ghedini, 2011). In particular, lactase is usually deactivated when exposed to the highly acidic gastric fluids

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present within the human stomach (He, Zhang, & Sheng, 2014).

The encapsulation of enzymes within porous matrices can improve their stability by creating a protective microenvironment or by acting as a physical barrier to the external environment (Kim, Grate, & Wang, 2006; Xu et al., 2008). At the same time, the porous matrices can be designed to allow small molecules (such as co-factors, substrates, and products) to easily diffuse into them and reach the catalytic site of the encapsulated enzyme, which enables the enzyme to maintain its activity even after encapsulation (Coviello, Matricardi, Marianecci, & Alhaique, 2007; Es, Vieira, & Amaral, 2015). Enzymes can be encapsulated within porous matrices using physical entrapment, physical interactions, or chemical bonding (Betancor, Luckarift, Seo, Brand, & Spain, 2008; Böyükbayram, Kıralp, Toppare, & Yağcı, 2006). The encapsulation of enzymes in hydrogels is particularly attractive for application in the food industry because these semi-solid materials can be fabricated from food-grade ingredients using simple processing operations (Zhang, Zhang, Chen, Tong, & McClements, 2015b). Enzymes are physically entrapped in the hydrogel network during the sol-gel transition, which is a relatively mild process that tends to preserve the structural integrity and activity of the enzymes. After fabrication, the enzymes can be retained within the hydrogel matrix by ensuring that its pore size is appreciably smaller than the enzyme dimensions, or by ensuring that there is a sufficiently strong attraction between the enzymes and the hydrogel matrix.

For many applications it is desirable to utilize hydrogel beads, rather than bulk hydrogels to encapsulate and deliver bioactive substances (Coviello et al., 2007). Hydrogel beads (also known as microgels) consist of small particles (typically from 100 nm to 1000 μm) that can easily be dispersed in aqueous solutions. These beads can be fabricated from food-grade biopolymers (typically polysaccharides and/or proteins) using simple processing operations, such as the injection-gelation method (Gombotz & Wee, 2012; Onwulata, 2012; Shewan & Stokes, 2013; Zhang, Zhang, Zou, & McClements, 2016). Typically, the formation of hydrogel beads requires two-steps that may occur consecutively or simultaneously: particle-formation and particle-gelation. For the injection-gelation method, particle-formation is performed by injecting an aqueous solution containing a biopolymer (gelling agent) and a bioactive agent (enzyme) into a gas or immiscible liquid, and then particle-gelation is performed by exposing the particles formed to solution or environmental conditions that promote biopolymer gelation. This process leads to the formation of hydrogel beads with bioactives trapped inside a biopolymer matrix (Li, Hu, Du, Xiao, & McClements, 2011). A serious limitation of this approach for the encapsulation of acid-sensitive enzymes (such as lactase) is that small hydrogen ions (H^+) can easily diffuse through the biopolymer network inside the hydrogel beads, thereby inducing enzyme deactivation. For this reason, a number of research groups have tried to improve the acid-resistant characteristics of hydrogel beads, *e.g.,* by reducing the pore size of the biopolymer network or by coating the beads with biopolymer layers (Srivastava, Brown, Zhu, & McShane, 2005; Taqieddin & Amiji, 2004). However, these approaches are often unsuccessful because the hydrogen ions are so small that they can still easily penetrate through the biopolymer networks that make up the hydrogel core or shell.

The main objective of the present study was to develop an alternative approach to create hydrogel beads that would protect enzymes from acid-induced deactivation when exposed to gastric conditions. The enzyme (lactase) was co-encapsulated with magnesium hydroxide (a basic buffer) inside the hydrogel beads. $Mg(OH)_2$ was selected as a buffering agent because it is a widely used food-grade antacid, which is insoluble in water at neutral and basic pH values, but soluble at acidic pH values (Zhu, Mallery, & Schwendeman, 2000). When these buffer-loaded hydrogel beads are dispersed in an acidic solution, the pH inside them remains close to neutral for an extended period. This phenomenon occurs because as hydrogen ions (H^+) from the surrounding aqueous phase diffuse into the hydrogel beads they cause some of the $Mg(OH)_2$ to dissociate, which generates hydroxyl ions (OH^-) that neutralize any pH changes. Moreover, it has been shown that the presence of Mg^{2+} can increase the activity of lactase (Juers et al., 2009; Lo et al., 2010), which may be another advantage of co-encapsulation of this particular buffering agent with lactase. The hydrogel beads were fabricated by an injection-gelation method using an anionic polysaccharide (κ-carrageenan) as the gelling agent and a cationic mineral ion (potassium) as a cross-linking agent. This polysaccharide was selected because it was previously shown that relatively high encapsulation efficiency (around 63%) can be achieved by the carrageenan beads. In addition, β-gal encapsulated within potassium-carrageenan beads had a higher activity than the free enzyme due to the stabilization effects of K^+ ions on enzyme structure (Zhang, Zhang, Chen, & McClements, 2016). The ability of the buffer-loaded hydrogel beads to protect lactase from acid-induced deactivation was demonstrated by exposing them to simulated gastric conditions. Another objective of this study was to show that a quantitative ratiometric fluorescence method based on confocal laser scanning microscopy (CLSM) could be used to map the pH inside the beads before and after exposure to gastric conditions. This study provides information that can be used to facilitate the fabrication of hydrogel beads designed to encapsulate and deliver acid-labile enzymes and other bioactive agents.

2. Materials and methods

2.1. Materials

Fluorescein tetramethylrhodamine dextran (FRD) with an average molecule weight of around 70 kDa was purchased from Molecular Probes (Eugene, OR). Lactase (β-galactosidase) with a specific activity of around 2600 U/g was obtained from Sigma Chemical Co (St. Louis, MO, USA). Carrageenan was kindly donated by FMC Biopolymer (Viscarin SD 389, Philadelphia, PA). The reagents *o*-nitrophenol (*o*-NP) and *o*-nitrophenyl-b-*d*-galactosidase (*o*-NPG) were obtained from the Sigma Chemical Co. (St. Louis, MO, USA). All chemicals used were analytical grade. Double distilled water was used to prepare all solutions.

2.2. Hydrogel beads preparation

An aqueous κ-carrageenan (2% w/v) solution was prepared by dissolving the powdered ingredient in distilled water by stirring at 50 °C for an hour, and then reducing the temperature to room temperature with continuous stirring until fully dissolved. The κ-carrageenan solution was then mixed with β-gal solution to obtain a concentration of 1% κ-carrageenan and 130 U β-gal mixture with or without different amount of $Mg(OH)_2$ co-encapsulation. After continuously stirring, the mixtures were injected into 10% potassium chloride solution using a syringe or a commercial encapsulation unit (Encapsulator B-390, BUCHI, Switzerland) with a 150 μm vibrating nozzle to prepare the hydrogel beads. The encapsulation device was operated under the following conditions: frequency 800 Hz; electrode 750 V; and pressure 450 mbar. The formed beads were held in the K^+ solution for 30 min at ambient temperature to promote bead hardening.

2.3. Simulated gastric conditions

A simulated stomach model was used to investigate the