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Barley β-glucan cryogels as encapsulation carriers of proteins: Impact of molecular size on thermo-mechanical and release properties

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ABSTRACT

The potential use of barley β-glucan cryogels as encapsulation carriers for delivery-controlled release of proteins was explored. For cryogel preparation, mixed β-glucan (4.2% w/w)/protein (0.8%) aqueous dispersions were subjected to 10 freezing (−23 °C/24 h) and thawing (25 °C/24 h) cycles, using three purified barley β-glucan isolates differing in molecular weight, Mp (55, 140 and 320 kDa) and four proteins, selected as model core constituents; i.e. β-lactoglobulin (18 kDa), ovalbumin (43 kDa), bovine serum albumin (67 kDa), invertase (270 kDa). Dynamic rheometry revealed that the mechanical properties of the composite cryostructurates, were significantly affected ($p < 0.05$) by the molecular size of β-glucan and protein. With increasing polysaccharide molecular size the elastic modulus (G') and the melting enthalpy (ΔH) values of the cryogels decreased, while the melting temperature (Tm) of the gels (calorimetry) increased. Incorporation of the highest Mw protein in the β-glucan cryogels also increased ($p < 0.05$) the G'. Large deformation mechanical tests have shown an increase of compression modulus (E) and true stress (σ_{TR}) with increasing Mp of the polysaccharide, whereas the cryogel's swelling capacity, followed the reverse trend. Release kinetics of entrapped proteins was monitored spectrophotometrically in aqueous suspensions of the mixed cryogels at 37 °C. The amount and the apparent diffusion coefficient (D_m) of proteins released significantly ($p < 0.001$) decreased with increasing molecular size of both β-glucans and proteins. Overall, the observed variation in thermo-mechanical, swelling and diffusional properties of the composite cryogels may allow the design of tailor-made delivery and controlled release systems of polymeric ingredients such as bioactive proteins.

1. Introduction

Cryostructuration or cryogelation is a specific type of gelation that occurs upon cryogenic treatment of aqueous solutions of gelling hydrocolloids involving freezing and storage in the frozen state of the initial solutions followed by thawing of the systems that gives rise to the formation of gel-like polymer systems called cryostructurates or cryogels or cryotropic gels (Bencherif, Braschler, & Renaud, 2013; Lozinsky et al., 2003; Zhang, Zhang, & Wu, 2013). During this process firstly, there is an increase of polymer concentration in the remaining unfrozen liquid microphase (cryoconcentration) during freezing that allows approaching of the polymer chains and the formation of a weak gel structure via physical noncovalent interactions and secondly, an additional slow growth of the cross-linked network that takes place during thawing, resulting in gels with interconnected micro- and macropores developed upon ice crystal melting. Submission of the polymer dispersions to repeated freeze–thaw cycles can be used to tune the structural properties of cryogels (Zhang et al., 2013); i.e., with increasing number of freezing–thawing cycles, the mechanical strength and the thermal stability of the cryogels is improved, while their macroporosity is enhanced giving gels with different barrier properties compared to hydrogels prepared by traditional crosslinking techniques (e.g. covalent bonding).

The improved mechanical properties and the porous–spongy structure of these cryogels make them promising gelling delivery systems for applications in the biotechnology, food, pharmaceutical and other industries, as well in biomedicine as useful materials for implants, heart valve stent, and artificial skin and organs (Bencherif et al., 2013; Lozinsky et al., 2003). Synthetic polymers, such as polyvinyl alcohol (PVA), and many natural polymers, such as polysaccharides, have been explored as cryogel-forming materials (Zhang et al., 2013; Lozinsky et al., 2003; Lazaridou & Biliaderis, 2004). Natural cryogels fabricated from agarose, xanthan, starch polymers and their hydrolyzates, galactomannans, cereal β-glucans as well as derivatives of microbial β-glucans have been studied; moreover, composite cryogels based on PVA and

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polysaccharides, such as sodium alginate, gellan, chitosan and carboxymethylcellulose have been also investigated.

Cryogels as well as other hydrogels fabricated from polysaccharides by other processes (e.g. chemotropic, ionotropic, chelatotropic, thermotropic, and psychrotropic gels) have been previously explored for pharmaceutical, nutraceutical and food applications (Gombotz & Wee, 2012; Lozinsky et al., 2003; Sinha & Kumria, 2001; Zhang et al., 2013). They are biocompatible, non-immunogenic, non-toxic, friendly to the environment due to the fact that are biodegradable materials and are prepared under mild conditions involving a renewable, non-toxic solvent (water). Moreover, the ability of natural hydrogels to easily degrade under different pH and ionic strength conditions or by colonic bacteria (enzymic degradation) established them as cell immobilization systems as well as ideal carriers of drug and bioactive compounds for controlled release of bioactive compounds at certain target sites of the gut. Additionally, most of the used polysaccharides for hydrogel making are indigestible carbohydrates, and therefore exert all the health benefits of dietary fibers.

In the present study, cryogels made from barley β-glucans were studied as encapsulation matrices. Mixed linkage (1→3), (1→4) β-D-glucans, that occur as structural cell wall polysaccharides in barley and oat grains, are linear homopolymers composed of D-glucopyranosyl units (Lazaridou, Biliaderis, & Izydorczyk, 2007). Their primary structure consists of cellulose-like oligomers interlinked via single (1→3) linkages with most of the cellulose segments being trimers and tetramers, while longer cellulosic oligosaccharides are also present to a lesser extent. Further to their ability to form viscous dispersions, cereal β-glucans can be gelled either by isothermal curing of aqueous dispersions (4–12%) at temperatures above zero (5–45 °C) or by cryostructuration via repeated freeze–thaw cycles of relatively dilute dispersions (1–4%) (Lazaridou et al., 2007; Lazaridou & Biliaderis, 2004; Lazaridou, Vaikousi, & Biliaderis, 2008). The formation of these gels involves extended junction zones stabilized via H-bonding among chain segments with consecutive cellotriose units. The thermo-mechanical properties of cereal β-glucans cryogels have been found to be governed by the number of freezing–thawing cycles, the polysaccharide concentration in the initial dispersion, as well as the molecular weight and primary structure of the β-glucan (Lazaridou & Biliaderis, 2004; Lazaridou et al., 2008).

So far, β-glucan gels have been used as encapsulation matrices of small molecules, such black currant antocyanins (Xiong, Melton, Easteal, & Siew, 2006). However, β-glucan cryogels prepared by the freezing–thawing process have not been explored in this context, yet. Usage of β-glucan cryogels as carriers of polymeric compounds, such as proteins seems to be an interesting approach due to the high structural diversity encountered in these systems which is reflected on their mechanical strength, thermostability and macroporosity, as well their potential as a colon-specific controlled release systems because of the ability of β-glucans to be fermented by the colonic flora (Lazaridou & Biliaderis, 2004; Lazaridou et al., 2008; Mitsou, Panopoulou, Turunen, Spiliotis, & Kyriacou, 2010).

Proteins are important functional biopolymers for food, drug and nutraceutical systems, since they can be structural materials, enzymes, antibodies, receptors and hormones (e.g. growth factors). However, oral delivery of proteins is restricted due to their hydrolysis by proteolytic enzymes in the stomach and small intestine (Sinha & Kumria, 2001); thus, the protective role of non-digestible polysaccharides for peptides or proteins in the upper gastrointestinal (GI) tract combined with their degradation by bacteria at the colon make the polysaccharide gels promising vehicles for protein delivery. Research on protein encapsulation by using polysaccharide hydrogels as carriers has been increasing; the systems that have been studied were fabricated by various polysaccharides, such as alginates, dextran, arabinoxylans, chitosan and starch derivatives (Berlanga-Reyes et al., 2009, 2014; Berlanga-Reyes, Carvajal-Millan, Lizardi-Mendoza, Islas-Rubio, & Rascon-Chu, 2011; Bertz et al., 2013; Carvajal-Millan, Guilbert, Doublier, & Micard, 2006; Carvajal-Millan, Guilbert, Morel, & Micard, 2005; Carvajal-Millan, Surget, Rouau, Guilbert, & Micard, 2003; Dragan, 2014; Gombotz & Wee, 2012; Hennink, Talsma, Borchert, De Smedt, & Demeester, 1996; Meyvis, De Smedt, Stubbe, Hennink, & Demeester, 2001). On the other hand, the investigation of potential use of cryogels as protein carriers has been limited to PVA and mixed PVA/carboxymethyl chitosan cryostructurates (Bajpai & Saini, 2006; Li, Du, Tang, & Wang, 2009; Peppas & Sott, 1992).

In addition to the prospective use of β-glucan cryogels as suitable carriers for bioactive protein molecules delivery, they also exhibit inherent physiological activities. Both barley and oat β-glucans display well documented health benefits as they have been clinically proven to reduce plasma cholesterol and to attenuate postprandial glucose and insulin levels in the blood plasma (Lazaridou et al., 2007; Tosh, 2013; Whitehead, Beck, Tosh, & Wolever, 2014). As a result, the Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) approved health claims for the reduction of coronary heart disease risk with a daily consumption of 3g of barley β-glucans (FDA, 2005; EFSA, 2011a). Moreover, EFSA has recently issued health claims concerning the reduction of postprandial glycemic responses (EFSA, 2011b) and the increase of faecal bulk after cereal β-glucan consumption (EFSA, 2011c).

The objective of this work was to develop encapsulation systems for the delivery and controlled release of proteins, using as carriers physically crossed-linked cryogels made by barley β-glucans, well known polysaccharides for their bioactivity. The impact of molecular size of both proteins and barley β-glucans on thermo-mechanical and swelling properties of the β-glucan cryogels as well as the release kinetics of the entrapped protein components were explored.

2. Materials and methods

2.1. Materials and molecular characterization of barley β-glucans

For preparation of cryogels, three highly purified barley β-glucan isolates, differing in molecular weight were used in this study as wall materials. These samples were prepared by acid hydrolysis from a high molecular weight β-glucan isolate obtained from whole barley flour gifted by the Mills of Crete S.A. (Souda, Chania and Greece). The isolation-purification and acid hydrolysis protocols were described in detail elsewhere (Irakli, Biliaderis, Izydorczyk, & Papadoyannis, 2004; Vaikousi, Biliaderis, & Izydorczyk, 2004). Briefly, following grinding (0.5 mm screen) and refluxing (aqueous ethanol 80% v/v, 2 h × 85 °C) to inactivate endogenous β-glucanases in the whole barley flour, aqueous extraction (10% solids, 2 h × 50 °C) was performed. The purification step involved digestion (2 h × 90 °C, pH 4.5) of the extract with a thermostable α-amylase (Termamyl 120 L, Novozymes A/S, Bagsvaerd, Denmark) for starch degradation, as well as adsorption of contaminating proteins on Fuller's earth particles (Sigma-Aldrich, St. Louis, MO, USA) and finally precipitation of β-glucans (72 h × 4 °C) by $(NH_4)_2SO_4$ (45% saturation); then, the precipitate was resolubilized and submitted to exhaustive dialysis using cellulose membranes (Sigma-Aldrich, St. Louis, MO, USA; molecular weight cut-off = 14,000) for removal of salt and the residual starch hydrolyzates. The β-glucan component was isolated from the dialyzate by precipitation with two volumes of ethanol, re-suspension in 2-propanol, filtration and drying (at 40 °C). Samples of