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Spontaneous co-assembly of lactoferrin and β-lactoglobulin as a promising biocarrier for vitamin B9

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ABSTRACT

The design of biocarriers for protection and controlled delivery of bioactives represents a challenge for developing functional foods. We investigated the potentiality of heteroprotein Beta-lactoglobulin (BLG) and Lactoferrin (LF) co-assemblies as biocarriers for vitamin B9 (B9). Using different B9:protein mixing ratios, B9-LF-BLG co-assemblies were obtained and assessed by turbidity and phase contrast microscopy. Kinetics of their formation and stability were monitored. B9 entrapment efficiency was evaluated. Two types of B9-LF-BLG co-assembly were identified: aggregates (at low and high protein concentrations), and heteroprotein coacervates (at intermediate protein concentrations), both exhibiting different kinetics and stability over time. Compiling screening maps of B9-LF-BLG co-assemblies and B9 entrapments, we evidenced that B9-LF-BLG coacervates exhibited higher performance as B9 biocarrier, with an optimal entrapment of ≈10 mg B9/g protein. Therefore, such co-assembly displays useful potentialities as vitamin biocarriers for the design of natural functional foods, offering enhanced health benefits, yet without resorting to non-food additives.

1. Introduction

Our food diet is often described as our first medicine. In recent years, there has been a growing demand from consumers for food products offering health benefits (Annunziata & Vecchio, 2011; Biesalski et al., 2009). To meet this demand, the food industry has sought to enhance the nutritional qualities of traditional food products by enriching them with bioactive compounds such as polyphenols, fatty acids or vitamins. It has led to the creation of a whole range of new functional food products (Betoret, Betoret, Vidal, & Fito, 2011). However, the design of these products requires to overcome two main technological challenges. Firstly, the bioactive compounds may be poorly soluble into the desired food product, thereby limiting the feasibility of product enrichment (Diarrassouba, Garrait, et al., 2015). Secondly, the bioactive compounds are often very sensitive to the conditions applied during food processing and storage, thereby limiting their actual bioavailability as intact constituents of the functional food products (Hosseini, Emam-Djomeh, Sabatino, & Van der Meeren, 2015). A way to overcome these two main issues and to ensure the effective delivery of the bioactive compounds is to load, protect and release the bioactives by means of structures called biocarriers (Shimoni, 2009). As a result, the design of these biocarriers appeared as a key challenge to develop functional food products (Chen, Remondetto, & Subirade, 2006; Lacatusu et al., 2013). In addition to their demand for functional food products, consumers tend also to favor food products with minimal amount of additives. As a result, it could be relevant to use directly some components of the targeted food product as biocarrier materials for the bioactives. This approach has the potential to ensure the design of functional and natural foods, enabling additive-free food products, well-known in the food industry sector as “clean label” or “green product” (Diaz, 2013). Following this approach, we address here the exploitation of milk components to create biocarriers for bioactives in order to design functional and natural dairy products, offering enhanced health benefits.

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Among food components, food proteins are biopolymers that appear suitable for the protection of several bioactives due to their capacity to establish various types of interactions with other compounds (Diarrassouba, Remondetto, et al., 2015). In addition, food proteins are generally recognized as safe (GRAS), which make them interesting biopolymers for the conception of biocarriers (Chen et al., 2006). In this regard, milk proteins have been specifically studied for the design of biocarrier systems, due to their versatility and excellent functional properties (Tavares, Croguennec, Carvalho, & Bouhallab, 2014). Many strategies were developed to use milk proteins as biocarriers such as formation of simple complexes with ligands, formation of gel networks by means of covalent, hydrophobic or hydrogen interactions, and formation of supra-molecular structures through electrostatic interactions (Bouhallab & Croguennec, 2014; Diarrassouba, Remondetto, et al., 2015; Schmitt, Aberkane, & Sanchez, 2009). Among milk proteins, Beta-lactoglobulin (BLG) and Lactoferrin (LF) exhibit various characteristics that make them good candidates for the design of biocarriers for bioactive compounds. BLG is a globular acidic protein (pI = 5.2) and constitutes the major whey proteins. BLG has been identified to bind various ligands either hydrophobic such as curcumin, vitamin E (Eratte, Wang, Dowling, Barrow, & Adhikari, 2014; Liang, Tremblay-Hébert, & Subirade, 2011; Teng, Li, & Wang, 2014), fatty acids, or hydrophilic such as vitamin B9 (Pérez-Masiá et al., 2015; Zhang, Liu, Subirade, Zhou, & Liang, 2014). Lactoferrin (LF) is an iron-binding glycoprotein folded into two symmetrical lobes (the N-lobe and C-lobe). LF is a basic protein (pI = 8.6) and thus carries positive electric charges at neutral and acidic pH. LF is a multifunctional protein with immunomodulatory, antimicrobial and antioxidant properties. Furthermore, recent studies have reported the ability of BLG and LF to spontaneously co-assemble to form two types of supra-molecular structures: aggregates or heteroprotein complexes called “coacervates” through a process of complex coacervation (Anema & (Kees) de Kruif, 2014; Tavares, Croguennec, Hamon, Carvalho, & Bouhallab, 2015). The shape of co-assembly depends strongly on the total protein concentration and protein stoichiometry of the system (Anema & (Kees) de Kruif, 2014; Bouhallab & Croguennec, 2014; Yan et al., 2013). Heteroprotein aggregation is often described as a kinetically controlled process. The resultant aggregates are typically fractal objects, which form often irreversible supra-molecular structures (Bouhallab & Croguennec, 2014; Yan et al., 2013). Complex coacervation is a spontaneous co-assembly occurring between two oppositely charged biopolymers and leading to phase separation (Bungenberg de Jong, 1949; Overbeek & Voorn, 1957). It generates the formation of two separated liquid phases: the dilute phase, rich in solvent and poor in biopolymers and the dense phase, concentrated in biopolymers, namely, complex coacervates (Schmitt et al., 2009). Complex coacervates are often reported as microspheres due to their μm size dimension and their spherical shapes. Moreover, they are often suggested as interesting biocarriers for biocative compounds (de Vos, Faas, Spasojevic, & Sikkema, 2010; Ezhilarasi, Karthik, Chhanwal, & Anandharamakrishnan, 2013; Gouin, 2004; Tavares et al., 2014). Complex coacervation has been mostly studied for proteins/polysaccharides systems (de Kruif, Weinbreck, & de Vries, 2004; Schmitt & Turgeon, 2011) and its potential applications for encapsulation as well (McClements, 2015; Ron, Zimet, Bargarum, & Livney, 2010; Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998). Nevertheless, few studies have been done to determine whether heteroprotein complex coacervation can be used for encapsulation. In this regard, complex coacervation between BLG and LF appears as good candidate as biocarrier for bioactives.

Among bioactive compounds, the vitamin B family is of great interest owing to its vital importance and biological role (Lucock, 2000). Folic acid, currently known as vitamin B9, is involved in several biochemical processes: DNA synthesis and repair, cell division and co-enzyme in carbon metabolism pathways, including the biosynthesis of several amino acids (Araújo et al., 2015). B9 has been recognized to provide several health benefits such as preventing cardiovascular diseases (Adank, Green, Skeaff, & Briars, 2003), colon cancer (Sanjoaquin, Allen, Couto, Roddam, & Key, 2005), or congenital malformations during pregnancy (Lucock, 2000; Moat et al., 2004). Nevertheless, humans cannot synthesize B9 and therefore must find sufficient sources of B9 in their daily diet (Basset, Quinlivan, Gregory, & Hanson, 2005). B9 deficiency is relatively common among the vulnerable populations such as elderly people or pregnant women (Mills & Signore, 2004). As a result, there is a need for developing foods and drinks enriched in B9 (Gregory, 2001). However, this appears as a technological challenge due to the poor solubility of B9 at acidic pH. Hence, to successfully enrich acidic food products with B9, the design of specific biocarriers for this vitamin is required. Furthermore, in literature, numerous studies and reviews can be found on the design of biocarriers for lipophilic bioactive compounds such as fatty acids, polyphenols, flavor compounds or oil-soluble vitamins (Champagne & Fustier, 2007; de Vos et al., 2010; Fang & Bhandari, 2010; Matalanis, Jones, & McClements, 2011; McClements, Decker, & Park, 2009; Xiao, Liu, Zhu, Zhou, & Niu, 2014). Nevertheless, the design of biocarriers for hydrophilic bioactive compounds such as B9 has been the subject of little attention and stays rather specific (He et al., 2015; Liang, Leung Sok Line, Remondetto, & Subirade, 2010; Pérez-Masiá et al., 2015). Here we examine in details the potentiality of the spontaneous co-assembly between LF and BLG at pH 5.5 as biocarriers for the hydrophilic vitamin B9, given that B9 interacts with LF (Tavares, Croguennec, Lê, et al., 2015). In this previous work, we showed that LF can bind until 10 mol of FA throughout electrostatic interactions following a two-step mechanism: interaction and subsequent self-association of the complexes. Here, co-assembly between LF and BLG in the presence of B9 was investigated across a range of physico-chemical conditions and the co-assembly yields were evaluated. The formation kinetics of B9-LF-BLG co-assemblies were monitored. The final morphology and the biocarrier efficiency of formed B9-LF-BLG co-assemblies were determined.

2. Materials and methods

2.1. Stock solutions

Lactoferrin (LF) from bovine milk, purity 90% and iron saturation level of 10%–20% according to the manufacturer's specifications, was purchased from the Fonterra Cooperative Group, New Zealand. LF powder was used without modification. Beta-Lactoglobulin (BLG) powder was obtained from a confidential industrial source. Its composition (w/w) was: protein 93.5%, moisture 4% and ash <1.8%. Protein purity was determined by reversed-phase HPLC and no proteins other than BLG were detected. BLG powder was dispersed in deionized water (45 g/L), adjusted to pH 4.6 with 1 M HCl and kept at 30 °C for 5 min in order to precipitate non-native forms of BLG. The dispersion was centrifuged at 20,000 g at room temperature for 10 min (Heraeus Biofuge Primo, Thermo Scientific, Waltham, MA, USA). BLG suspension was then freeze-dried and stored at −20 °C until use.

LF and BLG stock solutions were prepared by solubilizing the protein powders in milli-Q water and their pH were adjusted at pH 5.5 using 1 M HCl solution. The protein solutions were filtered through a 0.45 μm and a 0.2 μm membrane (cat. no. 4612, Pall Corporation, Ann Arbor, MI, USA). The exact proteins concentrations were determined by absorbance at 280 nm (spectrometer