Food Hydrocolloids 48 (2015) 38–46

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Interactions between different forms of bovine lactoferrin and sodium alginate affect the properties of their mixtures

Huma Bokkhim [a], Nidhi Bansal [a], Lisbeth Grøndahl [b], Bhesh Bhandari [a, *]

[a] The University of Queensland, School of Agriculture and Food Sciences, Brisbane, QLD 4072, Australia
[b] The University of Queensland, School of Chemistry and Molecular Biosciences, Brisbane, QLD 4072, Australia

ARTICLE INFO

Article history:
Received 3 November 2014
Received in revised form
29 December 2014
Accepted 31 December 2014
Available online 20 January 2015

Keywords:
Lactoferrin
Sodium alginate
Complexation
Zeta-potential
Viscosity
Gel electrophoresis
Denaturation temperature

ABSTRACT

The interactions between different forms (apo-, native- and holo-) of lactoferrin (Lf) and sodium alginate at different ratios in aqueous solution in the pH range of 4–7 were evaluated. Fourier transform infra-red (FTIR) spectra of freeze dried mixtures showed shifts only in the bands arising from the carboxylate groups of alginate relative to physical mixtures; indicating intermolecular interactions involving COO^- moieties of alginate. Circular dichroism (CD) spectroscopy showed that Lf retained its tertiary structure in the Lf-alginate mixtures. In the pH range of 4–7, the zeta-potential of Lf-alginate solutions was significantly less negative than that of alginate indicating charge compensation. Native-PAGE results indicated that the extent of binding of Lf by alginate was dependent of the form of Lf with apo-Lf displaying a higher binding affinity. At natural pH, the Lf-alginate mixtures generated higher viscosities than their respective sodium alginate controls indicating the existence of intermolecular interactions between the two components. A mixture of native-Lf and sodium alginate showed the highest increase in the viscosity while increasing level of iron saturation in Lf showed an inverse effect on viscosity. DSC analysis showed that the thermal denaturation temperature of native- and holo-Lf can be enhanced upon interaction with alginate in solution.

1. Introduction

Intermolecular interactions, mainly electrostatic interactions between proteins and polysaccharides, are explored in the food and pharmaceutical industries for the development of successful protein delivery systems (Peinado, Lesmes, Andrés, & McClements, 2010). These interactions are influenced by the physico-chemical properties of the proteins and polysaccharides as well as environmental factors (pH, ionic strength, protein-to-polysaccharide ratio, temperature and mixing process) and can be manipulated to fabricate protein/polysaccharide complexes with a new set of properties in comparison to the proteins and polysaccharides alone (Benichou, Aserin, & Garti, 2002; Schmitt & Turgeon, 2011; Ye, 2008). As detailed by Tolstoguzov (1991), the interaction between proteins and polysaccharides may lead to either their co-solubility, incompatibility (phase separation), or complexation (soluble or insoluble). The charges on the proteins/polysaccharides, presence of oppositely charged side groups (acidic and basic) and availability of charged patches in the polyions can play decisive roles in complex formation. Soluble complexes can form when the net charges of proteins and polysaccharides are very different (Ye, 2008).

The glycoprotein Lactoferrin (Lf) possesses a broad spectrum of functional properties towards humans and animals such as cellular growth regulation and differentiation, intestinal iron homeostasis, host defense against microbial infection and inflammation, regulation of myelopoiesis, immunomodulatory and anti-oxidant activities and protection against cancer (Conneely & Ward, 2004; Guo, Pan, Rowney, & Hobman, 2007). In order to exploit these benefits, Lf is being increasingly used in health products. However, these functional properties of Lf are affected by different factors during production, storage, transport and consumption such as heat, salts, pH and enzymes (Abe et al., 1991; Eriksen et al., 2010; Naidu, 2006; Onishi, 2011; Steijns, Brummer, Troost, & Saris, 2001). Lf is a cationic protein with positively charged regions most prominently at the N-terminus, as well as in an inter-lobe region between the C- and N-lobes close to a connecting helix (Moore, Anderson, Groom, Haridas, & Baker; 1997) and as such it has a high isoelectric point (pI ~ 8.0–9.0) (Baker, 2005; Brisson,

* Corresponding author. Tel.: +61 7 3346 9192.
E-mail addresses: h.rai@uq.edu.au (H. Bokkhim), n.bansal@uq.edu.au (N. Bansal), l.grondahl@uq.edu.au (L. Grøndahl), b.bhandari@uq.edu.au (B. Bhandari).

http://dx.doi.org/10.1016/j.foodhyd.2014.12.036
0268-005X/

Britten, & Pouliot, 2007; Bokkhim, Bansal, GrØndahl, & Bhandari, 2013; Ye & Singh, 2007). A substantial amount of research on Lf has been conducted by the pharmaceutical sector regarding its biochemical characterization and biological activities. In the food sector, studies have focussed on digestion and thermal stability mostly in Lf's natural form in different food systems. Lately, several researchers have explored Lf's technological properties (emulsifying and stabilizing) in oil-in-water emulsions (Bengoechea, Jones, Guerrero, & McClements, 2011; Bengoechea, Peinado, & McClements, 2011). In order to optimise stability and achieve safe delivery of Lf, its encapsulation in a high heat and acid stable matrix such as alginate is a potential avenue for new product development. The full potential of such a system can be reached only with the detailed knowledge of the intermolecular interactions between Lf and alginate and their effect on the properties of the mixture which can influence the fabrication of a delivery system and the release property of Lf.

Sodium alginate is an anionic polysaccharide extracted from the brown algae which is composed of polymeric sequences of (1–4) linked β-D-mannuronate (M) and α-L-guluronate (G) residues (Draget, 2009). Alginate molecules possess ion exchange property because of the presence of the carboxylic groups in both the M and G residues and have a high affinity for di- and tri-valent ions and cationic protein molecules molecules (Zhao, Li, Carvajal, & Harris, 2009). Electrostatic interactions between the negatively charged alginate polymer and positively charged proteins have been studied for lysozyme and chymotrypsin where gelling of the mixtures were observed (Wells & Sheardown, 2007) and for heat treated Lf particles where turbidity, dynamic light scattering and electrophoretic measurements were used to probe effect of pH and ionic strength (Peinado et al., 2010). In addition, alginate has been used to encapsulate vascular endothelial growth factor (Gu, Amsden, & Neufeld, 2004) leading to its sustained release from the alginate matrix. The interactions between the protein and alginate are mainly controlled by the charge density of the protein and the type of alginate used (e.g. M/G ratio, molecular mass) (Turgeon, Schmitt, & Sanchez, 2007). During the process encapsulating a protein, the functional properties of the protein might be altered due to e.g. electrostatic interactions with the alginate matrix (McClements, 2006). It has been reported that with some proteins such as transforming growth factor-beta (TGF-β1), irreversible complex formation can occur between the protein and alginate molecules resulting in protein inactivation. In such cases additives that protect the protein from the alginate polymer should be added to retain protein activity (Gombotz & Wee, 1998).

This study aims to investigate the interactions between different forms of Lf (apo-, native- and holo-) and alginate in aqueous solution at the natural pH of the proteins and in the pH range of 4–7. As different forms of Lf demonstrate different physico-chemical properties (Bokkhim et al., 2013), it is important to investigate how those properties affect the intermolecular interactions in the Lf-alginate mixtures. For many application of proteins, it is important to evaluate if the intermolecular interactions in the protein-polyelectrolyte complex affects the secondary and/or tertiary structure and thus functional properties of the protein, and in this study, this was evaluated using Fourier transform infra-red (FTIR) and circular dichroism (CD) spectroscopy. A measure of the binding affinity of different forms of Lf to alginate was evaluated using gel electrophoresis. zeta-potential measurements were done in order to evaluate complex formation and the properties of the mixtures were investigated through the use of viscosity, as well as DSC for the thermal properties of the protein in the mixtures. The research findings will provide fundamental information regarding the potential benefits and application of Lf-alginate mixtures.

2. Experimental section

2.1. Materials

Two forms of bovine lactoferrin (NatraFerrin), native- and apo-forms with iron saturation levels approximately 13 and 1% were provided by MG Nutritionals®, Burnswick, Australia. Sodium alginate (PE 12001-13.8 EN), GRINDSTED® Alginate FD 155 (M/G ratio 1.5) was donated by Danisco Australia Pty. Ltd., Sydney, Australia. The molecular mass was determined by U-tube viscometry using and the appropriate Mark-Houwink constant (Vold, Kristiansen, & Christensen, 2006; Vold, Kristiansen, & Christensen, 2007) and found to be 140 kDa. Bis (2-(hydroxymethyl) iminotris-[hydroxymethyl] methane) (bis-tris) (purity > 98%), potassium chloride, sodium hydroxide, sodium acetate (trihydrate), Trizma® base and glycine were purchased from Sigma Aldrich Co., Castle Hill, Australia (purity > 99%). Acetic acid (99%), hydrochloric acid (concentration ~ 31.5%) and methanol (99.8%) were from Labtek Pty. Ltd., Brendale, Australia. Sodium dodecyl sulphate (SDS) and glycerol, both of analytical grades were bought from Amresco, Solon, USA and Ajax Finechem Pty. Ltd., Taren Point, Australia respectively. The dyes, bromophenol blue and Coomassie brilliant blue G-250 were from Bio-rad, Gladesville, Australia. Millipore water was used for all experiments. All chemicals used in this study were of analytical grade. Lactoferrin having 50% iron saturation level and holo-Lf were prepared in the laboratory according to the method described by Bokkhim, Tran, Bansal, GrØndahl, and Bhandari (2014).

2.2. Methods

The Lf-alginate mixtures (2% w/w) at 1:1 mixing ratio were prepared in Millipore water, allowed to stand at room temperature (22 ± 2 °C) overnight and freeze dried (Christ, ALPHA 1-4 LSC, Osterode, Germany). Control samples of dry mixed Lf and alginate (1:1) were also analysed. Infra-red spectra were recorded on an FTIR 100 series Perkin–Elmer spectrometer fitted with a deuterated triglycine sulphate (DTGS) detector using the Universal attenuated total reflectance (ATR) mode. Spectra were recorded at ambient temperature (22 ± 2 °C) on solid samples at a resolution of 4 cm^{-1} and a scan number of 32 over the range of 4000–650 cm^{-1} with air as background. FTIR spectra of freeze dried Lf samples were similar to that of commercial as received samples (data not shown).

The structural conformation of Lf in the Lf-alginate solutions was studied by CD spectroscopy. The spectra of Lf, alginate and Lf-alginate mixtures (1:1) in aqueous solution were recorded in aqueous solution with Millipore water as the background in the wavelength region 250–350 nm using a Jasco J-710 spectrometer with a J-700 Spectra manager software. Lf and alginate samples were diluted to 0.25% while Lf-alginate mixtures were diluted to 0.50% for analysis. The measurements were made at ambient temperature (22 ± 2 °C) and the ellipticities were expressed as Milli degrees.

Surface charge properties of the Lf, alginate and Lf-alginate mixtures were measured using NanoS Zetasizer based on electrophoretic mobility of the particles. The solutions used for the measurements were acetate buffer (pH 4 and 5) and bis-tris buffer (pH 6 and 7) all with a final ionic concentration of 1 mM potassium chloride as well as water resulting in natural pH (the pH which is achieved upon dissolution of the protein). Solutions of Lf (apo-, native- and holo-) and sodium alginate were prepared in appropriate media (1% w/w). Lf was dissolved at constant stirring for 2 h at room temperature using a magnetic stirrer. Sodium alginate was dissolved using high shear homogeniser (IKA® RW 20 digital, USA) at 600 rpm for 30 min. The alginate solution was then heated in a water bath at 40 °C for 90 min to remove any trapped air bubbles. The Lf and alginate solutions were mixed in a ratio of 1:1 using the