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Effects of heat-treated β-lactoglobulin and its aggregates on foaming properties

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ABSTRACT

The effects on foaming properties of the aggregates formed by heating concentrate beta-lactoglobulin solutions (55 mg mL^{-1}, pH 6.8) at 85 °C from 1 to 15 min were investigated. Structural characteristics (size and molecular conformation), hydrophobicity and protein aggregates proportion were also studied. All tested methods pointed at 3 min of heating as a critical time, in terms of conformational changes and aggregation processes. At this time, the most significant conformational changes took place: non-native monomers were present and the greatest amount of dimers and trimers was produced, which was proved with the results of gel densitometry of SDS-PAGE, fluorescence quenching and circular dichroism tests. Foamability and foam stability were both improved by pre-heating the protein. A constant proportion among beta-lactoglobulin species (monomer 51%, dimer 33% and trimer 16%), regardless the protein concentration, led to the same results on foaming properties, confirming the link with structural changes. Aggregates formed by heating beta-lactoglobulin up to 10 min produced more stabilized foams, slowing down disproportionation, because of the formation of stiffer films. The increase in surface hydrophobicity was considered a decisive factor in the improved foamability and hydrophobic interactions improved the foam stability trough the rapid formation of a viscoelastic film.

1. Introduction

In food industry, foams are mainly stabilized by proteins. A number of papers have been devoted to the characterization of the foaming behaviour of proteins under a variety of conditions (Foegeding, Luck, & Davis, 2006; Murray, 2007).

Proteins from aqueous solutions adsorbed spontaneously to air/water interface where their free energy is lower than in the non adsorbed state. During adsorption process, proteins unfold and establish intermolecular interactions with other proteins at the interface, or close to the interface, leading to the formation of an interfacial film of proteins. Compared to low molecular weight surfactants, proteins are less effective to reduce the air/water interfacial tension but they form an interfacial film exhibiting viscoelastic properties that are thought to improve the resistance of the foam under stress or aging conditions (Damodaran, 2005; Foegeding et al., 2006).

Whey proteins, milk proteins that remain soluble after rennet or acid precipitation of caseins, have been increasingly utilized as a source of protein as well as a functional ingredient in many formulated foods. Heating induces denaturation/aggregation of whey proteins, modifying their functional properties. The majority of the studies on whey protein aggregation have been confined to model systems which use purified beta-lactoglobulin (β-LG), because it is the most abundant protein among whey proteins and readily purified (De la Fuente, Singh, & Hemar, 2002).

Under physiological conditions, β-LG associates as a non-covalent dimer. It is thought that β-LG native dimer is in rapid equilibrium with β-LG native monomer (McKenzie, 1971). β-LG monomer is a single polypeptide chain of 162 amino acids, with a molecular weight of 18.3 kDa and its structure involves eight strands of anti-parallel β-sheets and one α-helix, as it was determined by X-ray crystallography (Papiz et al., 1986). Each monomer has two disulfide bonds (Cys66–Cys160 and Cys106–Cys119), which stabilize the protein tertiary structure and one free sulfhydryl group at position Cys121 buried within the protein structure at pH > 7.5.

Using pre-heated β-LG solutions (1.0 mg mL^{-1}, pH 7.0) at 80 °C during different periods of time (5–30 min), Kim, Cornec, and Narsimhan (2005) found that thermal treatment enhanced foam stability. These authors did not characterize the species in solution. However, taking into account the low protein concentration they used and the temperature and time of heating, it can be inferred from previous works (Croguennec, O'Kennedy, & Mehra, 2004; Manderson, Hardman, & Creamer, 1998; Prabakaran & Damodaran, 1997; Schokker, Singh, Pinder, Norris, & Creamer,

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1999) that the species of β-LG which are responsible for this particular behaviour were native monomers, non-native monomers and oligomers of low molecular weight (dimers and trimers).

On the other hand, Rullier, Novales, and Axelos (2008) heated β-LG solutions of different concentrations (1, 2, 8 and 10 mg mL^{-1}) at 80 °C for 24 h, in order to obtain aggregates of high molecular weight. Their results showed that these aggregates varied between 35 and 197 nm and were not able to improve the foaming properties.

However, there are some differences between the experimental conditions in which those cited works were carried out and the conditions used in several industrial processes, e.g.: the protein concentrations currently used in food industry are greater, i.e. ≥50 mg mL^{-1} (McIntosh et al., 1998) and most of the industrial heat treatments applied to the samples are not as drastic as the long time of heating used by Rullier et al. (2008). In that sense, the aim of the present work was to investigate the effect on foam properties of the aggregates formed by heating concentrated β-LG solutions (55 mg mL^{-1}, pH 6.8) at 85 °C for periods of time from 1 to 15 min, conditions which are similar to those in industrial processes. Structural characteristics (size and molecular conformation), hydrophobicity and protein aggregates proportion were also studied.

2. Materials and methods

2.1. Heat treatment of β-LG

A stock 55 mg mL^{-1} β-LG solution was prepared in 20 mM phosphate buffer at pH 6.8. Aliquots from this solution were placed in small glass tubes and heated in a water bath at 85 °C. The heating times varied from 1 to 15 min. The samples were cooled to room temperature and analyzed as it is described in the following sections. The concentration of β-LG solutions was verified through the measurement of absorbance at 280 nm and calculated with the use of Beer's law with an extinction coefficient of 0.966 mL mg^{-1} cm^{-1}.

2.2. Electrophoresis

SDS-PAGE of native and heated β-LG was performed as Laemmli (1970) described, using a stacking gel of 10% and a running gel of 15%. The gels were run under nonreducing conditions to avoid cleavage of intermolecular disulfide bonds formed during the heating treatment. After electrophoresis, gels were stained with Coomassie Brilliant Blue R250 and scanned using a Hewlett–Packard ScanJet 5p connected to a computer. To quantify the relative intensities of the stained protein bands, the pixel densities of digitized images were analyzed using software developed by our group (Palazolo, Rodriguez, Farruggia, Picó, & Delorenzi, 2000). The molecular weight of each protein band was matched to known standard proteins.

In order to identify the β-LG species which are responsible for the foaming behavior obtained by Kim et al. (2005), a β-LG solution 1.0 mg mL^{-1} was heated at 80 °C, with heating times varying from 5 to 30 min, and also studied by SDS-PAGE.

2.3. Circular dichroic spectral measurements

Circular dichroism (CD) of β-LG solutions was measured using a Jasco J-810 automatic recording spectropolarimeter (Japan), with an integration time of 2 s and 2 nm bandwidth. Four scans at a rate of 50 nm min^{-1} were carried out and their results were averaged for the wavelength range employed. In the experiments in far UV (190–250 nm), 25 μM protein solutions were assayed in 0.1 cm path length cell. In the near UV (250–320 nm), 164 μM protein solutions were assayed in 1 cm path length cell. The CD data were reported as mean residue ellipticity ($[\theta]_{MRW}$) in units of deg cm^2 $dmol^{-1}$, using a mean residue weight of 113 g.

The CD spectra of the native and heat-treated protein solutions were compared to assess any conformational changes. The far UV spectra were analyzed to infer different proportions of α-helix, β-sheet and random coil using CONTIN software that compared the spectrum with the spectra of 16 standard proteins of different conformations (Chang, Wu, & Yang, 1978). The near UV CD spectra were used to characterize the tertiary structure of proteins mainly because of the constrained asymmetries in the environment of the aromatic amino acids (Greenfield, 1996).

In order to compare these spectra with those of chemically denatured β-LG, solutions of this protein in 8 M urea, without any heating treatment, were also assayed.

2.4. Measurement of β-LG surface hydrophobicity using acrylamide

Three milliliters of a sample, 10 μM in β-LG, was placed in the cell of a Jasco FP-770 spectrofluorometer, and the fluorescence intensity (F_0) was measured at 337 nm, using excitation at 295 nm. Aliquots of 7 M acrylamide, used as fluorescence quencher, were sequentially added in the cell content, and the new fluorescence intensities were measured (F). Acrylamide concentrations ranged from 0 to 0.2 M. The F_0/F ratio was plotted versus the quencher concentration (Stern–Volmer plot). In the used range, this plot was linear and the Stern–Volmer equation can be expressed as

$$\frac{F_0}{F} = 1 + K_{app}[\text{acrylamide}] \quad (1)$$

K_{app} is an apparent constant because β-LG has more than one tryptophanyl residue that can be quenched by acrylamide. The initial slope of Stern–Volmer plots, K_{app}, is an index of protein hydrophobicity.

Two other methods were tested to a further study of surface hydrophobicity: binding *cis*-parinaric acid (CPA) or 1-anilino-8-naphthalenesulfonate (ANS) to the protein. Their results completely agreed with the fluorescence quenching method and led to similar conclusions (Moro, Gatti, & Delorenzi, 2001). The fluorescence quenching of β-LG in urea 8 M without any heating treatment was also studied, in order to compare chemically and thermally denatured structures.

2.5. Foaming properties

Foams were formed using a bubbling apparatus (Hagolle, Relkin, Popineau, & Bertrand, 2000; Loisel, Guéguen, & Popineau, 1993). Native and heated β-LG was diluted to 0.1% (w/v) in 20 mM phosphate buffer, pH 6.8. Determinations were made in a transparent acrylic tube (3.5 cm × 20.0 cm) equipped with a pair of electrodes located at the base of the column and with a porous disk through which air, at a flow rate of 5 mL s^{-1}, was passed and forced through the liquid (V_{init} = 10 mL), creating foam. Bubbling stopped when the foam reached a fixed volume of 115 mL (V_f). During the test, the conductivity and the volume of foam were recorded by a computer and a digital camera Olympus DS-580 4.0 mega pixels.

Conductivity measurements at different times (C_t) and with reference to the initial conductivity (C_{init}) were used to calculate the volume of liquid in the foam (V_{LF}) (Chevalier, Chobert, Popineau, Nicolas, & Haertle, 2001; Loisel et al., 1993):

$$V_{LF} = V_{init}\left[1 - \frac{C_t}{C_{init}}\right] \quad (2)$$