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Structural properties of condensed ovalbumin systems following application of high pressure

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ABSTRACT

The effect of high pressure on thermomechanical and physicochemical properties of ovalbumin samples up to 80% (w/w) solids is presented and compared to conventional thermal treatment. Results from small deformation dynamic oscillation in shear, modulated differential scanning calorimetry (MDSC) and Fourier transform infrared spectroscopy (FTIR) revealed that pressure-treated ovalbumin maintains its native conformation in condensed systems of 80% (w/w) solids, whereas its structure has been irreversibly changed in the aqueous environment of 20% (w/w) solids, and partially altered at intermediate levels of solids (30–60%, w/w). That was rationalized on the basis of specific rearrangements between sulfhydryl and disulphide bonds following application of high pressure and the high hydrophobicity of the ovalbumin molecule. Cooling to subzero temperatures results in vitrification and the formation of a matrix with glassy consistency for both atmospheric and pressurised materials. Application of the method of reduced variables and the combined WLF/free volume theoretical framework are able to predict the glass transition temperature of condensed ovalbumin preparations.

1. Introduction

High hydrostatic pressure processing applied to foodstuffs has become of interest recently both in academia and industry. The effects of high pressure have been reported on four main areas: inactivation of microorganisms, enzymatic activity, protein conformation, and thermodynamic phase changes of materials depending on applied pressure/temperature regime and duration of the experimental treatment (Messens, Van Camp, & Huyghebaert, 1997). As pointed out in most of the studies, high hydrostatic pressure can affect protein systems at levels of secondary, tertiary and quaternary structures *via* driving the dissociation and unfolding/disassembly of protein's conformational chemistry (Gross & Jaenicke, 1994; Mozhaev, Heremans, Frank, Masson, & Balny, 1996; Smith, Galazka, Wellner, & Summer, 2000).

Ovalbumin is a monomeric phosphoglycoprotein with a molecular weight of 45 kDa and isoelectric point of 4.5, comprising four sulfhydryl groups and one disulfide bond buried in the protein core (Iametti et al., 1998; Mine, 1995). The three dimensional structure of ovalbumin is mainly arranged by α-helical reactive loop, which is 41% of the molecule, 34% β-sheet, 12% β-turns and 13% random coils (Ngarize, Herman, Adams, & Howell, 2004). Molecules have a tendency to aggregate under various conditions including high pressure. However, previous investigations revealed that the extent of ovalbumin denaturation by high pressure is much less than for either heat or chemicals. The pressure-induced egg white gels are also softer and more elastic than heat-induced counterparts (Hayashi, Kawamura, Nakasa, & Okinaka, 1989).

High pressure induces a decrease in solubility and buried SH content in ovalbumin being accompanied by an increase in turbidity and surface hydrophobicity, which depends on pH, protein concentration, ionic strength, and extent of pressurisation (Van der Plancken, Van Loey, & Hendrickx, 2007). Studies on the solubility of ovalbumin at neutral pH indicate the formation of insoluble aggregates after pressurization, as a function of protein concentration and treatment intensity, due to modification in the tertiary structure of the protein (Iametti et al., 1998). Using spectrofluorometry, it was demonstrated that the native conformation of ovalbumin remains largely stable after pressurization at 400 MPa at low protein concentration, although the α-helix structure was reduced from 41% to 29% (Hayakawa, Linko, & Linko, 1996; Messens

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et al., 1997). FTIR experiments also indicate rearrangements in the secondary structure of pressurized ovalbumin and formation of intermolecular hydrogen bonds (Mine, 1995).

Most of the aforementioned studies have been focussed on the qualitative and quantitative description of changes observed in pressure-treated ovalbumin systems at a relative low level of solids content, typically up to 20% (w/w). There is scanty information on the effect of high-pressure on globular systems reaching higher level of solids (up to 80%), which of course is of great industrial interest for inclusion in high solid formulations of powders, snacks, breakfast cereals, etc. Recent studies on the application of high pressure in condensed materials have been carried out in whey proteins and immunoglobulins extracted from whey proteins (Dissanayake et al., 2012; George et al., 2013). It was demonstrated that at these high levels of solids, systems undergo a pressure induced glass transition, which has been contrasted with the conventional temperature effects.

There is no relevant data describing the effect of high pressure at ambient temperature on a range of ovalbumin gels from low to intermediate and high levels of solids. In this study, we make an effort to characterize thoroughly the application of high hydrostatic pressure on the structural properties of such ovalbumin preparations through sensitive and small deformation thermomechanical analysis. This is further assisted by wide angle X-ray and FTIR techniques to identify the conformational arrangements in ovalbumin secondary structure following application of high pressure up to 600 MPa. In addition, the current investigation utilises certain theoretical modelling in order to provide physical significance to the viscoelastic properties of the material at subzero temperatures. Molecular understanding was pursued by contrasting the structural properties of high solid atmospheric (i.e. thermally treated) and pressurized ovalbumin materials in terms of the glass transition temperature.

2. Experimental protocol

2.1. Extraction of ovalbumin from egg white

Ovalbumin was extracted from chicken egg whites using a four-stage crystallization technique according to the method of Kekwick and Cannan (1935). A solution of Na_2SO_4 (40% w/w) (Sigma Aldrich, Australia) was prepared by dissolving anhydrous salt in warm water. Fresh chicken eggs were collected from a local market in Melbourne, Australia, their volume was measured and an equal volume of the salt solution was added. The mixture was stirred for 2 h and the precipitate was discarded using centrifugation at 6500 *g* for 10 min at 25 °C. The liquid phase was filtered and a solution of 0.2 N H_2SO_4 was slowly added to the filtrate, with the latter being stirred mechanically until the pH reached 4.6–4.8. Stirring was continued and anhydrous Na_2SO_4 was added slowly until a permanent opalescence developed. Once the crystallisation of protein became evident, the mixture was kept at room temperature overnight, and the crystalline material was separated using centrifugation at 6500 *g* for 10 min at 25 °C.

The resultant was redissolved in a volume of water, which was approximately equal to the original volume of egg white. Recrystallisation was then effected by addition of anhydrous Na_2SO_4 accompanied with stirring. After two stages of further recrystallisation, the final product was brought into solution and dialysed in deionized water for 24 h at room temperature. The dialysate was freeze dried and stored at 5 °C for further experimentation. Three replicates of the freeze dried material were analysed using the Bradford method to identify the protein content, which was found to be 91.5%.

2.2. Preparation of ovalbumin samples

Ovalbumin protein dispersions were prepared by dissolving freeze-dried powder in deionized water at neutral pH and room temperature to provide concentrations of approximately 10% (w/w). Solutions were stirred for approximately 2 h using a magnetic stirrer to ensure proper dissolution, and then stored overnight at 4 °C. Following this, a series of concentrations for the ovalbumin samples (20–80%, w/w) were prepared by dehydrating this original material using a vacuum rotary evaporator at 40 °C (±1 °C).

Prepared samples were placed in plastic bags and then vacuum sealed using a vacuum sealer. Vacuum bags were then placed in a holding basket, loaded into a stainless steel pressure vessel and treated with high hydrostatic pressure at 600 MPa at about 21 °C (the temperature did not exceed 40 °C during experimentation) for 15 min using a cylindrical vessel apparatus of high pressure with 35 L volume and 70 mm plunger diameter (Quintus Press-QFP 35L, Avure Technologies, Kent, WA, USA). Demineralised water served as the pressure transmission medium and the pressure build up was performed gradually at about 100 MPa per 20 s until the desired pressure level was attained. In order to compare the results of pressurized ovalbumin with heat treated counterparts, the thermal treatment was performed by subjecting the ovalbumin samples to a heating ramp to 85 °C at atmospheric pressure. Pressurized and heat-treated samples were stored at 4 °C for characterisation of molecular, thermal and structural properties.

2.3. Experimental analysis

2.3.1. Sodium dodecylsulphate polyacrylamide gel electrophoresis

SDS-PAGE was performed according to the method of Laemmli (1970) in a Bio-Rad mini-Protein electrophoresis cell at a constant voltage (100 V) with a gel concentration of 6%. Ovalbumin samples for SDS-PAGE were prepared by mixing with sample buffer (60 mM Tris–HCl, 2% SDS, 14.4 mM β-mercaptoethanol, 25% glycerol, 0.1% bromophenol blue, pH 6.8). Freeze dried powders of ovalbumin were resolved on a 12.5 separation gel, stained with 0.1% Coomassie Brilliant Blue for 30 min and were stored overnight. The solution was homogenised by 30 s sonication cycles for 3 min. Two different concentrations of non-denatured ovalbumin samples (50 and 100%) and five different concentrations of heat denatured protein samples (10, 40, 50, 70 and 90%) were prepared from the stock material (ovalbumin powder as 100%). Following migration, the protein fractions were stained with 0.1% Coomassie Brilliant Blue.

Fig. 1 depicts the SDS-PAGE profile of extracted ovalbumin from egg white at non-denatured and thermally treated conditions. In both cases, ovalbumin exhibits a single band around a molecular weight of 45 kDa. This result indicates high purity for the extracted proteinaceous fraction and is according to experience (Le Maire et al., 1990; Moon & Song, 2001).

2.3.2. Rheological measurements

Atmospheric and pressurized ovalbumin samples were examined using the technique of small-deformation dynamic oscillation in shear. A controlled strain rheometer (TA Instruments, Ltd., New Castle, DE, USA) was employed to obtain measurements. Steel parallel-plate measuring geometries were employed (40 and 20-mm diameter) with automatically controlled gap. Samples containing 10–80% (w/w) total solids were loaded onto the Peltier and allowed to equilibrate at 25 ± 0.5 °C for 2 min. The exposed edges of samples were covered with a silicone fluid from BDH (100 cs) to minimize variation in the water content. Measurements involved heating of the samples from 25 to 85 °C, keeping at 85 °C for 20 min followed by cooling to 5 °C at a constant rate of 2 °C/min and a frequency of 1 rad/s. Further observations were carried out with

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