



## Structural behaviour in condensed bovine serum albumin systems following application of high pressure



Sobhan Savadkoohi<sup>a</sup>, Anna Bannikova<sup>a</sup>, Stefan Kasapis<sup>a,\*</sup>, Benu Adhikari<sup>b</sup>

<sup>a</sup> School of Applied Sciences, RMIT University, City Campus, Melbourne, Vic. 3001, Australia

<sup>b</sup> School of Health Sciences, University of Ballarat, Mt. Helen Campus, Ballarat, Vic. 3353, Australia

### ARTICLE INFO

#### Article history:

Received 18 July 2013

Received in revised form 3 October 2013

Accepted 6 November 2013

Available online 16 November 2013

#### Keywords:

Bovine serum albumin  
High hydrostatic pressure  
High-solid systems  
MicroDSC  
FTIR  
Mechanical  $T_g$

### ABSTRACT

The present study shows that application of high hydrostatic pressure of 600 MPa for 15 min at ambient temperature on condensed bovine serum albumin systems (BSA) with up to 80% w/w solids content has a limited effect on the conformational structure of the protein, as compared to thermal treatment. This was demonstrated throughout the experimental concentration range using small-deformation dynamic oscillation, differential scanning calorimetry and infrared spectroscopy. BSA possesses seventeen disulfide linkages per molecule, which constitutes a stable arrangement with high energy requirements for substantial structure alteration. Upon cooling, pressurised materials undergo vitrification and networks exhibit comparative mechanical strength to that of thermally treated counterparts. The mechanical manifestation of the glass transition region and glassy state for atmospheric and pressurised samples was examined by the method of reduced variables and the combined framework of WLF/free volume theory producing disparate predictions of the glass transition temperature for the two types of polymeric network.

© 2013 Published by Elsevier Ltd.

### 1. Introduction

In the last ten years, there has been a marked increase in the application of high pressure processing (HPP) in the food industry worldwide to create “fresh” fruit juices with enhanced natural flavour retention and higher content of natural vitamins, compared to conventional thermally treated products. In terms of protein functionality, which is the subject of interest in this work, pressure processing can lead to denaturation and different states of viscosity, aggregation or gelation, depending on the protein system, treatment temperature, physicochemical environment of the solution and magnitude or duration of the applied pressure (Patel, Singh, Havea, Considine, & Creamer, 2005).

The effect of high hydrostatic pressure on the structure of proteins in aqueous solution has received considerable attention over the last few years (Dumay, Kalichevsky, & Cheftel, 1994; Hayakawa, Kajihara, Morikawa, Oda, & Fujio, 1992). These studies have confirmed the view that, since formation of ion pairs or hydrophobic bonds is accompanied by a substantial positive volume change, high pressure treatment has a disruptive effect on intermolecular electrostatic and hydrophobic interactions. On the other hand, hydrogen bond formation is associated with a small (usually negative) volume change, and so hydrogen bonding is relatively insen-

sitive to pressure. This means that high pressure may be able to disrupt the secondary, tertiary and quaternary structure of globular proteins but has no effect on the primary structure (Dumay et al., 1994).

Bovine serum albumin (BSA) is a protein component of whey and blood. Its physicochemical and structural properties have been well-characterised, making it a desirable model system for food related research (Leibman, Tiktopulo, & Privalov, 1975). In terms of thermal processing, the formation of a gel network takes place at moderate operating temperatures (80 °C for 30 min). Pressure-induced unfolding of BSA can rapidly lead to aggregation at pressures above 400 MPa, depending on protein concentration and treatment time, but there is no subsequent gelation in samples subjected to high pressure of 400 MPa for 30 min. Using spectrofluorometry, it was demonstrated that the conformation of BSA remains fairly stable at pressures of 400 MPa and a low protein concentration. This pressure stability of BSA is positively correlated with the high number of disulphide bonds stabilizing its three-dimensional structure (Michnik, 2003; Hayakawa et al., 1992).

The application of high pressures affects the secondary structure of BSA, with the changes mainly consisting of a decrease in the  $\alpha$ -helical structure, accompanied by a slight increase in  $\beta$ -sheet structure (Hosseini-Nia, Ismail, & Kubow, 2002). Most studies report that pressures between 800 and 1000 MPa are required to bring upon a considerable alteration to the secondary structure of the protein. Hayakawa, Linko, and Linko (1996) reported a

\* Corresponding author. Tel.: +61 3 99255244; fax: +61 3 99255241.

E-mail address: [stefan.kasapis@rmit.edu.au](mailto:stefan.kasapis@rmit.edu.au) (S. Kasapis).

decrease in the BSA  $\alpha$ -helix content of up to 50% at 1000 MPa at 30 °C after 10 min. These structural modifications of the protein under pressure can be irreversible depending on pH, protein concentration and accompanying thermal treatment or shear.

Literature thus far has reported on relatively dilute aqueous systems, i.e. up to 10% or 20% (w/w) solids content, and demonstrated the effect of the level and time of pressurisation on reversible or irreversible conformational change in the protein structure (Hayakawa et al., 1992; Hosseini-Nia et al., 2002; Patel et al., 2005). The present investigation employs several physicochemical techniques and theoretical modelling for the characterisation of the structural properties of atmospheric and pressurised condensed BSA systems, reaching a solids content of up to 80% (w/w) in preparations. In these high-density systems, protein macromolecules are unable to rearrange themselves into regularly ordered forms and instead exhibit an amorphous rubbery or glassy consistency (Kasapis & Mitchell, 2001).

The passage from the rubbery to glassy state is in the nature of a second-order thermodynamic transition, describing changes in state but not in phase (Kasapis, 2008). Headway in this type of research is achieved by adapting theoretical frameworks from the “sophisticated synthetic polymer science”, which allows for the determination of viscoelastic functions in relation to time or temperature of observation. Molecular and macroscopic properties of concentrated BSA matrices have been thus examined in this work using FTIR, microDSC and dynamic oscillatory rheometry in shear, where very little, if anything, has been reported in the literature in terms of the pressure effects.

## 2. Materials and methods

### 2.1. Materials, sample preparation and treatment

Bovine serum albumin (BSA) was a lyophilized powder from Sigma–Aldrich, Castle Hill, NSW, Australia. It had purity of more than 98% based on agarose gel electrophoresis, the molecular weight was about 66 kDa and the pH was between 6.8 and 7.2 in a 1% (w/w) solution of 0.15 M NaCl.

Dispersions of 10–30% (w/w) solids were prepared by mixing the powder in deionized water at neutral pH and ambient temperature. These were stirred for approximately 2 h using an IEC magnetic stirring at 510 rpm, to ensure proper dissolution. They were stored overnight at 4 °C to achieve thorough hydration and the removal of air bubbles. A series of concentrations for the BSA protein samples (40%, 45%, 50%, 55%, 60%, 65%, 70%, 75% and 80%, w/w) were prepared by dehydrating the original material using a rotary evaporator at 40 °C ( $\pm 1$  °C) and all samples were made in triplicate to allow for experimental replication.

Samples were treated with high hydrostatic pressure at ambient (initial) temperature (22 °C) for 15 min using the cylindrical chamber of a high pressure unit, with a 35 litre volume and 70 mm plunger diameter (Quintus Press – QFP 35L, Avure Technologies, Kent, WA, USA). The pressure medium was demineralised water, the pressure build up was performed at about 100 MPa per 20 s and the accessible pressure range extended to 600 MPa, which was sufficient to induce considerable structural effects on the materials. The recorded temperature profile during HPP showed that during compression to 600 MPa the temperature increased by about 20 °C. The maximum temperature (about 42 °C) was not high enough to induce thermal denaturation of BSA during pressurisation. Atmospheric samples, and pressurised counterparts (following HPP treatment), were subjected to physicochemical experimentation over a broad temperature range of –70 to 90 °C (Dissanayake et al., 2012; Kasapis & Mitchell, 2001).

### 2.2. Methods of analysis

#### 2.2.1. Small-deformation dynamic oscillation

Small-deformation dynamic oscillation in shear was used to analyse the BSA samples, being pressure or thermally treated, with a controlled strain AR-G2 rheometer (TA Instruments, New Castle, DE, USA). Standard steel parallel plate geometries of 10, 20 and 40 mm were needed to obtain measurements of samples containing 10%, 20%, 30%, 40%, 50%, 60%, 70% and 80% (w/w) total solids. Thermal gelation of the BSA samples was carried out by applying small amplitude oscillatory measurements at a constant strain of 0.1–0.001% and an angular frequency of 1 rad/s. About 3.1 ml of sample was introduced onto the Peltier plate of the instrument and a thin layer of silicon oil was placed on the gap between the two measuring plates to minimise evaporation. Prior to analysis, samples were equilibrated at 25 °C for 2 min. Samples were then heated from 25 to 85 °C at a rate of 2 °C/min, kept at that temperature for 20 min under observation and further monitored with an increasing frequency from 0.1 to 100 rad/s at 85 °C to examine the nature of formed gels. Thus, the low-amplitude oscillatory measurements of the real ( $G'$ ; storage modulus) and imaginary ( $G''$ ; loss modulus) parts of the complex shear modulus ( $G^* = G' + iG''$ ), and  $\tan \delta$  ( $G''/G'$ ) were recorded at atmospheric pressure (Dissanayake et al., 2012; Kasapis, 2012). A similar experimental protocol was adopted for materials at this solid content that were previously subjected to high pressure. All experiments were performed in triplicate and effectively overlapping traces were reported.

The investigation of vitrification phenomena using small amplitude oscillatory measurements of 80% (w/w) BSA samples at atmospheric pressure or pressurised at 600 MPa for 15 min was obtained at a constant strain of 0.001% and angular frequency of 1 rad/s. In doing so, the 80% BSA samples at atmospheric pressure were thermally denatured by heating from 25 to 85 °C at a rate of 2 °C/min, kept at that temperature for 20 min and then cooled down at 2 °C/min to subzero temperatures. Frequency sweeps were also carried out from 0.1 to 100 rad/s and strain of 0.001% at the low temperature end of the experimental routine, at constant temperature intervals of 4 °C to facilitate theoretical modelling of the molecular processes responsible for the observed viscoelastic behaviour in the glass transition region and glassy state. Pressurised samples of 80% BSA were cooled from 25 °C to subzero temperatures at the above controlled scan rate followed by frequency sweeps to match the analysis implemented for the atmospheric pressure counterparts. In the high solid (80%) regime, there was no ice formation at subzero temperatures, which allowed for treatment of the results with the glass transition theory.

#### 2.2.2. Micro differential scanning calorimetry

Thermal analysis of the BSA samples was measured using a micro differential scanning calorimeter VII (Setaram, France). The calorimetric furnace of Micro DSC VII was made of a double aluminium wall, with the experimental and reference vessels being inserted into the furnace via two cylindrical cavities. The minimum and maximum temperatures that could be reached using a Peltier device were –20 and 120 °C, respectively. The purging rate of nitrogen gas was 50 ml/min. Samples of around 850 mg were accurately weighed into aluminium pans and hermetically sealed. A pan with water of equal weight was used as the reference. The 10%, 20%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75% and 80% (w/w) BSA samples at atmospheric pressure were analysed by equilibrating at 25 °C, ramping the temperature to 100 °C at a rate of 1 °C/min and then cooling to 20 °C at 1 °C/min. Similar experimental settings were utilised for the pressure treated samples at the same concentrations. The enthalpy of denaturation ( $\Delta H$ ) and denaturation temperature ( $T_d$ ) were determined using the instrument software after manually setting the start and end

Download English Version:

<https://daneshyari.com/en/article/7599075>

Download Persian Version:

<https://daneshyari.com/article/7599075>

[Daneshyari.com](https://daneshyari.com)