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Thermomechanical effects of co-solute on the structure formation of bovine serum albumin

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ABSTRACT

The effect of glucose syrup on the structural properties of bovine serum albumin has been addressed in preparations from low to high solids. Fifteen percent protein was mixed with the co-solute at concentrations up to 65% and subjected to thermal treatment to examine the changes in phase and state transitions. Thermomechanics were the working protocol being carried out with micro differential scanning calorimetry and small deformation dynamic oscillation. Results argue that protein molecules have been extensively stabilised by the addition of a co-solute, recorded *via* a delayed thermal denaturation. Further, increasing the glucose syrup enhanced polymer–polymer interactions leading to stronger networks following thermal denaturation of the globular protein. Condensed BSA/glucose syrup mixtures, i.e. at 80% solids, were cooled at subzero temperatures to exhibit a considerable state of vitrification. Molecular relaxation phenomena were successfully followed using theoretical concepts from synthetic polymer research to yield the mechanical glass transition temperature.

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1. Introduction

Bovine serum albumin (BSA) is a fraction of whey protein exhibiting important structural and textural characteristics (Hines & Foegeding, 1993). Even though BSA is present in less than 10% in whey protein, along with β-lactoglobulin, it provides a range of diverse functional properties to food products, from viscous fluids to hard and cohesive gels (Hiller & Cheeseman, 1979; Zeigler & Foegeding, 1990). Its functional properties, as for other globular proteins, are dependent on the molecular structure, presence of counterions, solvent quality, thermal processing and applied mechanical stress.

BSA is a relatively large protein molecule, made up of 580 amino acid residues containing 17 disulphide bonds and a free thiol group at position 34 in the peptide sequence (Kinsella & Whitehead, 1989). Its globular conformation includes 54% α-helix and 40% β-structure (both β-sheets and β-turns). The high percentage of the stable helical structure in BSA is expected to deliver molecular stability to external stresses, such as hydrostatic pressure (Kinsella, Whitehead, Brady, & Gringe, 1989). The mechanism of thermal protein denaturation has been a well-established process involving two molecular paths. First, conformational changes in the protein, due to the application of sufficient heat induce unfolding of some polypeptide segments. There is a transition from the native to progel state where intramolecular hydrogen bonding and electrostatic forces of the native state engage in intermolecular bonding. During the subsequent phase, protein–protein interactions intensify, with buried sulfhydryl groups initiating disulphide-sulphydryl interchange reactions, which contribute to network formation (Bernal & Jelen, 1985).

Earlier, we reported on the effect of co-solute on whey protein isolates, which is predominantly an exhibition of β-lactoglobulin in both mechanical and thermal observations (George, Lundin, & Kasapis, 2013). Beta-lactoglobulin contains approximately 10–15% α-helix and 50% β-sheets, as estimated by infrared spectroscopy and circular dichroism (Creamer, Parry, & Malcolm, 1983). Even though the content of the β-sheets is relatively low in native BSA, when the temperature of the system is raised, a decrease in the α-helix is observed followed by an increment in the concentration of β-sheets before aggregation (Clark, Saunderson & Suggett, 1981). This led to the hypothesis that β-sheet hydrogen bonding plays a major role in protein aggregation. Wang and Damodaran (1991) demonstrated that addition of 0.5 M $NaClO_4$ or NaCl to BSA enhances the stability of the protein with a sequential increase in β-sheets, along with a decrease in the α-helical content. The stability of protein by the addition of $NaClO_4$ and NaCl was confirmed by the elevation in denaturation temperature from 64 to 75 °C and 70 °C, respectively.

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Sugars have been classified as kosmotropic agents by contributing to the stability and structure of protein-water interactions. Stabilisation of intermolecular interactions in protein molecules results in high denaturation temperature and an elastic network following thermal treatment. At high enough concentrations, addition of amorphous sugars, for example, high dextrose equivalent glucose syrup, prevents ice formation in systems cooled at subzero temperatures. Instead, systems should develop a glassy consistency, which is characterised by the strength and brittleness. The rubber-to-glass transformation is one of the underlying mechanisms that underpin the development of soft confections, boiled down sweets and ice-cream. The present study attempts to understand the effect of glucose syrup as a co-solute on the structural behaviour of bovine serum albumin with a solid content of up to 80% in formulations.

2. Materials and methods

2.1. Materials

2.1.1. Bovine serum albumin

The product was purchased from Sigma Aldrich Private Ltd., New South Wales, Australia. According to the supplier, the molecular weight of the lyophilised powder of bovine serum albumin was 66 kDa with a purity of 98% as observed by electrophoresis.

2.1.2. Glucose syrup

The material was a product of Cerestar, (Manchester, UK) with a dextrose equivalent of 42. The moisture content of the sample was 18% (w/w), and the percentage of solids refers to the dry solids. The polydisperse nature of this glucose syrup sample was confirmed by gel permeation chromatography (Kasapis & Shrinivas, 2010).

2.2. Methods

2.2.1. Sample preparation

Samples were formulated using a fixed concentration of protein (15%, w/w) with the glucose syrup content ranging from 0% to 65% (w/w). The amount of glucose syrup required was mixed with 10 mM $CaCl_2$ using a magnetic stirrer until a clear solution was obtained. BSA was then added piecemeal to the glucose syrup solution and stirred for 2 h until it was properly dispersed into the system. Samples were refrigerated overnight to ensure proper hydration prior to usage.

2.2.2. Rheology

Samples were analysed for their viscoelastic characteristics as a function of temperature, time and applied deformation. Systems from low to intermediate level of solids (15% to 65% total solids) were measured using a MCR 301 rheometer (Anton Paar, Virginia, USA) using bob and cup geometry with dimensions of 26.66 and 28.66 mm in internal and external diameter, respectively. Samples were loaded at 25 °C, heated to 85 °C at 1 °C/min, kept there for 60 min followed by cooling to 5 °C at the same scan rate. A solvent trap was placed throughout the experimentation to reduce the moisture loss from the sample. A constant angular frequency of 1 rad/s and a strain of 0.1% were applied during the experiment. This helped to characterise the protein structure based on the values of storage modulus (G') and loss modulus (G'') in shear.

High solid samples (80%, w/w) were analysed using an AR-G2 (TA instruments, Newcastle, DE) equipped with an environmental test chamber. This expanded the experimental temperature range to −90 °C by purging liquid nitrogen at a controlled rate. Apart from the denaturation and subsequent aggregation of the protein molecules, the cooling facility helped to investigate the relaxation of the polymeric networks at subzero temperature. Samples were analysed using parallel plate geometry of 5 mm diameter, with a thin layer of silicone oil covering the edges to minimize moisture loss during the temperature sweep. Samples were heated to 85 °C at 1 °C/min followed by an isothermal run at the same temperature for 60 min. Once a mature protein gel was obtained, it was cooled to subzero temperatures at 1 °C/min. To visualise the sample within a broad timescale, frequency sweeps from 0.1 to 100 rad/s were performed at an interval of 4 °C during heating from subzero to ambient temperatures.

2.2.3. Differential scanning calorimetry

Thermal events in the protein molecules, typically unfolding of the globular conformation at relatively high temperatures, were observed using differential scanning calorimetry, a technique that probes first order thermodynamic transitions of the proteinaceous material as an endothermic peak. The instrument (Q2000; TA instruments, Newcastle, DE) was fitted with a refrigerated cooling system (RCS 90) with a temperature range of −90 to 560 °C. Samples from low to high solid contents (15–65% total solids) were loaded in hermatic aluminium pans (~10 mg) and were scanned between 25 and 95 °C at a modulation rate of 0.53 °C every 40 s. For systems with 80% (w/w) total solids, the experimental temperature range was extended to −90 °C to observe molecular relaxations in the material, which are typical of a second order transition. This was shown as a variation in the heat flow signal arising from changes in heat capacity as the material passes from the glassy to the rubbery state.

3. Results and discussion

3.1. Mechanical approach to characterise the BSA/glucose syrup networks

The presence of a small molecule co-solute in sufficient amounts should alter the patterns of denaturation and polymeric aggregation of globular proteins upon thermal treatment. To validate this hypothesis and examine the possible effects in some detail, small deformation dynamic oscillation in shear was carried out

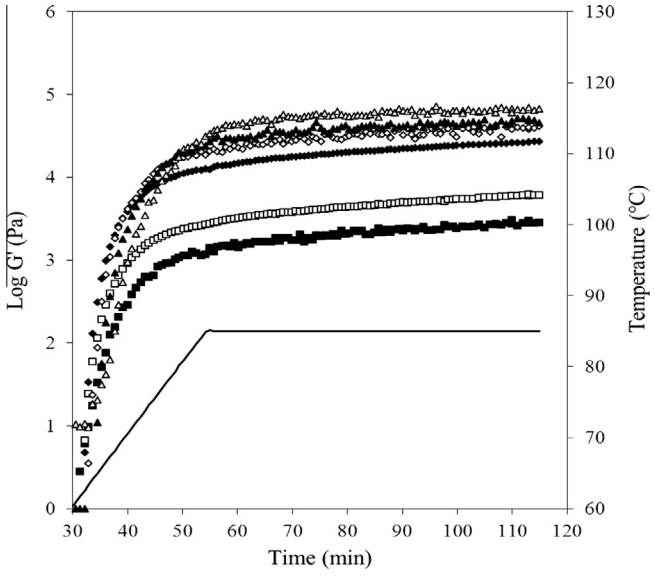

Fig. 1. Trend in the development of storage modulus for 15% bovine serum albumin with 0 (■), 10 (□), 20 (♦), 30 (◇), 40 (▲) and 50% (Δ) glucose syrup during heating to 85 °C followed by an isothermal run at the same temperature for 60 min (scan rate: 1 °C/min; frequency: 1 rad/s; strain: 1%).

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