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Studies on plasma protein interactions in heat-induced gels by differential scanning calorimetry and FT-Raman spectroscopy

Eduard Dàvila[a,*], Dolors Parés[a], Nazlin K. Howell[b]

[a]*Institut de Tecnologia Agroalimentària—CeRTA, Universitat de Girona, Escola Politècnica Superior, Av, Lluís Santaló s/n, 17071, Girona, Spain*
[b]*School of Biomedical and Molecular Sciences, University of Surrey, Guildford, Surrey GU2 7XH, UK*

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Abstract

Protein–protein interactions during the heat-induced gelation of plasma proteins were investigated by means of differential scanning calorimetry (DSC) and FT-Raman spectroscopy. The deconvolution of DSC curves revealed a specific interaction between fibrinogen and albumin, which involved changes in disulphide bonds, buriedness of hydrophobic groups and β-sheet content, as described by FT-Raman spectroscopy. On the other hand, mixtures of albumin and globulins showed specific interactions involving disulphide bonds and hydrophobic residues to a lesser extent, which could be responsible of the distinct physical attributes between heat-induced gels formed from single fractions and mixtures previously reported. In addition, an inhibitory effect of albumin on globulin's aggregation was observed in mixtures when albumin was the dominant fraction, although no major structural changes were involved in this process.

Keywords: FT-Raman spectroscopy; Differential scanning calorimetry; Heat-induced gelation; Albumin; Globulins; Fibrinogen; Serum; Plasma proteins; Protein interactions

1. Introduction

Proteins play an important role in the texture of food products owing to functional properties such as gelation, emulsifying or foaming ability. Since heating induces protein denaturation, aggregation and eventually the formation of a gel that gives consistency to the food product, many proteins act as gelling or thickening agents when a thermal treatment is applied (Ziegler & Foegeding, 1990). The thermal behaviour and textural properties of many proteins of interest to the food industry have been widely studied. However, these attributes vary in the presence of other proteins due to protein–protein interactions, because changes in the formation of disulphide bonds, hydrophobic interactions, hydrogen bonds or Van der Waals forces can lead to either an improvement or a loss of functionality (Dalgleish & Hunt, 1995). Therefore, studies about these types of interactions are especially relevant for the development of functional ingredients.

Protein–protein interactions are common phenomena during food processing that can modify the gelation properties of single proteins (Dickinson & McClements, 1996; Howell, 1992, 1995). For example, Howell and Lawrie (1984) and Dàvila, Parés, Cuvelier, and Relkin (2006, unpublished) described synergistic effects between blood plasma fractions concerning the strength of whole plasma protein gels. Comfort and Howell (2003) found that actomyosin strands interacted with wheat protein to enhance the strength of salt-soluble meat proteins/soluble wheat proteins mixtures, but when both systems were mixed at 10:10 ratio, phase separation occurred. The same authors also described precipitation in soya/whey mixtures at a 5:1 fractions ratio (Comfort & Howell, 2002).

These processes can be used to improve the functionality of single proteins, i.e., to enhance or develop textural attributes or reduce working concentrations. There is abundant literature concerning the gelation properties of blends of proteins from different origins at various proportions, describing the best relative ratios to optimise textural attributes. By contrast, there is relatively limited information about the variation of protein proportions

*Corresponding author. Tel.: +34 972 41 89 41; fax: +34 972 41 83 99.
E-mail address: eduard.davila@udg.es (E. Dàvila).

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within naturally occurring protein mixtures, examples of which are whey, soya, egg and blood plasma proteins. Indeed, it is reasonable to consider the possibility of modifying the protein contents of existing natural mixtures to find particular proportions that enhance their functionality. Thus, a natural-blend protein product could be reformulated and steered to specific food requirements.

Blood is a common by-product of the meat industry with interesting applications due to its diverse and useful functional properties, especially the ability of plasma proteins to form heat-induced gels (Hermansson, 1982; Howell & Lawrie, 1984; Oshodi & Ojokan, 1997; Parés, Saguer, Saurina, Suñol, & Carretero, 1998). Plasma contains a complex mixture of proteins, which can be classified into three major groups, namely albumin, globulins and fibrinogen. Albumin is a globular protein with a molecular weight of 69 kDa, representing up to 60% of the total protein content. Globulins are a heterogeneous group of globular proteins mainly containing α, β and γ immunoglobulins, with a wide range of molecular weights and comprise 40% of the protein content. Fibrinogen is a fibrous protein of 340 kDa made by three pairs of non-identical polypeptide chains forming two identical subunits that represents around 3% of blood plasma proteins (Putnam, 1975).

The behaviour of plasma under heat-induced gelation has been previously studied (Dàvila, Parés, & Howell, 2006; Howell & Lawrie, 1984; Parés et al., 1998). As mentioned above, it has been proven that protein fractions influence textural properties of gels; however, there is a lack of information at a molecular level concerning the contribution of each fraction in the protein–protein interaction phenomena. This could be investigated by studying the thermal properties of individual fractions and their mixtures, complemented by the use of spectroscopic techniques to directly investigate protein–protein interactions within plasma. In the present study, differential scanning calorimetry (DSC) and FT-Raman spectroscopy, both widely used in the analysis of foods and food components, were utilised to elucidate the interactions between the three main protein groups of blood plasma during heat-induced gelation.

2. Materials and methods

2.1. Blood plasma proteins

Blood was obtained from an industrial slaughter house using sterile bleeding containers containing sodium citrate as anticoagulant (1 w/w final concentration). Plasma was separated by centrifuging blood at 2520*g* at 4 °C for 15 min (Sorvall RC 5C Plus, DuPont Co., Newtown, CT) and decanting. Plasma protein fractions were separated by the salting out method, using a saturated solution of ammonium sulphate in 10 mM Tris/EDTA pH 7.4 at 4 °C as precipitating agent. Three protein fractions (fibrinogen, globulins and albumin) were isolated from plasma at progressive salt saturation (20%, 60% and 70%, respectively). Protein precipitates were collected by centrifugation (10,000*g* at 4 °C for 15 min) and washed with a solution of ammonium sulphate at the same saturation used for precipitation prior to a second centrifugation. Final protein precipitates were dissolved in 10 mM Tris/EDTA pH 7.4 and, together with aliquots of whole plasma and serum (fibrinogen-removed plasma) were exhaustively dialysed against milli-Q water with a membrane of 12–14 kDa pore diameter (Medicell International Ltd., London, UK). Dialysed protein solutions were frozen at −80 °C and freeze-dried in a Virtis Unitop SQ freeze dryer (The Virtis Co., Gardiner, NY). Purity of proteins was confirmed by SDS-PAGE electrophoresis and the proximate composition was determined. Protein content of all samples was >90%. Dried proteins were vacuum-packed and stored in chilled conditions until use.

2.2. Experimental design

Samples of plasma, serum, albumin, globulins and fibrinogen were studied, as well as mixtures of albumin and globulins at fractions ratio 2:1, 1:1 and 1:2, respectively. Interactions in protein mixtures were investigated by comparison of experimental and theoretical data. Theoretical DSC curves and Raman spectra were constructed for protein mixtures from the sum of the data of single fractions, multiplied by the proportion of each fraction present in the protein mixture being analysed. Moreover, differences between plasma and serum samples were used to elucidate the contribution of fibrinogen during the gelation process.

2.3. Preparation of samples

Protein solutions 6% and 15% (w/w) for DSC and FT-Raman experiments, respectively, were prepared in distilled deionised water containing 50 mM phosphate buffer at pH 7.0. Solutions were continuously agitated for 2 h to reach ionic equilibrium, prior to experimentation. For Raman analysis, aliquots of 15% (w/w) protein solutions (1 mL) were poured into NMR tubes (5 mm diameter, precision grade, Aldrich Chemical Company, Milwaukee, WI) and heated in a water bath at 80 °C for 45 min to form gels. Gels were immediately cooled to room temperature and stored overnight at 4 °C to age. All experiments were performed in triplicate.

2.4. DSC measurements

Heat-induced conformational changes of 6% (w/w) protein solutions were monitored by DSC (DSC822, Mettler Toledo, Switzerland), using 160 μL aluminium pans with phosphate buffer in the reference cell. Samples were held at 35 °C for 1 min and scanned between 35 and 100 °C at 3 °C min^{-1} scan rate. Peak temperatures at maximum heat flow or shoulders (T_p) and the global