H3. MILK PROTEINS AGGREGATION UNDER HIGH PRESSURE STUDIED BY SMALL ANGLE NEUTRON SCATTERING

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The food scientist is commonly confronted with the challenge of modifying the formulation of a food product. The objective may be to enhance the taste, texture or appearance of the food, to obtain a product with longer shelf-life or healthier image, or to improve manufacturing efficiency by incorporating cheaper ingredient or adopting a new processing technology. The speed with which these objectives can be accomplished depends on the level of fundamental understanding that exists on the key physico-chemical factors affecting products properties. In the case of foods colloids, it is especially important to understand how the interfacial and aggregation behaviour of polymer constituents (polysaccharides, proteins, pectins...) are affected by processing conditions (heat, drying, freezing, shear forces), or by molecular interactions with other constituents (fat, hydrocolloids, aroma, water...). One of our goal is to improve insights into such factors by taking advantage of polymer science concepts and neutron scattering technique applications to such systems, to the systematic study of model food systems [1].

In foodstuffs, proteins are very often used for their functional properties. Most of the time their abilities to act as emulsifiant, gelation or foaming agents, are related to their structure. Processing foods under high pressure often results at the molecular level in structural changes of the protein [2]. Experimental and theoretical approaches indicate that one of the underlying mechanism of pressure unfolding is the penetration of water into the protein, several intermediate states of the protein have been shown to exist, with their properties depending on the experimental conditions [3]. The isolation of folding intermediates is crucial to understand protein misfolding and protein aggregation. β-lactoglobulin (BLG) is the main protein constituent of the milk whey from ruminant. This protein is an important functional protein in foods, as it is the major component of many dairy gel and emulsions. A basic challenge of this study was to better understand the mechanism of pressure unfolding, dissociation and aggregation of BLG. We used pressure as a physicochemical perturbation to establish experimental conditions under which a different mechanism of aggregation might occur. From the small-angle neutron scattering (SANS) measurements the overall conformation of the β-lactoglobulin was studied at pH 7 on the dimeric form of the protein in a pressure range going from 50 to 300 MPa. These measurements were done "on-line" by gradually increasing the pressure. We can determine whether the dissociation of the dimeric units occurs and if the aggregation mechanism involves the monomeric form of the protein. To determine the pressure effects on the protein interactions and the variation of the value of the actual radius of gyration, the SANS measurements were performed at different protein concentrations.

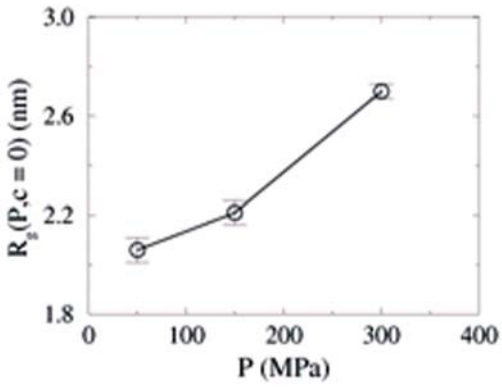

Figure 1. Value of the actual radius of gyration, Rg (c = 0) as function of pressure, P. Measurements as been made on PACE spectrometer. The range of the wave-number was between 0.07 and 0.74 nm^{-1}. A sapphire–anvil cell specially designed to perform SANS measurements has been used (M. Bonetti, SPEC, CEA, Saclay)

One of the questions addressed in this work was to know whether or not the aggregation process induced by applying pressure involves the dimeric unit or monomeric unit of the protein. This has been answered by the analysis of the evolution of the radius of gyration as a function of applied pressure (Figure 1). Our analysis shows that no dissociation of the dimmer occurs in the 50-150 MPa pressure range as our measured radius of gyration (Rg = 2.20 nm) is far away from the monomeric form (Rg = 2.06 nm). Increasing pressure up to 150 MPa leads to a swollen state of the protein that gives rise to an increase of the radius of gyration by about 7 %.

The measurements show an aggregation process occurring above 150 MPa, irreversible aggregates are formed at pressure around 300 MPa. This aggregation occurs between swollen dimeric units of the protein, which is very different that for heat-induced gel that occurs between unfolded monomeric units. Different parameters could lead to this swollen state of the protein after applying pressure: hindrance of water inside the protein matrix and/or change in the hydrogen bonds network and/or breaking down the electrostatic bonds and some of the protein hydrophobic interactions. Within this pressure range, the observation of the second virial coefficient (A_2) indicates that the interaction between macromolecules weakens although it remains repulsive (Figure 2).

It can be stated that a pressure value around 150 MPa leads to a swollen state of β-lactoglobulin and that at pressure around 300 MPa the protein begins to form irreversible aggregates. In the future it will be interesting to see the repercussion of this aggregation between dimeric units on the gels properties (rheological and neutron scattering studies).

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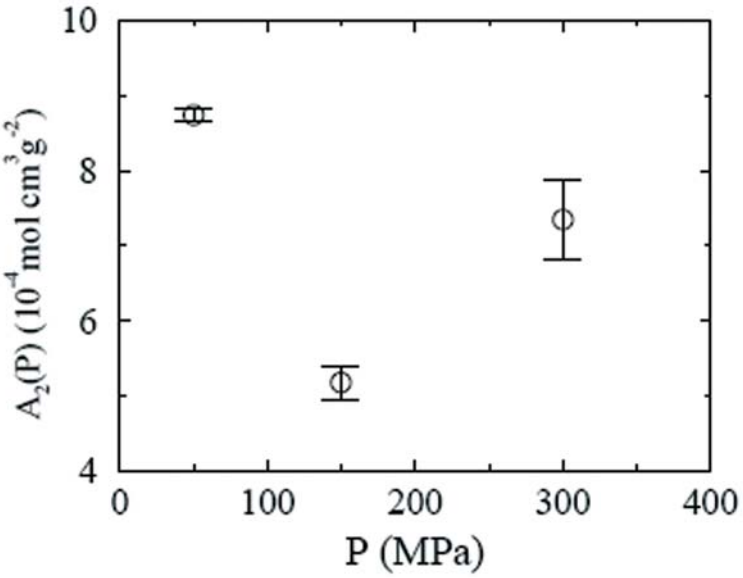

Figure 2. Second virial coefficient as function of pressure