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Interaction of transglutaminase with adsorbed and spread films of β-casein and κ-casein

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ABSTRACT

Enzymes can be used to enable a specific and controlled approach for structural modifications of protein networks in food technology. Enzymatically induced cross-links between proteins in the continuous phase and/or at interfaces result in better stabilisation and enhanced material properties in foams and emulsions. In this work the interfacial properties of β-casein and κ-casein films were investigated with a special focus on the mechanism of transglutaminase (TG) induced cross-linking at the air/water interface. The surface rheology results showed that for the enhanced interfacial strength the order and timing of TG addition matters: TG reaction was most effective when the enzyme was applied during adsorption of proteins to the interface. Differences observed between enzymatic cross-linking of β-casein and κ-casein at the air/water interface verified the importance of molecular structure and close packing for formation of an elastic protein network.

1. Introduction

Proteins are used widely to stabilise foams and emulsions, chiefly in foods but also in other applications [1–3]. Proteins are unique in their interfacial behaviour in that they often form strong viscoelastic films at interfaces, compared to that for surfactants and emulsifiers which form weak or fluid films [4]. The viscoelastic protein films confer a degree of stability to the interface, mechanically stabilising the interface to prevent coalescence between droplets and bubbles in emulsions and foams. The strength of the interface can also affect other properties of the dispersions such as bulk rheology and sensory properties. Therefore the ability to manipulate the interfacial rheology could enable control of the functional properties of emulsions and foams [4].

Caseins cover 80% of the total protein in bovine milk proteins, and they refer to a number of individual proteins in the casein fraction of milk. β-Casein is the most surface active of the casein proteins, and it has excellent emulsion stabilising properties. It has a largely disordered structure, which results in a very weak interfacial film. Its stabilising properties stem from its unique electro-steric repulsion properties. κ-Casein is a minor casein protein, but it has a greater degree of secondary structure than β-casein. It is thought to be more resistant to disruption and displacement from the interface by surfactants, which may play a role in the functional properties of caseinate based ingredients. Caseinates tend to form weak interfacial films, as they are dominated by β-casein. Therefore strategies that could enhance the surface visco-elasticity of caseins would benefit their functional properties [5,6].

One elegant and controlled approach for inducing modifications towards better stabilisation and desired material properties is enzyme-aided structural engineering. In this approach enzymes specifically cross-link protein network by inducing covalent intra- and intermolecular bonds between proteins [7]. Transglutaminase (TG) is an enzyme which crosslinks proteins by catalysing the formation of a covalent bond between a free amine group (e.g., protein- or peptide-bound lysine) and the gamma-carboxamid group of protein- or peptide bound glutamine [8]. Milk proteins are rich of glutamine and lysine and hence, TG is considered to be the most efficient enzyme for cross-linking milk proteins. It is used in the food industry to enhance the strength of protein gels. It has been shown to enhance the surface viscoelasticity of interfacial protein

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films, as the adsorption process usually unfolds the protein sufficiently to allow the enzyme access to the side groups to facilitate the crosslinking reaction [7,8]. Enzymatic cross-linking of caseins has been reported to increase surface rheological parameters of the air/water [9–12] and oil/water interface [13,14].

Although it has been shown that the interfacial strength can be enhanced in the presence of TG, the mechanism of action is not clear. The aim of this study was to investigate the interactions between TG and surface protein films. By gaining a better understanding of this process will enable improved strategies to be developed to maximise the functionality of protein stabilised emulsions and foams.

2. Materials and methods

2.1. Proteins and chemicals

Samples of β-casein (from bovine milk, minimum 90% lyophilised, essentially salt-free) and κ-casein (from bovine milk, minimum 70%) were purchased from Sigma (C6905-1G) and Biochemika Fluka (22087, WA12146), respectively. Sodium caseinate (Na-caseinate, protein content 90%, fat content max. 1.5%) was obtained from Kaslink Foods (Finland). Casein samples were used without further purification. β-Casein samples were prepared at pH 7 in 10 mM sodium phosphate buffer at a concentration of 0.001% from stock solutions. κ-Casein and Na-caseinate samples were prepared in a similar manner. These solutions were equilibrated for 1 h before measurements. TG was commercial Ca^{2+}-independent product (Activa® WM by Ajinomoto Inc., Japan) purchased from Vesantti Oy (Finland). TG was fractionated according to Lantto et al. [15] to make it free of maltodextrin and activity was determined at 37 °C according to Folk [16]. The measured activity of the enzyme preparation was 8764 nkat ml^{-1}. TG was prepared in a 10 mM sodium phosphate buffer at pH 7. The concentrations were such that they corresponded to an activity of 70 nkat ml^{-1} in the 500 μl injection volume for the surface dilatational rheology experiments, or 45 nkat ml^{-1} in the 2 ml injection volume for the surface shear rheology experiments. An excess amount of enzyme was used per g of caseins (50 000 nkat g^{-1} protein for both shear and dilatational experiments) in order to balance the effect of dilution on the catalytic activity and to see the effect of cross-linking within 2–3 h in RT. All other reagents used were of analytical grade.

2.2. Surface rheology

Surface dilatational rheological properties of caseins before and after enzymatic cross-linking at the air/water interface were studied using an in-house built ring trough unit. Further explanation of the technique can be found in Kokelaar et al. [17]. In the dilatation experiments the ring was oscillated at a frequency of 0.1 Hz and a $\Delta A/A$ of typically 4%. Measurements were taken every 30 s, and change in surface tension (surface pressure) was monitored of which the dilatational surface modulus (E), and surface viscosity at room temperature were calculated.

The surface shear rheological properties of casein films were measured using an AR2000 controlled stress rheometer (TA Instruments, Crawley, UK) at room temperature. In this instance the measuring geometry was a large bicone (60 mm diameter) placed at the air/water interface. The AR2000 was used in controlled strain mode, the applied frequency of oscillation of the bi-cone was 1 Hz and the deformation of the surface equivalent to 1%. The bi-cone set up assumes a concentric cylinder geometry of finitely small sides, hence the relative radii of the bicone and outer cylinder determine the factors for calculating elastic and loss moduli (G' and G'') as well as viscosity at the interface.

Both surface dilatational and surface shear rheology experiments were repeated at least twice and the same trends were repeatedly observed upon addition of enzyme. The presented results are individual sets of measurements and the estimated experimental errors are shown in figure captions for each measurement type.

2.3. Adsorbed protein films

Typically a dilute solution of β-casein was allowed to adsorb to the air/water interface for either a known period of time, or until the surface pressure at the air water interface remained relatively constant. Once this point had been reached TG was added into the subphase and measurements were continued for a further 2 h. In the dilatational experiments the TG was added to the subphase outside the ring geometry to prevent surface disruption on addition, whereas in the shear measurements on the AR2000 a syringe needle was permanently held at the edge of the dish containing the liquid with its tip under the surface of the solution. Again this prevented disruption of the surface when the TG was added. The films were monitored for a further 2 h.

2.4. Spread protein films

In some experiments a concentrated casein solution was spread onto the air/water surface as opposed to sample being allowed to adsorb. In these experiments sufficient casein was added to generate a known surface pressure at the interface. Addition of TG into the subphase was performed in the same manner as described above.

2.5. Perfused protein films

In a series of experiments the dilute casein solution was allowed to adsorb to the air/water interface then the subphase was perfused with buffer to remove any casein from the bulk solution prior to addition of the TG into the subphase. This allowed the monitoring of the TG surface bound casein, and comparison with the surface behaviour of solution reacted caseins.

3. Results and discussion

TG is a widely used cross-linking enzyme for milk proteins, as they are rich in target amino acid residues for TG [7,8]. The resulting cross-linked interfacial film has been shown to be stronger than in the absence of enzymatic cross-linking [4]. In this work, casein films were formed at the air/water interface either by adsorption or as spread films, and the effect caused by TG was studied in order to understand the mechanism of TG action better. Both surface shear and surface dilatational rheology were determined as they both impart different information about the surface film. The shear measurements are a direct mechanical measurement of the interfacial film, and are sensitive to intermolecular interactions. Surface dilatational measurements are in response to a compression/expansion stress, and tend to be more sensitive to the composition and structure of the surface film.

3.1. Adsorbed β- and κ-casein films and their response to cross-linking by TG

Initial measurements involved monitoring the adsorption of the protein films for 1 h before addition of TG into the subphase. The impact on the surface rheological properties was monitored for a further 2 h. Fig. 1 shows the surface tension response during adsorption and after addition TG by dilatation. In all cases the addition of TG did not cause any additional reductions in the surface tensions of the proteins studied, which implies that no new proteins were

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