The relation between protein structure, interfacial rheology and foam formation for various milk proteins

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ABSTRACT

A number of purified milk protein preparations (caseins as well as whey proteins, including naturally occurring genetic variants) were investigated for their interfacial and foaming properties. Relations were found between the structure of a protein and its ability to spread and form a coherent film at the air-water interface, as well as with the foaming properties.

INTRODUCTION

Many food products are foams, and the controlled incorporation of air into a food matrix is often done in order to impart desirable textural characteristics and ensure a good flavour release. Milk proteins can be used to stabilize such food foams, and it is thus of interest to elucidate how protein structure and interfacial properties (such as surface pressure and the rheological properties at the interface) relate to the final foam properties (volume and stability).

MATERIALS AND METHODS

Protein preparations

β-Lactoglobulin (β-Lg, genetic variants A and B) and α-Lactalbumin (α-La) were isolated as described by Kristiansen et al[1].

β-casein (β-CN), genetic variants A1 and A2 and α_{s1}-casein, (α_{s1}-CN), genetic variant B, were prepared from crude casein obtained from milk from individual cows with known genetic variants. The cows belonged to the herd of the Experimental Dairy Farm at the Swedish University of Agriculture, Uppsala, Sweden. Fractionation of the casein was performed using ion-exchange chromatogra-phy in a manner similar to Cayot et al[2].

Solutions

Stock solutions (1%, 0.01% or 0.001%) were prepared by dissolving the various proteins in imidiazole buffer (20 mM, pH 7.0) and storing overnight at 5°C. The solutions were frozen and stored at -20°C until use. Prior to use, they were thawed overnight at 5°C.

Surface pressure

After thawing, the 0.01% protein solution was filtered through a 0.45 μm microfilter and kept refrigerated until use. A micro Langmuir trough (Molecular Photonics Ltd., DH1 3LE Durham, UK) was used to obtain measurements of surface pressure (Π) during compression of a monolayer of protein. The subphase consisted of the same buffer as in the stock solutions. In order to obtain a monolayer, 60 μL of 0.01% protein solution was carefully spread on clean subphase using a Hamilton

pipette and Π was recorded during compression at 22°C using a barrier speed of 0.023 mm/s. Isotherms of Π vs A (area/molecule, Å^2) were used to quantify the extent of protein spreading at the interface.

Interfacial rheology

The rheological properties at the air-water interface were monitored using an interfacial rheometer (CIR-100, Camtel, Royston, SG8 9AZ, UK). After thawing, 15 mL 0.001% protein solution was measured into a glass measuring dish. The De Noüy ring was placed at the air-water interface after 15 min tempering at 22°C.

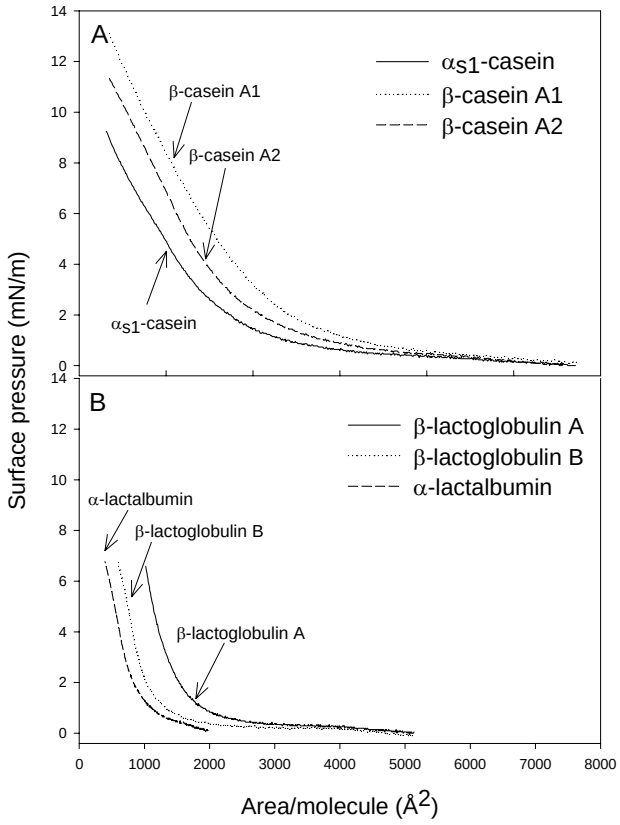

Figure 1. Isotherms of surface pressure vs area/molecule for purified caseins (A) and whey proteins (B). n=3

Measurements were done in triplicate (standard deviations in the range 2-15%) as time sweeps (24 hrs) at a frequency of 3 Hz and an angular displacement of 2.5 mrad.

The CIR-100 measures the interfacial properties by placing a De Noüy ring at the interface and submitting it to small angle oscillatory shear. At frequencies above 2 Hz the interfacial elasticity and viscosity are derived using the principle of normalized resonance.

Foaming properties

Measurements were performed at room temperature (22°C) using 1% protein solutions. The foam was made using an Ultra-Turrax homogeniser in a set-up very similar to what has been described by Huang et al[3]. 1.5 mL of sample was transferred to a graduated syringe (B-D Plastipak 0806 91022; Bie & Berntsen, DK-2610, Rødovre, Denmark) fitted with a short piece of tubing and closed with a clip. Homogenisation was performed for 60 s, and the height of the foam was read immediately after homogenisation and at frequent intervals thereafter until it had collapsed.

Foam overrun was calculated according to the formula

$$O(t) = \frac{100\ V_{tot}(t)}{V_0} - 100$$

where O(t) is the foam overrun (%) at time t, $V_{tot}(t)$ is the total foam volume (mL) at time t and V_o is the initial volume (mL) of the liquid sample. Five measurements were done on each sample and the standard deviation ranged from 6-15%.

RESULTS AND DISCUSSION

Surface pressure

As shown in Fig. 1, major differences were observed between the caseins and the whey proteins in terms of surface pressure. This is not surprising, as caseins are highly flexible and unstructured ('soft') proteins, whereas the whey proteins are globular with a highly ordered tertiary structure ('hard'). Flexible proteins will more readily change conformation at an interface than the more

structured proteins[4] and different behaviour in terms of interfacial adsorption and subsequent molecular rearrangement can be expected between the two different types of proteins.

It is evident from our results that the interfacial tension starts to increase at a higher molecular area for the caseins than for whey proteins. Thus the more flexible caseins take up a larger area at the interface, whereas the globular whey protein can be expected to retain much of their initial structure. Martin et al.[5] only found a limited (~10%) change in conformation (from β–sheet to random coil) for β–Lg at the air-water interface. It is also noteworthy that the slope of the increase in Π is smaller for the caseins than for the whey proteins, presumably indicating a softer, more compressible interfacial layer in the case of the caseins.

It is interesting that differences are found between the caseins as well as the whey proteins. β–CN spreads more extensively at the interface than α_{s1}-CN, and this can be explained by the structural differences between these two casein species: β–CN adsorbs with an extensive hydrophobic region (160–170 amino acid residues) anchored at the surface and a hydrophilic tail (40–50 residues) protruding into the aqueous phase[6]. Distribution of hydrophilic and hydrophobic residues is more random in α_{s1}-CN than in β–CN and a loop-like conformation is suggested for adsorbed α_{s1}-CN[6], hence a smaller interfacial area/molecule can be expected, as indeed observed. A slight difference was also found between the Π-A isotherms of the two genetic variants of β–CN, and it would appear that the A1 variant spreads somewhat more at the air-water interface than the A2 variant. The two variants differ in that the A1 variant has a histidine residue in position 67, whereas the A2 variant has proline. Proline residues disrupt secondary structure, and the additional proline in the A2 variant is situated within a region of several others, forming a hinge between the polar C-terminal and the primarily hydrophobic N-terminal region. Possibly the presence of a proline instead of histidine within this hinge (the A2 variant), provides for a less extensive part of the hydrophobic domain to be adsorbed, thus explaining why the A2 variant of β–CN is less space filling than the A1 variant.

Concerning the whey proteins, the values found for the area taken up by an adsorbed molecule are similar to what has been found previously for an air-water interface[7]. The difference between α–La and β–Lg presumably reflects a slightly higher unfolding of β–Lg during adsorption. The observed differences between the A and B variants of β–Lg are corroborated by the differences in thermal stability of these two variants. The A variant has valine in stead of arginine in position 118, close to one of the disulphide bonds that stabilizes the tertiary structure of β–Lg. This results in the A variant being somewhat more flexible and heat sensitive[8], and it can thus be expected to adsorb to an air-water interface in a somewhat more unfolded state, as indicated by our results (Fig. 1).

Interfacial rheology

Fig. 2 shows the development of the interfacial elastic modulus with time, and major differences are evident between the caseins and the whey proteins as well as within these groups. Both variants of β–CN rapidly adsorb to the air-water interface, resulting in an initial modulus of 0.3-1.2 mN/m, which is equivalent to previous results[4]. Marked differences occur, however, with time. The curve for the A2 variant exhibits a peak followed by a decrease to a steady state value, whereas the modulus for the A1 variant steadily increase with time (Fig. 2 A). The initial peak has been observed in other studies on β–CN with unresolved genetic composition[9] and has been attributed to collapse phenomena, possibly caused by steric interference

between the dangling, polar tails. Why the A1 variant does not exhibit such behaviour is unclear, but could perhaps be related to the more extensively adsorbed hydrophobic domain, as indicated in the results obtained from Π-A isotherms (Fig. 1). α_{s1}-CN, in

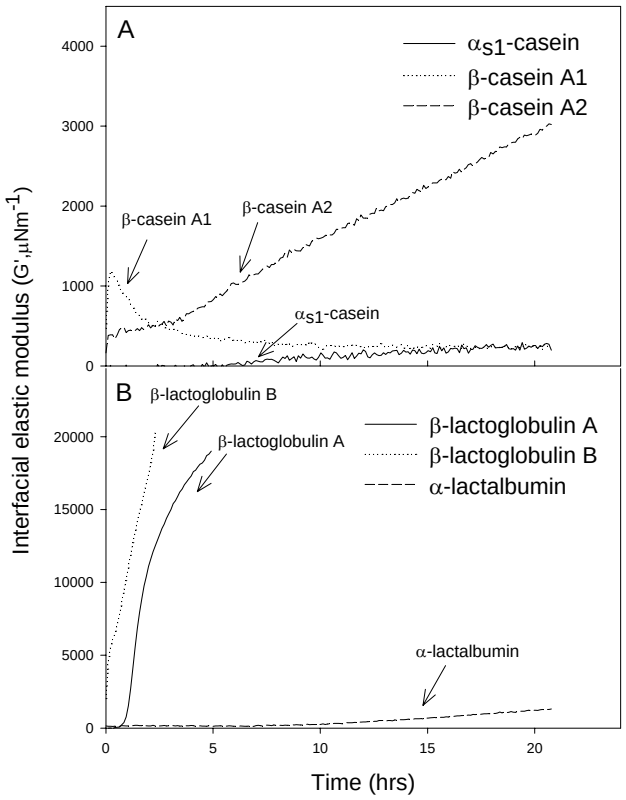

Figure 2. Interfacial elastic modulus vs time for purified caseins (A) and whey proteins (B). Note the difference in y-axis scale between A and B. n=3.

contrast to the β–CNs, does not readily form a mechanically strong interfacial layer and only after 5 hrs an appreciable elastic modulus is achieved. The interfacial elastic modulus can be assumed to depend mainly on lateral interactions between protein molecules. The distribution of hydrophobic and hydrophilic domains is much more uneven in α_{s1}-CN than in β–CN, resulting in a polar loop extending into the water phase, thus hindering formation of inter-molecular interactions at the interface. β–CN, on the other hand, can assume to be primarily adsorbed through the extensive hydrophobic N-terminal domain, hence providing opportunity for lateral hydrophobic interactions at the interface. A consequence of this is the fact that β–CN replaces α_{s1}-CN in mixtures made up from the two casein species[10].

In the case of the whey proteins the importance of lateral interactions at the interface is even more striking. β–Lg possesses a free –SH group that can react to form intermolecular disulphide bonds e.g. resulting in gels upon heating. Undoubtedly the unfolding that takes place at the interface results in a similar bonding, effectively forming a gel like layer at the interface as indicated by the very rapid increase in interfacial elastic modulus (Fig. 2, B). In fact, the value of the interfacial elastic modulus exceeds the upper limit of the instrument (20 mN/m) within just a few

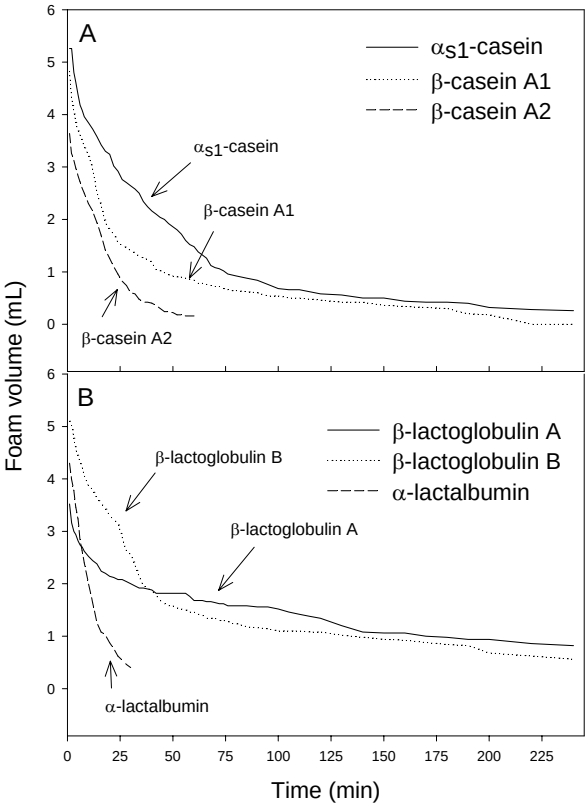

Figure 3. Foam volume plotted against time for purified caseins (A) and whey proteins (B). n=5.

hours. α–La, on the other hand, does not readily form intermolecular bonds as it does not possess a free –SH group, and in addition, as described above, less readily unfolds (α–La is stabilized by 4 disulphide bridges and as well as by binding calcium).

The differences between the A and B variant of β–Lg, with adsorption of the B form resulting in a more rapid increase in interfacial elastic modulus does not correlate well with the observation, that the a form more readily unfolds at the interface (Fig. 1). However, it could be speculated that the specific unfolding of the B variant at the interface provided for better formation of intermolecular disulphide bonds.

Foaming properties

Fig. 3 illustrates the foaming behaviour of the protein preparations used. Also in this case, the differences between caseins and whey proteins are distinct, with β-Lg providing for the most stable foam. In contrast to the A1 variant, β-CN A2 does not possess good foaming properties, nor does α-La.

Is it then possible to relate the interfacial properties to the macroscopic foam properties in milk protein foams?

It would appear from our results on the foaming properties of caseins, that it is not necessarily a prerequisite for a protein to form a strong elastic layer at the interface in order to produce a voluminous and stable foam. At least not in the case of α_{s1}-CN, which produces a more voluminous and stable foam than both of the β–CNs investigated (Fig. 3), but which does not readily form a strong interfacial layer. It must be stressed, though, that foam formation in our case was facilitated by whipping and thus took place under high shear conditions, whereas the measurements of the interfacial rheological properties were made under quiescent conditions. It is not improbable, that shear can induce α_{s1}-CN to form intermolecular interactions that will be able to stabilise the formed foam. When it comes to β–CN, the A1 variant exhibited the best foaming properties, which appears in accordance with the results from the measurements of surface pressure and interfacial rheology. It would thus appear that the A1 variant spreads more extensively at the interface and facilitates a faster build up of a coherent interfacial layer, thus resulting in a foam that is both more voluminous and has increased stability compared to the A2 variant.

It also appears from Fig. 3 that the foaming properties of whey proteins are related to their structure. α–La, more highly structured than β–Lg, is adsorbed in a less unfolded state and lacks the ability to form lateral interactions, thus producing a rather voluminous but unstable foam. β–Lg, on the other hand, produces very stable foams, presumably due to the ability (of both genetic variants) to form intermolecular disulphide bonds at the interface. The B variant, which most rapidly forms a strong interfacial layer, also produces an initially more stable foam than the a variant.

CONCLUSION

We have found substantial differences in the interfacial properties between caseins and whey proteins, as well as between individual protein species. In general, the expected differences between soft, unstructured protein such as caseins, and hard, highly structured proteins such as whey proteins, were found. These differences were also reflected in the foaming properties of the proteins, with β–Lg resulting in the most stable foams. α_{s1}-CN appears to be an exception, as it proved able to form a voluminous as well as a rather stable foam, but this could possibly be due to relating high shear conditions (whipping) to instrumental measurements made under quiescent conditions.

In addition to the differences found between the main groups of milk proteins studied, major differences between genetic variants of the individual proteins were

found, highlighting that minor changes in the primary sequence of a protein can have dramatic effect its behaviour.

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