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On the link between surface rheology and foam disproportionation in mixed hydrophobin HFBII and whey protein systems

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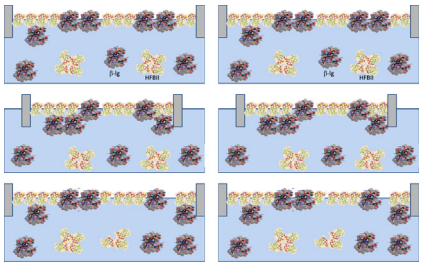
HIGHLIGHTS

- Higher HFBII-fraction needed for HFBII-dominance in spread vs. adsorbing layers.
- HFBII becomes more dominant upon sequential deformation cycles of a mixed surface.
- We propose that multi-layer formation at the surface explains the above behaviour.
- Foam coarsening corresponds well to dilational properties of adsorbing layers.

GRAPHICAL ABSTRACT



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ABSTRACT

Here we study the surface dilational properties of spread and adsorbed layers of whey protein isolate (WPI) and hydrophobin HFBII at air/water interface using Langmuir trough and relate them to foam ability and stability. In spread and adsorbed systems, a gradual increase in modulus with the fraction of HFBII on the surface or in the bulk is observed and we can identify distinct regimes of WPI-dominated and HFBII-dominated behaviour. The dominance of HFBII is further substantiated by visual observation of microscopic wrinkles appearing on the surface of the trough. When comparing spread to adsorbed systems, it was found that a higher HFBII fraction is needed to obtain HFBII dominant behaviour in spread layers than in case of adsorbing layers (f_{HFBII} = 0.6 and 0.2 respectively). Furthermore, our results indicate that the HFBII-contribution to the interfacial behaviour becomes more pronounced upon sequential large scale compression/expansion cycles. In order to explain this non-trivial behaviour, we propose that there is multi-layer formation at the interface, having a top layer enriched in HFBII and bottom layer enriched in WPI. It is also concluded that coarsening stability in foams corresponds more closely to the surface dilational properties measured in adsorbing layers than those in spread layers. Finally, it was observed that in mixed systems of HFBII and WPI, the coarsening process levels off, which corresponds to the increased dominance of HFBII in mixed WPI:HFBII-layers upon large surface deformation that is occurring at the surface of shrinking bubbles.

1. Introduction

Hydrophobins are a class of highly surface-active proteins produced by filamentous fungi. Their biological function is to mediate the formation of (aerial) hyphae, spores and fruiting bodies, during which a large interface is formed between hydrophilic and hydrophobic environments (i.e. cell material and air) [1].

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Hydrophobins consist of 100 ± 25 amino-acids and have a characteristic pattern of 8 cysteine residues, resulting into 4 intramolecular disulfide bridges [2], making the protein molecule very compact and rigid. The hydrophobin tertiary structure shows clearly distinguished hydrophobic and a hydrophilic regions [3–5]. Based on their aqueous solubility and hydropathy patterns hydrophobins can be divided in two classes [4]. Class I hydrophobins are the most surface active, but have very low water solubility and thus are difficult to use in practical applications. Class II have a bit lower surface activity, (when compared to class 1 hydrophobins, but still extraordinary when compared to any other protein classes) combined with good water solubility. In the case of class II hydrophobin, HFBII, the unique combination of small molecular weight (7.2 kDa) and 4 disulfide bridges, prevents the rearrangement of the hydrophobic part into the protein core and keeps it exposed to the surrounding media. Thus in aqueous environment HFBII behaves as natural Janus particle [4], having distinct hydrophilic and hydrophobic surface patches, which in turn leads to a distinct amphiphilic behaviour and bulk and surface self-assembly.

Recently, the surface properties of HFBII were studied by Cox et al. [6], and were used to explain the extremely low rate of air dissolution in simple air bubble clusters. In the follow up work Cox et al. [7] have demonstrated that HFBII can be used to produce liquid foams, which are stable for the period of several months. For this reason these molecules have been described as air structuring proteins. Such stability is exceptional, since in most cases liquid foams will steadily coarsen by the disproportionation process [7,8]. Because of this, one often depends on bulk elasticity to stabilise foams, limiting the amount of applications, e.g. in bread, ice cream or mousses and bavarois-type of dessert [9].

Now, the exceptional stability to disproportionation in HFBII-stabilised foams can be explained by extraordinary surface properties: HFBII has an extremely high desorption energy and it appears as a compact, stiff, and sticky particle [8,10]. As a result, HFBII surface layers have outstanding surface shear and dilatational moduli measured in both oscillating and steady dilatation regimes [8,11]. Moreover, they show wrinkle formation upon continuous compression, related to bending elasticity of the surface layers [10].

When it comes to applying these air structuring proteins into consumer products, they will almost inevitably encounter the presence of other ingredients that are also surface active, for example proteins. It is known for example that the surface properties of mixed protein and/or emulsifier systems can be drastically different from that of pure systems and this can either lead to stabilisation or destabilisation of emulsions or foams [12–14]. Recently, also data have been published on the surface shear rheology of mixed layers of HFBII and β-casein, providing a detailed hypothesis on the layer build-up of such layers [15].

As an exploration to investigate the impact of other proteins on the functionality of HFBII as a stabilizer against foam disproportionation, this paper is dedicated to mixed systems of HFBII and whey protein isolate (WPI). In WPI the main protein is β-lactoglobulin: A representative and well studied globular food protein with a molecular weight of approximately 18 kDa, an iso-electric point of 5.1 and ability to unfold upon adsorption to interfaces [8,16–20].

The goal of the study is to correlate the behaviour of mixed layers of known compositions to the foaming behaviour of mixtures. To this end, we chose to approach these extremes in three steps: The first step is to study the dilational surface properties of layers of aqueous HFBII:WPI mixtures, which were spread over a pure air/water interface, which sets the initial composition of proteins at the interface. As both proteins are relatively large (they are macromolecules) and have significant surface activity and attachment energy, it is expected that there is no (or negligible) protein desorption to the bulk. As a result the protein concentration and composition is expected remain the same during compression/expansion cycles. In the second step we work with adsorbing systems where we prepare aqueous solutions of both proteins with known bulk composition and leave this system to spontaneously adsorb at air/water and equilibrate with the bulk. It is clear that in this case the bulk and surface compositions might differ due to different affinity of the proteins to the interface. When we start the compression/expansion cycle to probe the surface dilatational rheology, the surface composition could also change due a number of reasons. There can be accumulation of protein at the surface during the expansion cycle, rearrangement of proteins at the interface and subsurface with possible multi layer formation and to a much lower extent protein desorption during the compression cycle. As result during this process the system could get enriched with the most surface active protein on the top layer and depleted of less surface active one, which can possibly form a second layer below the interface. Furthermore, in order to correlate these to disproportionation stability, we chose to apply large deformation dilatational rheology for which theoretical linkage to disproportionation has been established and verified [8,9,21]. Our the final step was to assess foam formation and foam disproportionation stability of mixed protein solutions and try to correlate foam properties with the surface dilatational rheology of both adsorbed and spread layers.

2. Materials and methods

2.1. Materials

Class II Hydrophobin HFBII (Mw 7200 Da) was obtained from VTT Biotechnology Finland and was extracted from *Trichoderma reesei* as described elsewhere [22,23]. Stock solutions were stored in the freezer and thawed, diluted and treated in an ultrasonic bath for 30 s before use. For the Langmuir trough experiments, whey protein isolate 'Bipro' was obtained from Davisco (USA), which exists for the majority (approximately 50 wt%) of β-lactoglobulin. Xanthan gum, Ketrol RD, was purchased from CPKelco (San Diego, USA).

2.2. Methods

2.2.1. Small and large deformation dilatational Surface rheology using a Langmuir trough

For surface dilatational rheology experiments at the air/water surface we used a MicroTroughX Langmuir trough, produced by Kibron Inc. (Espoo, Finland). The dimensions of the trough are 230×55 mm ($A = 11800$ mm^2) with a sub phase volume of approximately 15 ml. Surface tension is measured in the middle of the trough using an ultra sensitive Kibron sensor, small diameter (0.51 mm) special alloy wire. The sensitivity is better than 0.01 mN/m.

For spread systems, stock solutions of 1 mg/ml HFBII and 1 mg/ml WPI were prepared and mixed to several mixing ratios. After cleaning the Langmuir trough extensively, it was filled with approximately 15 ml clean deionised (Millipore) water, which was de-aerated under vacuum, with the barrier settings at 3000 mm^2. After cleaning the interface between the barriers with a syringe, the surface was expanded to 10000 mm^2. The surface tension probe was applied to the interface and calibrated. Aliquots of approximately 2 μl protein solution were spread on several spots on the surface the surface using a clean 10 μl glass micro syringe with needle until a total volume of 15 μl was applied. Surface pressure normally rose to roughly 1 to 3 mN/m, while spreading the protein. After equilibrating the surface for 15 min, the area was compressed to 3000 mm^2 at a linear velocity of 5 mm/min while recording surface tension as a function of time. Subsequently 5 expansion compression cycles were applied between surface