Enzymatic modification of adsorbed β-lactoglobulin layers at air/water interface: characterization by interfacial rheology

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ABSTRACT

In this study, we aimed to follow the effect of enzymatic modifications attained by a commercial *S. mobarensis* transglutaminase (TG) and *T. reesei* tyrosinase (TrTyr) on the strength of adsorbed β-lactoglobulin (BLG) films at the air/water interface. The results show that film strength as measured by surface shear elastic modulus decreased upon enzyme treatment of the interfacial film. Neither of the enzymes was surface active on a protein covered interface indicating that the modulus decrease was due to modification of adsorbed BLG molecules.

INTRODUCTION

Many proteins readily adsorb to air or liquid interfaces because of their amphiphilic character and form a characteristic surface film, the strength of which is dependant on the nature and state of the protein, type of the interface and environmental conditions. Whey proteins for example, when adsorbed at the air/water interface, form a film with viscoelastic character that can be characterized by interfacial rheological methods. The mechanical properties of the interfacial layer can be tailored for increased stability of the air bubbles against coalescence or disruption in liquid foams. Enzymatic cross-linking of proteins has been reported to increase surface dilatational rheological parameters in milk protein foams.[1] Transglutaminase (TG) is a well-studied enzyme for protein cross-linking. Recently, *T. reesei* tyrosinase (TrTyr) has gained interest for its ability to oxidize proteinyl tyrosines and create intra- and inter-molecular protein cross-links.[2]

BLG is a globular protein which is stabilized by two disulfide bridges. In its native state, the molecule is resistant to enzymatic reactions due to its compact structure which hinders reactive sites. A change in protein fold is required for enzymatic modifications. Once adsorbed to air/water interface, the BLG molecule is reported to go through a structural re-arrangement.[3] We aimed to analyze if such an arrangement would allow increased enzymatic reactivity of the adsorbed BLG molecules leading to formation of inter-molecular covalent bonds at the interface.

MATERIALS AND METHODS

Bovine BLG (90% by PAGE, mixture of A and B variants) was purchased from Sigma (St. Louis, MO, USA). BLG stock solution (1 mg/mL) was prepared in sodium phosphate buffer at pH 6.8 by 1 h stirring at room temperature. T. reesei tyrosinase (TrTyr) was produced and characterized at VTT. The commercial TG preparation (Activa® WM, Ajinomoto Inc., Japan) was supplied by Vesantti Oy (Helsinki, Finland) and further purified at VTT.

Interfacial Rheology

The surface shear rheological properties of BLG at the air-water interface were measured using a stress controlled rheometer (AR-G2, TA Instruments, U.K.) equipped with a Pt-Ir du Noüy ring (13 mm diameter). BLG stock solution was diluted to 0.05 mg/ml in the same buffer and equilibrated at room temperature for 1 h before rheological measurements. The instrument was mapped prior to each measurement. Sample (50 mL) was placed in a glass dish of 60 mm diameter and the du Noüy ring was placed onto the surface according to the manufacturer's directions. Surface shear moduli were followed at room temperature at 0.1 Hz and constant strain of 0.5%, which was measured to be in the linear viscoelastic region. After a time sweep of 1 h, the measurement was paused for 10 min and the prepared enzyme dilutions providing a dosage of 10 000 nkat/g for both TrTyr and TG were injected to the subphase in 50 μL portion. Only buffer was injected in the case of control. After that, time sweep continued for 8 h. Injection caused a reversible disturbance in BLG film.

Surface pressure

The surface pressure of adsorbed BLG layers was measured with a KSV film balance (Minimicro series, KSV Instruments) using a platinum Wilhelmy plate at room temperature. The same dish and same amount of sample that was used for rheology measurements were used and the similar time spans and enzyme injection manners were attained. Injections caused a reversible disruption in surface film as observed by surface pressure.

RESULTS AND DISCUSSION

The interfacial shear rheological methods generally do not have good reproducibility[4]; however, qualitative data can be achieved. As explained above, the protein adsorption and film formation was followed for 1 h for BLG alone before injection of the enzyme in each measurement. That way, a representative film was formed and the effect of any enzymatic action could be reliably assessed as negative or positive. Besides, adsorption of the substrate molecule before addition of the enzymes was a preferred order. Each measurement was replicated at least twice and the same trends were repeatedly achieved upon enzyme treatment.

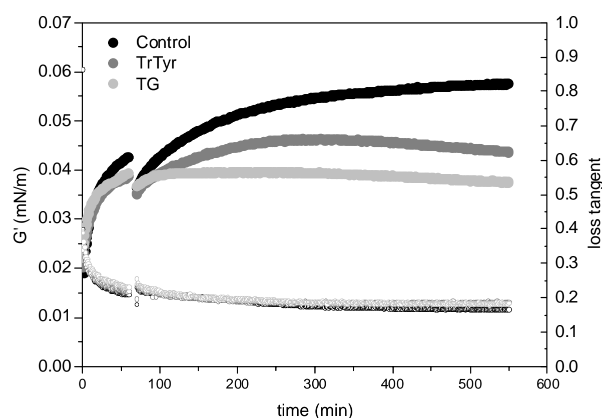

Figure 1. Changes in interfacial shear elastic modulus (G') (solid lines) and loss tangent (G''/ G') (dotted lines) in time.

The surface pressure measurements (Fig. 2) revealed that at the studied BLG concentration, adsorption of protein molecules to the surface was rather fast. The surface was saturated with BLG molecules instantaneously. However, G′ continued to increase and did not even stabilize in 1 h (Fig. 1). That infers ongoing physical interactions between tightly packed molecules. Enzymes were injected underneath such protein layer. Addition of TrTyr or TG to the subphase resulted in gradual disruption of the interfacial film as was observed by decreasing G′ in time (Fig. 1).

We can assume that the result is due to the enzymatic modifications directly on the already adsorbed BLG molecules as no further adsorption or desorption (in a

significant level) of molecules to or from the interface was observed after enzymatic reaction (Fig. 2).

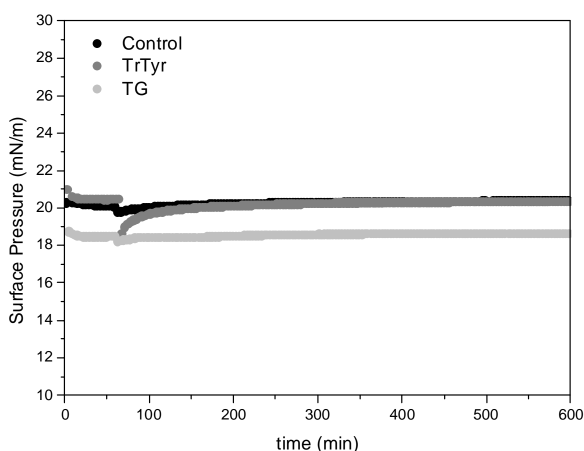

Figure 2. Changes in surface pressure upon adsorption of BLG solution and subsequent enzyme injection.

Many reports have been published on unfolding kinetics of globular proteins at interfaces with a common conclusion that partial unfolding upon self re-arrangement of adsorbed proteins may happen if the protein has a certain minimum area to expand. Most probably, on such a packed surface, the enzymes were not able to induce inter-molecular cross-linking due to rigidity of the molecules. However, intra-molecular links could be formed still. Hellman et al.[5] have recently shown that intra-moleculary cross-linked proteins in solution are locked in their globular fold. Such bonds created by both TrTyr and TG would then further impede the re-arrangement of adsorbed protein according to the interface during aging which would lead to diminishing of physical protein-protein interactions at the interface.

CONCLUSIONS

We suggest that the observed results are caused by mainly intra-molecular cross-linking of the adsorbed molecules leading to more rigid BLG molecules at the surface and impaired physical interactions in between. Consequences of such behaviour in stabilization of foams need to be elucidated.

ACKNOWLEDGMENTS

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