Shear rheology of mixed adsorption layers with hydrophobin vs. their structure studied by surface force measurements

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Summary

The hydrophobins form the most rigid adsorption layers at the air/water interface in comparison with all other investigated proteins. Here we studied the effect of different additives on the surface shear elasticity and viscosity, E_{sh} and η_{sh}.

If the additive is a globular protein, like β-lactoglobulin and ovalbumin, the surface rigidity is preserved and even enhanced. The experiments with foam films indicate that this is due to the formation of a bilayer structure at the air/water interface.

If the additive is the disordered protein β-casein or the nonionic surfactant Tween 20, this leads to softening of the HFBII adsorption layers. Possible explanation is the penetration of the hydrophobic tails of β-casein and Tween 20 between the HFBII molecules at the interface, which breaks the integrity of the hydrophobin interfacial elastic network.

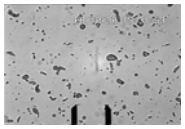
The analyzed experimental data for the surface shear rheology of various protein adsorption layers comply with a viscoelastic thixotropic model, which allows one to determine, E_{sh} and η_{sh}, from the measured storage and loss moduli, G' and G''.

Special properties of hydrophobins

The hydrophobins represent a class of amphiphilic proteins that at air/water and oil/water interfaces form adsorption layers with very high surface elasticity and viscosity, E_{sh} and η_{sh}, among all of the investigated proteins.

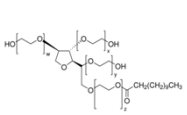
The Hydrophobin HFBII forms fast solidifying adsorption layers and upon shaking, the protein solution forms bubbles with irregular (non-spherical) shape.

Another valuable property of hydrophobins is that they are rather "sticky" molecules. Evidence for this is that when two HFBII adsorption monolayers are brought in close contact, they stick to each other and form a self-assembled bilayer (S-bilayer) with significant energy gain.

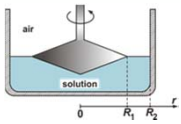


Materials and Methods

	HFBII	β-lactoglobulin (BLG)	ovalbumin (OVA)	β-casein
Structure				
Molecular weight	7.2 kDa	18.4 kDa	45 kDa	24 kDa
Disulfide bonds	4	2	1	0

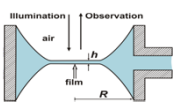


Nonionic surfactant Tween 20
M_w = 1.228 kDa

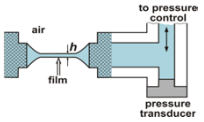


Rotational rheometer was used in two regimes:
- Fixed-rate-of-strain regime
- Oscillatory regime

Scheludko-Exerowa (SE) cell



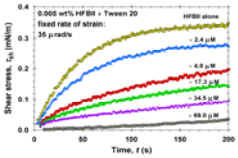
Mycels-Jones (MJ) cell (thickness vs. pressure)



Experimental results and discussion

Fixed-rate-of-strain regime

Addition of Tween 20 reduces the elasticity of the HFBII adsorption layers.

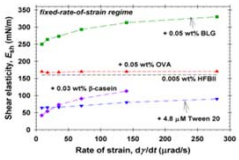


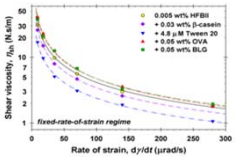
Maxwell model:

$$\frac{1}{E_{sh}}\frac{d\tau_{sh}}{dt}+\frac{\tau_{sh}}{\eta_{sh}}=\frac{d\gamma}{dt}$$

$$\tau_{sh}=\eta_{sh}\dot{\gamma}\,[1-\exp(-\frac{E_{sh}}{\eta_{sh}}t)]$$

$$\tau_{sh}=g_t\tau,\quad g_t\equiv\frac{1}{4\pi}\left(\frac{1}{R_1^2}-\frac{1}{R_2^2}\right)$$



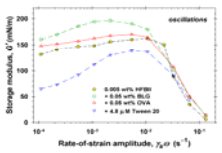


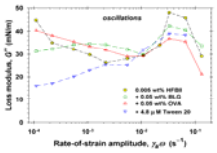
It is possible to replace a part of HFBII with OVA, without decreasing the high elasticity of the adsorption layer.
The addition of β-casein and Tween 20 reduces the elasticity of the HFBII adsorption layers.

Oscillatory regime

$$\gamma=\gamma_a\sin(\omega t+\phi)$$

$$\frac{\tau_{sh}}{\gamma_a}=G'\sin(\omega t+\phi)+G''\cos(\omega t+\phi)$$





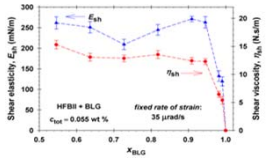
The special behavior of the HFBII + BLG mixture

So far, x_{BLG} = 0.91 = fix.

To investigate the role of x_{BLG} we carried out experiments for $0.5 \le x_{BLG} \le 1$ at fixed total protein concentration of 0.055 wt%.

At x_{BLG} = 1 – no detectable rheological response

At x_{BLG} = 0.946 – transition to a state with rather low values for E_{sh} and η_{sh}



Viscoelastic thixotropic model

$$\frac{1}{E_{sh}}\frac{d\tau_{sh}}{dt}+\frac{\tau_{sh}}{\eta_{sh}}=\frac{d\gamma}{dt}$$

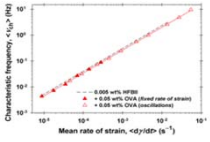
$E_{sh}=E_{sh}(|\dot{\gamma}|)$ and $\eta_{sh}=\eta_{sh}(|\dot{\gamma}|)$

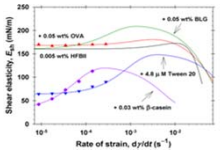
characteristic frequency: $\nu_{ch}(|\dot{\gamma}|)=\frac{E_{sh}(|\dot{\gamma}|)}{\eta_{sh}(|\dot{\gamma}|)}$

in fixed-rate-of-strain regime: $\nu_{ch}(|\dot{\gamma}|)=Q\,|\dot{\gamma}|^m$ (modified Herschel – Bulkley law)

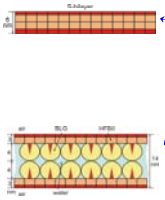
in oscillatory regime: $\langle\nu_{ch}\rangle=Q\langle\dot{\gamma}\rangle^m$ where $\langle\dot{\gamma}\rangle=\mu\gamma_a\omega$, $\mu=\left[\frac{\Gamma(m/2+0.5)}{\pi^{1/2}\Gamma(m/2+2)}\right]^{1/m}$

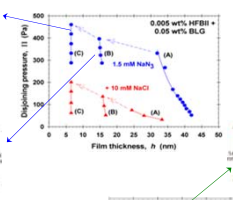
$$\langle E_{sh}\rangle=\frac{G'^2+(m+1)G''^2}{G'}\qquad\langle\eta_{sh}\rangle=\frac{G'^2+(m+1)G''^2}{G''\omega}$$





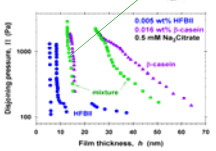
Experiments with thin liquid films



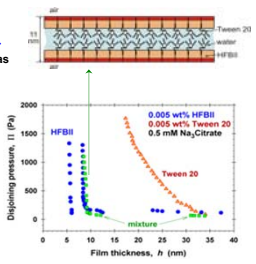


The addition of BLG and OVA increases both E_{sh} and η_{sh}.

The addition of β-casein and Tween 20 decreases both E_{sh} and η_{sh}.



When the additive is a globular protein (BLG or OVA), the resulting film has a two-layered structure.



If the additive is the disordered protein β-casein or the nonionic Tween 20, a part of its molecule is incorporated in the adsorption layers, thus strongly affect their rheology.

Conclusions

1) The addition of the globular proteins BLG and OVA leads to increase of both E_{sh} and η_{sh} – the added protein forms a second layer under the HFBII monolayer, thus enhancing the interfacial rheology;
2) The addition of the disordered protein β-casein and of the nonionic surfactant Tween 20 leads to decreasing of E_{sh} and η_{sh} – the hydrophobic tails penetrate between the HFBII molecules at the interface and disrupt the integrity of the interfacial elastic network;
3) Up to 94.6% of the HFBII can be replaced with BLG, without decreasing the surface rigidity.

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