Milk proteins on smooth and porous surfaces

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Casein micelles consist of four different types of caseins (α_{S1}-, α_{S2}-, β- and κ-casein) and colloidal calcium phosphate. Under native conditions, the sizes of casein micelles are broadly distributed between 50 nm and 500 nm. The major whey protein in cow milk is ß-lactoglobulin. Changes in the site of casein micelles in the presence of ß-lactoglobulin have been reported in temperature-dependent experiments [1]. It was found that those changes were highly correlated with the level of denaturated ß-lactoglobulin associated with the casein micelles.

We investigated milk protein deposits on smooth and porous surfaces using grazing incidence small angle X-ray scattering (GISAXS) at the beamline BW4. GISAXS allows estimating the size of casein micelles in films as well as studying their internal substructure [1,2]. Experiments were performed in a project aiming to explore the structure and formation of deposits on membranes during the micro-filtration of milk. In general, micro-filtration is used to separate the small whey proteins (ß-lactoglobulin) from the large casein micelles forming the deposit on the membrane. As a first approach, we deposited mixtures of both proteins on Si-wavers. Beside size-fractionated casein micelles only, the samples contained high and low contents of ß-lactoglobulin. In order to obtain homogeneous films we used the spin-coating technique. As a result, we obtained three casein films with different ß-lactoglobulin content. We performed GISAXS experiments of the dried films at different sample positions. After normalization to the incoming X-ray intensity, we averaged the GISAXS patterns of the same sample. For a detailed analysis, we took out-of-plane scans of the GISAXS patterns at the critical angle of the protein.

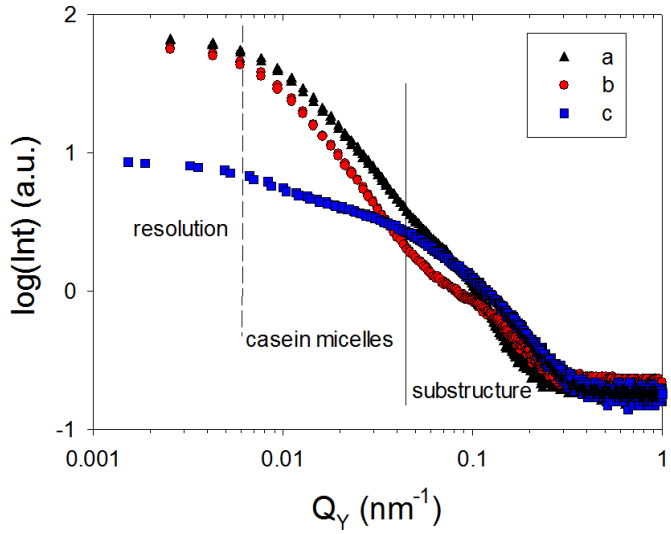

Figure 1: Out-of-plane scan of a two-dimensional GISAXS-pattern of casein films, with high (a), medium (b) content of ß-lactoglobulin and without (c) ß-lactoglobulin. The multi-level structure of casein micelles and the experimental resolution is indicated.

Figure 1 depicts the scattered intensity as a function of the Q_Y-vector for the three films. The scattering functions contain structural information about the overall size (at $Q_Y = 0.01$ nm^{-1}) and the inner structure (at $Q_Y = 0.1$ nm^{-1}) of the casein micelles. The dashed line denotes the resolution of the experiments.

The presence of ß-lactoglobulin led to an increase in the scattering intensity at low Q_Y-values, which could be a result of an increased scattering contrast between casein micelles and film matrix consisting of ß-lactoglobulin. Moreover, in comparison to the sample with medium ß-lactoglobulin content, the scattering function of the sample with high ß-lactoglobulin content shifted towards higher Q_Y-values. This could be due to a size decrease of the casein micelles when the concentration of ß-lactoglobulin molecules in the matrix increases.

Another aspect of the experiments concerned the shape of deposited casein micelles and their arrangements on surfaces. In general, the form and structure factor is difficult to access experimentally due to the large polydispersity of the casein micelles. We used size fractionated casein micelles to induce ordered film structures. Scanning experiments allowed finding film areas, which resulted in structured GISAXS-patterns. From such a pattern, an out-of-plane scan at an exit angle near the critical angle of the protein was taken and being depicted in Figure 2. Two symmetrically arranged side maxima were resolved by GISAXS. At present, we perform simulations to find out a suitable form and structure factor model which would best fit the GISAXS data.

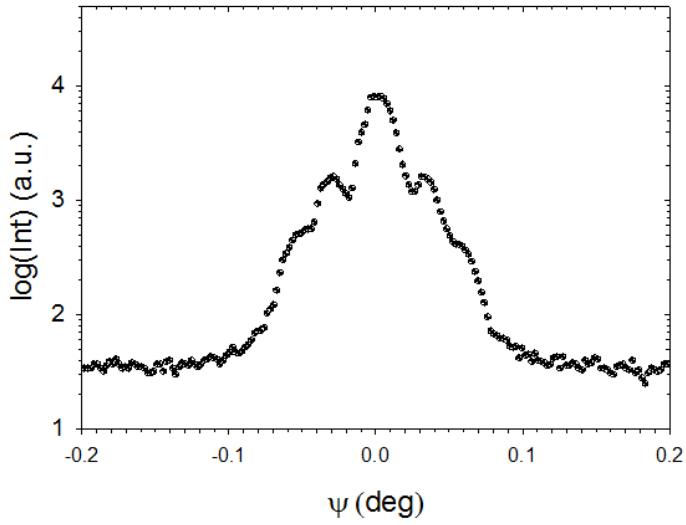

Figure 2: Out-of-plane scan of a two-dimensional GISAXS-pattern of a highly ordered casein films. Up to three symmetrically arranged side-maxima are clearly resolved by the experiment. The cut was taken near the critical angle of the protein.

With future experiments we will start to focus towards in-situ experiments.

References

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