Effect of enzymatic cross-linking on casein micelles thin film structure


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Milk and milk components are mainly used for related food products. Nevertheless, some of the milk constituents such as casein have alternative non-food applications. In ancient Egypt casein-based glue was first used. Milk casein proteins are also used for other technical applications [1] including plastic manufacturing [2], fiber textile [3], methane gas production [4]. Casein proteins in bovine milk include four main types: $\alpha_1$-casein (38 %), $\alpha_2$-casein (10%), $\beta$-casein (36 %) and $\kappa$-casein (13%), that are involved in formation of hydrated casein micelles of sizes on the order of 150-300 nm [5]. Various models [6] are proposed for casein micelle structure including coat-core, sub-micelles, and the internal structure. The coat-core model describes the micelle as an aggregate of casein proteins with inner layer composition different from the outer layer. The sub-micelles model proposed the micelle as roughly spherically uniform sub-micelles which are linked together via a calcium phosphate ion cluster. The internal structure model specifies the mode of aggregation of different caseins.

Müller-Buschbaum et. al. [7] investigated thin casein films prepared using spin coating method. The GISAXS results [7] of these casein films indicated a decrease of micelle size at the glass surface compared with the micelles in solutions (probed by dynamic light scattering). The micelles are arranged on the surface in a compact structure due to the moderate force applied by spin coating. This information on the micelle size and their spatial distribution on the solid supports are important for application such as labeling of glass containers. Varying the micelle sizes using different concentrations of calcium chloride [8] have also been tested and showed a decrease in the number of small sub-micelles and a corresponding increase in the number of large size micelles. In the present study, we aim to manipulate the micelle size distribution using the enzymatic cross-linking technique prior to casein protein thin film preparations.

Pasteurized skim milk was used to prepare the casein micelles via micro-filtration and ultra-filtration processes [9] to completely remove the whey proteins without varying the calcium, salts and lactose contents. The casein micelles crosslinking process was performed by incubation of casein protein solution (2.8 %) in transglutaminase enzyme (3 U g$^{-1}$) for different time periods up to 180 minutes. The transglutaminase enzyme incubation reaction was stopped by heating the samples after the specified times at 80°C for 2 min in order to inactivate the enzyme, followed by cooling in ice. One casein sample solution without crosslinking was used as a zero time sample. The casein micelles were applied by spin coating on precleaned glass microscopic slides.

The GIUSAXS experiments was performed at beamline BW4 of DORIS III storage ring at HASYLAB/DESY in high resolution set up to account for highly ordered scutections up to 10 micrometers resulting from casein micelles. The selected beam wavelength was 0.138 nm and the sample-to-detector distance was 13.2 m. The sample was placed horizontally on a goniometer and the scattered intensities passed through an evacuated flight tube and detected using a 2D detector (MARCCD; 2048 x 2048 pixel). Vertical cuts gives information on the structures perpendicular to the surface and the horizontal cuts contains informations on the in-plane surface structures. Figure 1a shows the 2D GIUSAXS scattering patterns measured from the casein micelles thin films. The incident angle was chosen to avoid the overlap between the specular and diffuse scattering contributions along the vertical detector directions. The specular reflection was shielded using a beam stop to protect the detector form the extensive high intensity damage.
Figure 1b shows the horizontal scans from different film prepared from casein micelle solutions crosslinked using transglutaminase enzyme for different time. In Figure 1b the incubation time increases from bottom to the top (0, 20, 40, 60, 80, 100, 120, 140, and 180 min.). At first sight, all out-of-plane cuts qualitatively look similar, however, a close look at the intensity of each cut near the resolution limit (very small \( q_y \)-values) indicates relatively higher fraction of large-scale structures that reveals an increase in the assembly of larger casein micelles with increasing the enzymatic cross-linking time. Thus as expected, the cross-linking mainly creates larger sized structures, which however are well resolved by the high-resolution GIUSAXS set-up.

A quantitative analysis of these data will be performed by data-fitting programs such as IsGISAXS. Moreover, these samples will be further investigated using GISAXS to test the larger \( q_y \) range (\( q_y > 0.1 \) nm\(^{-1} \)) for better understanding of the changes associated with the smaller sizes (submicelles < 30 nm) structures.

**Figure 1:** a) The 2D GIUSAXS scattering pattern of casein micelle thin film measured at BW4, b) log-log plot of the horizontal line cuts from the 2D GIUSAXS signals measured on casein films prepared by incubation in transglutaminase enzyme for different time periods. The time increases from the bottom (no enzyme incubation) to the top (180 minutes incubation) in steps of 20 minutes.

**References**