Effect of calcium concentration on the structure of casein micelles in thin films

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Caseins, a family of phosphoproteins, form the largest protein component in most milks of industrial significance. Today, it is generally accepted that caseins assemble into casein micelles, the aggregates in which they are found in milk. Casein micelles are poly-disperse, roughly spherical aggregates with diameters ranging between 150 and 300 nm. In contrast to conventional surfactant systems, casein micelles are heterogeneous, composed of four different proteins, alpha(s1)- and alpha(s2)-caseins, beta-casein, and kappa-casein [1]. Structure and stability of the casein micelles is still a matter of debate and different models are discussed in literature [2-5]. In the sub-micelle model, the caseins first aggregate via hydrophobic interaction into subunits of 15–20 molecules each, thereby creating a well defined sub-structure on a scale of 20 nm [6]. These units are linked by small calcium phosphate clusters, while other models deny the existence of casein sub-micelles and consider calcium phosphate clusters as seeds of micelle growth. Along this line, the dual binding model accounts for distinct hydrophilic and hydrophobic regions of the particular polypeptides. Analogous to diblock copolymers, the hydrophobic regions associate, stabilizing the core of the micelle and at the hydrophilic regions the colloidal calcium phosphate particles are attached.

A consensus of opinion exists that an outer hairy-layer of kappa-casein ensures the stability of the casein micelle through a steric stabilization mechanism and that calcium is essential for the micelle formation at all. The casein proteins divide themselves into two groups, the calcium-sensitive and the non-calcium sensitive, which prevent or inhibit also in mixtures the precipitation of the calcium-sensitive group by calcium. Kappa-casein is insensitive to calcium and alpha(s1)- and alpha(s2)-caseins and beta-casein are calcium sensitive. The main physiological task of the caseins is to dissolve calcium phosphate in neonates. Approximately 1 mM casein in milk binds 10 mM Ca₃PO₄ in aqueous solution, whose solubility is in the range of 10⁻⁶ mM.

Within the present investigation we focus on the effect of calcium concentration on the structure of casein micelles in thin films (prepared by spin-coating [7]). CaCl₂ was added, at room temperature, to casein micelles extracted from commercial-grade skim milk in a concentration range from 0 to 100 mM. As reference for the thin film investigation, bulk solutions were probed with static and dynamic light scattering experiments. To avoid problems related with dilution, native turbid solutions were analyzed at a concentration of 3%, using a backscattering technique, which records only light scattered from the surface of the sample. This procedure minimizes multiple scattering and yields approximate molecular parameters even of turbid solutions. The thin film investigation is based on grazing incidence small angle X-ray scattering (GISAXS) and complemented with optical microscopy and atomic force microscopy to picture the surface structure [7]. Due to the typical size of micelles on the order of 100 nm, these experiments addressing the mesoscopic structure require a high resolution. To probe nanostructured films GISAXS offers an opportunity to address large scale structures [8]. In principle, with the present set-ups available at the BW4 USAXS beamline of the DORIS III storage ring at HASYLAB/DESY [9] in Hamburg lengths up to more than 10 micrometers are accessible. This resembles a sufficient resolution to account for highly ordered structures resulting from casein micelles.

Extracted casein micelles of a desired concentration were dissolved in purified water. The pH of the solution was adjusted to 7.3. Casein films were prepared by spin-coating onto pre-cleaned glass slides. The cleaning was performed in base baths yielding a defined surface chemistry. The samples were freshly prepared in advance of the scattering experiment. Dry films were investigated. The GISAXS experiment was performed at a fixed wavelength of 0.138 nm and a sample-detector distance of 2.2 m a allow for a high resolution. To check for possible radiation damage, the total counting time was split into smaller slots and the resulting signals were compared.
presented concentration and pH range no signature of radiation damage was detected. The scattered intensity was recorded with a two dimensional detector which consists of a 2048x2048 pixel array. At the fixed angle of incidence 0.466° the major signals, namely the specular and the Yoneda peak are well separated and enable an easy detection of the GISAXS signal from the 2d intensity distribution via a horizontal cut. The horizontal slice contains only scattering contributions with an in-plane information. Because the flat glass surface gives no contribution with a marked intensity distribution to the diffuse scattering, the probed signal originated from the casein film. In case of c=100 g/l we assume bulk like films.

A detailed analysis of the type of structural order is in progress.

![Image of the graph and AFM images]

**Figure 1:** a) Double logarithmic plot of the horizontal line cuts from the two-dimensional GISAXS signals (dots) measured at casein films prepared at different Ca concentrations together with model fits (solid lines) as explained in the text. From the bottom to the top the added amount of Ca increases from 0 to 100 mM in steps of 10 mM (except 70 and 90 mM). The curves are shifted along the y-axis for clarity. The resolution limit towards large length scales is shown by the dashed line. b) AFM image on a 5*5 and c) on a 10*10 micrometer scan range.

**References**