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Report:

Introduction

The phase behavior of aqueous model protein solutions in the presence of multivalent salts was investigated. A reentrant condensation phase behavior has been found in solutions of bovine and human serum albumin (BSA and HSA) as well as in beta lactoglobulin (BLG) with several multivalent salts [1]. This means that the solutions become turbid at a salt concentration c* and reclarify at a higher salt concentration c**. In the second regime (i.e. the turbid regime) the behavior of the system depends on the nature of both protein and salt. We observe formation of long living clusters as well as formation of aggregates which immediately after preparation fall out of the solution. Furthermore e.g. in the BSA-YCl_3 system there is also liquid-liquid phase separation (LLPS) [2, 3]. In some of the protein salt systems this LLPS has a lower critical solution temperature (LCST). Phase separation and spinodal decomposition occurs when the solution is heated. In other systems we also observe the formation of protein crystals [4]. In order to understand the mechanisms behind aggregation and crystallization more deeply it is important to study all the different salt and protein systems in detail [5, 6].

Experimental and Results

For the measurements solutions of BSA and HSA with YCl_3 were prepared at room temperature with protein and salt concentrations in the LLPS region. This means that after preparation the samples in the quartz cuvettes separated into a protein-poor and a protein-rich phase. The samples were prepared two weeks before the beamtime and then kept in the cuvettes for equilibration. Both the protein-rich and –poor phases undergo further phase separation when heated. The BSA/HSA system with YCl_3 exhibits an LCST phase behavior.

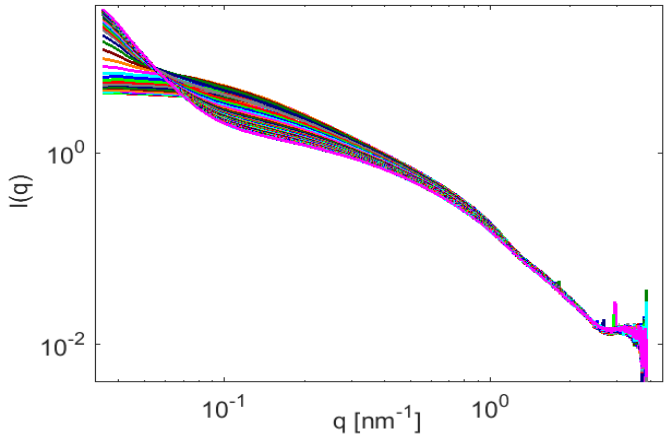

Fig1: Real-time SAXS profile for a sample with 175mg/ml BSA and 34mM YCl_3 after a temperature jump from 12 to 34°C (SD 2m).

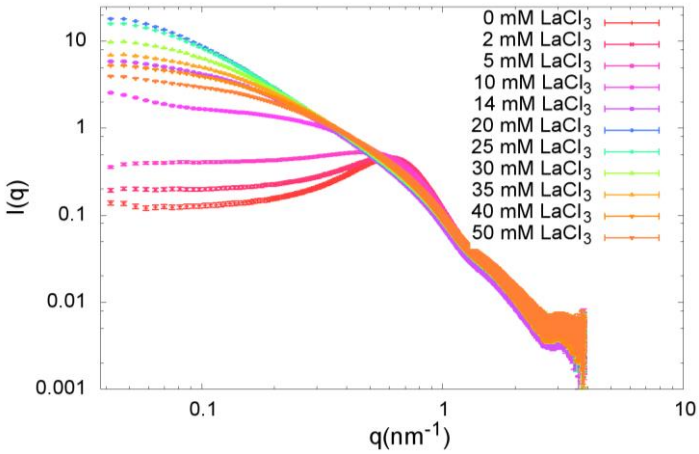

Fig2: SAXS profiles for samples of BSA 150mg/ml with different $LaCl_3$ concentrations.

During the beamtime SC3859 the spinodal decomposition during this further phase separation of the protein-rich phase was studied [7]. We performed temperature ramp measurements of the protein-rich phase of BSA and HSA with different YCl_3 concentrations, namely 175mg/ml BSA with 34, 36, 40, 44 and 46mM YCl_3 and 150mg/ml HSA with 22, 26, 30, 34, 38, 42 and 46mM YCl_3. The samples were transfered to a thermostated sample holder which was first set to 12°C (or 10°C in case of the 150mg/ml HSA samples with 38, 42 and 46mM YCl_3). When the measurement was started the temperature was set to 34°C. During the increase of temperature in the sample several (100-200) frames were recorded with an exposure time of 100ms each. The smple-to-detector distance was 2m. In Fig1 the recorded intensity is shown for the sample with 175mg/ml BSA and 34mM YCl_3. In the medium q-range the intensity decreases when the temperature is increased. Whereas at low q the intensity increases. The development of the intensity suggests the assumption of the development of a peak at lower q outside of the investigated window. With increasing salt concentration this peak seems to move to lower and lower q. In the measured HSA samples this trend is not as obvious yet the steepness of the low-q increase also diminishes for high salt concentrations.

The samples were also measured at a sample-to-detector distance of 30m. For details on these measurements see the report on experiment SC3858 [8].

In addition to the BSA/HSA system with YCl_3, a BSA system with another trivalent salt, $LaCl_3$, was investigated. The cation size of La^{3+} is larger than that of Y^{3+}. The special feature of this sytem is that there is no formation of massive aggreagtes but of long- lived clusters which do not precipitate until several hours after preparation. Therefore it is possible to, even in the second regime, measure the whole solution and not only the dense or dilute phase. These samples were prepared directly before the measurement. For each sample 20 frames were recorded with an exposure time of 50ms each.

The result of these measurements is shown in Fig2. At low protein concentrations the low-q intensity is below 1. This means that the system is repulsive. With increasing salt concentration the low-q intensity also increases and the system becomes attractive. When the system approaches c** this trend is reversed and the low-q intensity decreases with further increasing salt concentration. It is also observed that even well above c** (c** = 32±2mM at this protein concentration), the system does not become repulsive again.

References:

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