ION-SPECIFIC EFFECTS FOR TUNING THE PHASE BEHAVIOUR OF PROTEIN SOLUTIONS

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Protein phase behaviour is of importance in various areas of research such as structural biology, rational drug design and delivery, medicine (in particular protein condensation diseases), biotechnology, food science and cell biology. A particularly intriguing variety of phase behaviours can be induced in negatively charged, globular proteins in the presence of multivalent salts such as lanthanide (Ln) chlorides. These behaviours include reentrant condensation, crystallisation and cluster formation as well as liquid-liquid phase separation (LLPS) into a protein-rich and a protein-poor phase [1-3]. LLPS can occur upon a temperature decrease or increase, which is referred to as an upper or a lower critical solution temperature (UCST- and LCST-LLPS), respectively. Here, we present a challenging set of experiments investigating the complex phenomenon of LCST-LLPS in systems of bovine serum albumin (BSA) and multivalent salts from different perspectives including thermodynamic, (non-)equilibrium and spectroscopic studies.

First, the rather unusual phenomenon of LCST-LLPS in aqueous systems consisting of BSA and yttrium chloride (YCl_3) is characterised thermodynamically. Surface charge (zeta potential) and isothermal titration calorimetry (ITC) measurements show LCST-LLPS to be a hydration entropy-driven condensation [2]. This mechanistic explanation is corroborated by results obtained using extended X-ray absorption fine structure (EXAFS) spectroscopy. Based on the Y^{3+}-induced LCST-LLPS described above, the aspect investigated subsequently is the influence that the nature of the multivalent cations used has on this phase behaviour. The experiments focus on the three multivalent salts $HoCl_3$, YCl_3 and $LaCl_3$. A multi-technique approach including temperature-controlled UV-Vis absorbance and synchrotron small-angle X-ray scattering (SAXS) measurements shows that Ho^{3+} cations induce the strongest protein-protein attractions, while the interactions are weakest in the case of La^{3+}. The overall protein-protein and protein-cation interaction strengths can therefore be ranked according to the order $Ho^{3+} > Y^{3+} > La^{3+}$ [3]. Finally, the kinetics of LCST-LLPS of BSA in the presence of varying ratios of $HoCl_3$ and $LaCl_3$ is investigated using synchrotron ultra-small-angle X-ray scattering (USAXS). The growth of the characteristic length scale of the respective experimental systems as a function of time and temperature is found to be strongly influenced by the $HoCl_3/LaCl_3$ ratio. Notably, a higher volume fraction of $HoCl_3$ preferentially drives the samples into an arrested state even at low temperatures [4].

The present study thus shows how a careful choice of multivalent ions can be used to fine-tune protein-protein interactions and the resulting phase behaviour in solution. The results are of importance not only for a fundamental understanding of soft matter thermodynamics, but also for the design of so-called “smart” materials with implications for, e.g., drug delivery or water purification.

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