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Abstract:

The aggregation rate of B-Lactoglobulin (BLG) was studied using turbidimetry and dynamic light scattering in the range 5.8<pH,3.5 at a fixed ionic strength of 4.5 mM, and in the range 4.5 - 500mM NACl at a fixed pH of 5.0. The initial slope of turbidity vs time curve was used to define an initial rate. The highest initial rates of aggregation were observed in the pH range 4.50 to 4.75 but the increase in aggregation rate when the pH was reduced from 5.0 to 4.69 was large compared to its decrease when the pH was reduced from pH 4.69 to 4.20; i.e. the dependence of initial rate on pH was highly asymmetric. The rate of aggregation at pH 5.0 strongly increased with decrease in ionic strength I from 100 to 4.5 mM and was found to be nearly linear with 1/ I. QELS measurements at pH 5.22 and 5.40 at I = 4.5mM revealed that particle size increased with time. Eventual appearances of bimodal distributions showed fast and slow modes corresponding to the BLG dimer and to hydrodynamic diameter 100-800 nm. Measurements at 4.0 and 4.2 indicated the consumption of dimers in the first few minutes to form higher order aggregates. Electrostatic modeling via Delphi was used to visualize the electrostatic poetnetial around the BLG dimer in order to elucidate the pH and ionic strength dependence of BLG aggregation rates. The aggregation process appears to comprise firstly an initial fast consumption of dimer, whose dependence on pH and I arises from the interaction of the positive and negative domains of interacting dimers; and secondly, the slow formation of much larger aggregates with relatively little sensitivity to pH and I. The open-ended nature of BLG aggregation is thought to arise from the asymmetry of the dimer charge distribution in the range 4.2<pH<5.2.

Polyanions appear to inhibit aggregation. However, the role of polyanions in minimizing BLG aggregation was observed immediately after the addition of polyanioin to the protein.

Description:

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