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Analytica-2015 : Thermal denaturation and aggregation of bovine serum albumin- Borzova Vera Alexandrovna - Russian Academy of Sciences

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Thermal aggregation of Bovine Serum Albumin (BSA) was studied at fixed temperatures (60 °C, 65°C, 70°C and 80°C) using Dynamic Light Scattering (DLS), asymmetric-flow field flow fractionation and Analytical Ultra-Centrifugation (AUC). Thermal denaturation of the protein was characterized by differential scanning calorimetry. Analysis of the experimental data allowed us to propose the mechanism of thermal aggregation of BSA. Protein unfolding results in the formation of three forms of the non-native protein with different propensity to aggregation. Highly reactive form (Uhr) is characterized by a high rate of aggregation. Aggregation of Uhr leads to the formation of the primary aggregates. Lowly reactive form (Ulr) possesses a low ability for self-aggregation. The Ulr form is able to participate in the aggregation process by attachment to the primary aggregates produced by the Uhr form. Non-reactive form (Unr) remains in the non-aggregated state during prolonged heating. The Unr form was purified and characterized by fluorescent spectroscopy, AUC and DLS. Thermal aggregation of BSA was proposed as a test-system for quantification of the anti-aggregation activity of arginine and its derivatives. The dual effect of arginine derivatives on the initial rate of aggregation was observed. The determination of the order of aggregation with respect to the protein at 700C shows that the rate-limiting state of the general process of BSA aggregation is the stage of aggregation of the denatured protein molecules. Thus, the observed effects of arginine and its derivatives demonstrate their direct action on the stage of aggregation.

In the present work we studied the kinetics of thermal aggregation of BSA in the temperature interval from 60°C to 80°C using DLS and AF4. To control unfolding of BSA, DSC was used. Morphology of BSA aggregates was studied using transmission electron microscopy (TEM). The relationship between non-aggregated and aggregated forms in the preparation of BSA preincubated at 60°C was also characterized using analytical ultracentrifugation (AUC). To isolate the fraction of non-aggregated unfolded form of BSA, size-exclusion chromatography (SEC) was applied. Based on the analysis of the relationship between the portion of the aggregated protein (γagg) and portion of the denatured protein and the relationship between the light scattering intensity and γagg, a model of thermal aggregation of BSA has been developed. The model involves formation of the primary aggregates with participation of highly reactive unfolded form of BSA, growth of the primary aggregates as a result of the attachment of low reactive unfolded form of BSA and further sticking of the newly-formed secondary aggregates with formation of polydisperse amorphous aggregates.

Analysis of the relationship between the portions of aggregated and denatured protein allowed us to make a conclusion about the kinetic regime of thermal aggregation of proteins. Using this approach, we have demonstrated that the rate-limiting stage of BSA aggregation at 65°C is the stage of protein unfolding. At higher temperatures (for example, at 80°C) the stage of aggregation of the denatured protein molecules becomes the rate-limiting stage.

Biography

Borzova Vera Alexandrovna has completed her graduation from Moscow State University, Department of Biochemistry. She is working currently on her PhD thesis at A N Bach Institute of Biochemistry, Russian Academy of Sciences, Laboratory of Structural Biochemistry of Proteins, as a Research Scholar. She has published 4 papers in international journals with impact factor >3.

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