

## Fundamental Aspects of Conformational Lability of Proteins

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The distinctive feature of proteins is their lability. Proteins lose native structure under the action of physical and chemical factors resulting in disappearance of the biological activity. The denaturation processes, as a rule, are accompanied by aggregation of the denatured protein molecules. Over many years phenomenon of protein aggregation is of little scientific interest. In recent years, much attention is given to this problem in connection with the discovery of a great number of inherited diseases known as “conformational diseases”. These diseases are originated when certain proteins undergo structural alterations favoring their aggregation (Alzheimer’s and Parkinson’s diseases, Huntington’s disease, Machado-Joseph’s disease (spinocerebellar ataxia), prion encephalopathies, Charcot’s disease (amyotrophic lateral sclerosis), systemic amyloidosis and cystic fibrosis) [1-6]. Therefore the investigations of mechanisms of protein aggregation and a search for the agents possessing the anti-aggregation activity acquire especial actuality.

There is a specialized class of molecular chaperones, namely small heat shock proteins (sHsps), whose main function is suppression of protein aggregation [7,8]. Besides, certain low molecular weight compounds (amino acids, polyamines, cyclodextrins and others) may reveal anti-aggregation activity [9-11]. Different types of test systems are used for detection of the agents possessing the anti-aggregation activity. Among them are the test systems based on protein aggregation induced by the action of physical factors (heat) or chemical agents (acid, relatively low concentrations of denaturing agents such as urea or guanidine hydrochloride), the test systems based on protein aggregation induced by reduction of disulfide bonds in the protein molecules (dithiothreitol-induced aggregation of insulin,  $\alpha$ -lactalbumin, lysozyme, conalbumin or bovine serum albumin) and the test systems based on protein aggregation accompanying refolding of proteins which are transferred to unfolded state by the action of urea or guanidine hydrochloride. Analysis of the test systems used in the literature for the study of the effects of different agents on protein aggregation shows that they do not allow registering the true influence of the agents on the aggregation stage. The observed effects include the action of the agents on the stage of the formation of the intermediates prone to aggregation. In a test system based on heat-induced aggregation of proteins the aggregation stage is preceded by the stage of unfolding of the protein molecule, and an agent being tested may affect both the protein unfolding stage and the stage of aggregation of unfolded protein molecules. Such a situation is observed, for example, when studying the effect of  $\alpha$ -crystalline, a representative of the family of small Heat shock proteins (sHsps), on thermal aggregation of rabbit muscle glyceraldehyde-3-phosphate dehydrogenase [12] or glycogen phosphorylase *b* [13]. In a test system where aggregation accompanying refolding of the proteins denatured by guanidine hydrochloride or urea is registered an agent being tested may affect not only the aggregation stage but also the stage of the formation of aggregation-prone intermediate from completely unfolded state.

Thus, a strong demand arose for the elaboration of the test systems which allow the effect of the agents being tested directly on the stage of aggregation of protein molecules to be registered. To achieve these aims, Kurganov and coworkers [14-16] have elaborated the test systems based on aggregation of proteins irradiated by ultraviolet light.

The protein substrate is exposed to ultraviolet radiation at relatively low temperature. UV-irradiation causes the formation of denatured protein, the degree of denaturation being controlled using, for example, differential scanning calorimetry. The elevation of temperature till 37°C results in aggregation of ultraviolet light-denatured protein molecules. The chief merit of test systems based on aggregation of UV-irradiated proteins is that they enable testing the effect of various agents exclusively on the aggregation stage.

The cellular environment contains many volume-excluded macromolecules such as proteins, polysaccharides, nuclear acids and lipids, creating conditions of so-called “crowding”. Molecular crowding may significantly affect protein conformational transitions, protein folding, the rates of biochemical reactions and association (aggregation) of macromolecules [17-19]. It is significant that the test systems based on aggregation of UV-irradiated proteins allow registering “pure” effects of crowding on protein aggregation and anti-aggregation activity of molecular chaperones [14-16]. It was shown that the protective action of sHsps under crowding conditions was due to the formation of the complexes of dissociated forms of sHsp with a protein substrate [14].

The study of the mechanisms of protein misfolding and protein aggregation will serve as a basis for refinement of our knowledge of the nature of conformational diseases and elaboration of the methods of their treatment. The practical result of the investigations of the action of the agents possessing anti-aggregation activity with the use of new test systems is the sampling of the compounds which may find application in biotechnology for the elaboration of the methods of renaturation of recombinant proteins being isolated from inclusion bodies and methods of suppression of aggregation in therapeutic protein preparations as well as in medical practice for design of medicines suitable for suppression of adverse aggregation of proteins (for example, protein aggregation induced by ultraviolet irradiation).

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