

The Functioning of Chaperones Possessing the Anti-Aggregation Activity in a Crowded Medium

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Crowding conditions created by macromolecules (proteins, nucleic acids, polysaccharides) affect all the biochemical reactions in the cell including the processes of folding and misfolding of the proteins. Interaction of non-native forms of proteins leading to the formation of amorphous aggregates or amyloid fibrils is potentiated by crowding. There is a large body of data demonstrating stimulating action of crowding on protein aggregation in model systems [1-16].

One of the components of the protein quality control system is the family of small heat shock proteins (sHsps). The main function of sHsps is the suppression of aggregation of non-native forms of proteins. Oligomers of sHsps are composed from subunits with molecular mass of 12-43 kDa. sHsps are not capable of assisting the folding of newly synthesized and stress-denatured polypeptide chains, however they form complexes with non-native forms of proteins and transfer the latter to ATP-dependent chaperones providing protein folding or to proteasomes where proteolytic degradation of unfolded proteins occurs. One of the representatives of the family of sHsps is α -crystallin which reveals protective (anti-aggregation) action in eye lens. sHsp oligomers have dynamic quaternary structure. There are numerous experimental data demonstrating high rate of subunit exchange between oligomers formed by sHsp [17-24].

The anti-aggregation activity of sHsps has been thoroughly studied in model systems. However the problem of action of sHsps under crowding conditions has not received enough attention. When studying the effect of dextran with molecular mass of 68800 Da on dithiothreitol-induced aggregation of ovotransferrin at various temperatures, Carver and co-workers [25] showed that α -crystallin and A-crystallin were poorer chaperones under crowding conditions. Kinetics of protein aggregation was followed by measurement of the light scattering intensity at 360 nm.

The analogous result was obtained in our research into the action of α -crystallin on aggregation of UV-irradiated glycogen phosphorylase (Phb) in the presence of the following crowding agents: polyethylene glycol (PEG) with molecular mass of 20000 Da and trimethylamine N-oxide [9]. The increase in the concentration of the crowding agent results in the disappearance of the protective ability of α -crystallin. Chebotareva et al. [16] studied the effect of crowding agents (PEG and Ficoll-70) on the chaperone-like activity of α -crystallin with a test-system based on thermal aggregation of apo-Phb. It was also demonstrated that the anti-aggregation activity of α -crystallin was decreased in the presence of crowders. To register aggregation of UV-irradiated Phb or apo-Phb, the increment of the light scattering intensity at 632.8 nm was measured.

The fact that the protective action of α -crystallin disappears under crowding conditions was surprising because it became unclear how sHsps could realize their protective function in the crowded cell environment.

To elucidate the peculiarities of functioning of sHsps under conditions imitating the cell crowded medium, the interaction of α -crystallin with the target protein (UV-irradiated Phb) was studied using analytical ultracentrifugation and size-exclusive chromatography

[9]. The α -crystallin-target protein complexes resistant to aggregation under crowding conditions have been detected. These complexes are formed by the target protein and dissociated species of α -crystallin. The idea of forming a complex between dissociated forms of α -crystallin and a target protein followed by the assembly of these complexes into high-molecular-weight species was developed by Carver and co-workers [26]. When using Phb and glyceraldehyde 3-phosphate dehydrogenase as target proteins, we demonstrated the formation of complexes between the target substrate and dissociated forms of α -crystallin at elevated temperatures (48 and 45°C, respectively) [28-30]. It should be noted that according to the current view dissociation of sHsps is required for recognition and binding of structurally unstable proteins [31-37].

Using UV-irradiated Phb as a target protein we proposed the mechanism of functioning of α -crystallin under crowding conditions [9]. This mechanism is represented in figure 1. First of all, this scheme demonstrates that crowding stimulates aggregation of a target protein. Interaction of α -crystallin with a target protein results in the formation of the primary complex I and by this means prevents the target protein aggregation. The complexation process is rather fast. However, the primary complex undergoes a time-dependent structural rearrangement resulting in the formation of complexes II and III greatly differing in ability to aggregation. Complex II is formed by the dissociated species of α -crystallin and a target protein. It is significant

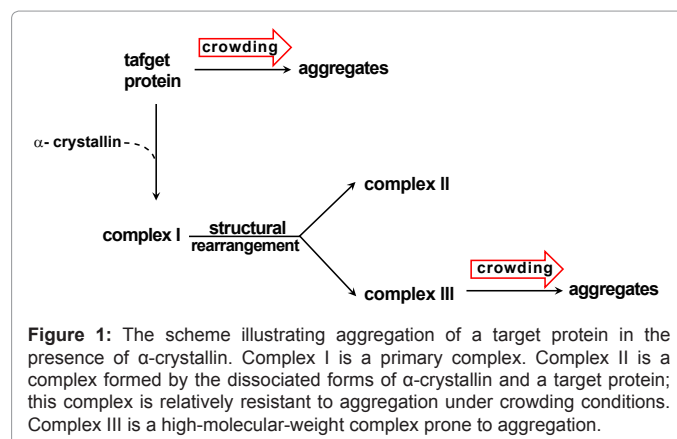


Figure 1: The scheme illustrating aggregation of a target protein in the presence of α -crystallin. Complex I is a primary complex. Complex II is a complex formed by the dissociated forms of α -crystallin and a target protein; this complex is relatively resistant to aggregation under crowding conditions. Complex III is a high-molecular-weight complex prone to aggregation.

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that this complex is relatively resistant to aggregation under crowding conditions. On the contrary complex III is a high-molecular-weight complex that readily aggregate in a crowded medium.

The fact that complex III is prone to aggregation under crowding conditions explains the paradoxical disappearance of the chaperone-like activity of α -crystallin registered by the measurement of the increase in the light scattering intensity. The disappearance of the chaperone-like activity in crowded solutions may be simply explained by the acceleration of aggregation of complex III. Aggregation of this complex masks the formation of complex II that provides the protection against aggregation of a target protein under crowding conditions. Thus, using analytical ultracentrifugation and size-exclusive chromatography allowed us to elucidate how sHsps can fulfill their protective function in the cell crowded medium. High mobility of the quaternary structures of sHsps provides the possibility of the formation of complexes involving a target protein and dissociated forms of sHsp. These complexes remain in a non-aggregated form under crowding conditions.

The structural rearrangement of the primary complexes (complexes I) deserves special attention. To obtain the time characteristics of complex I reorganization, the following approach may be used. Aggregation of a target protein is being registered in the presence of α -crystallin (or other sHsp). We select the interval of time where an initial increment of the light scattering intensity is not yet observed and add a crowding agent at definite time intervals. Analysis of the initial rate of aggregation of a target protein as a function of time corresponding to the moment of addition of a crowding agent allows estimating the time of half-conversion ($t_{1/2}$) for the structural rearrangement of the primary complexes. Using such an approach, we determined, for example, the $t_{1/2}$ value for the structural rearrangement of the primary complexes formed by UV-irradiated Phb and α -crystallin at 37 °C ($t_{1/2}$ = 6.2 min; N.A.Chebotareva, B.I.Kurganov, unpublished data). PEG with a molecular mass of 20 kDa was used as a crowding agent in these experiments.

In conclusion it should be stressed that the crowded environments inside cells play a critical role in the development of protein-aggregation related diseases and investigation of factors controlling protein aggregation is of great importance for the development of corresponding treatment modes [38,39].

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