Chaperone-Like Activity of HSPB5

Subjects: Biochemistry & Molecular Biology

Contributor: Natalia Chebotareva, Svetlana Roman, Valeriya Mikhaylova, Boris I. Kurganov

Small heat-shock proteins (sHSPs) are ATP-independent molecular chaperones that interact with partially unfolded proteins, preventing their aberrant aggregation, thereby exhibiting a chaperone-like activity. Dynamics of the quaternary structure plays an important role in the chaperone-like activity of sHSPs. However, relationship between the dynamic structure of sHSPs and their chaperone-like activity remains insufficiently characterized. Many factors (temperature, ions, a target protein, crowding etc.) affect the structure and activity of sHSPs. The least studied is an effect of crowding on sHSPs activity. In this work the chaperone-like activity of HSPB5 was quantitatively characterized by dynamic light scattering using two test systems, namely test systems based on heat-induced aggregation of muscle glycogen phosphorylase *b* (Ph*b*) at 48 °C and dithiothreitol-induced aggregation of α-lactalbumin at 37 °C. Analytical ultracentrifugation was used to control the oligomeric state of HSPB5 and target proteins. The possible anti-aggregation functioning of suboligomeric forms of HSPB5 is discussed. The effect of crowding on HSPB5 anti-aggregation activity was characterized using Ph*b* as a target protein. The duration of the nucleation stage was shown to decrease with simultaneous increase in the relative rate of aggregation of Ph*b* in the presence of HSPB5 under crowded conditions. Crowding may subtly modulate sHSPs activity.

Keywords: HSPB5 ; chaperone-like activity ; oligomeric states ; mixed crowding

1. Definition

HSPB5 (αB-crystallin) belongs to a superfamily of small heat shock proteins (sHSPs), which are ubiquitously expressed and play an important role in maintaining cellular proteostasis [1]. sHSPs bind non-native and misfolded proteins, keeping them from further aggregation and protecting the cell from toxic aggregates [1][2][3][4][5]. In addition to the exhibition of the anti-aggregation (chaperone-like) activity, these proteins are involved in many important processes in the cell, such as apoptosis, stabilization of cytoskeleton [6], regulation of muscle contraction, regulation of redox state [7], signal transduction, etc. [4][8][9][10]. Given these significant biological roles, the dysregulation of sHSPs is associated with cancer [11], cataract formation [12][13][14], and neurodegenerative diseases [5][10]. It is known that mutations in sHSPs have been directly linked to different myopathies including Charcot-Marie-Tooth disease and neuropathy [10][14][15]. Therefore, the processes of regulation of the functioning of sHSPs are very important.

2. Features

Among the ten human sHSPs, HSPB5 is one of the principle members. HSPB5 is widespread in all tissues, but its concentration in the eye lens is especially high (400 mg/mL), where it interacts with HSPB4 (αA-crystallin) and forms a native hetero-oligomeric complex, α-crystallin [16]. These proteins ensure the transparency of the eye lens [17], preventing the aggregation of other crystallins and thereby protecting the lens from the development of cataracts [12]. All sHSPs, such as HSPB5, in their structure have a central α-crystalline domain (ACD) with an ordered structure, which is flanked by two variable terminal regions, C- and N- terminal domains, with a partially disordered structure, intrinsically disordered regions (IDRs) [18]. The ACD domain plays an important role in the formation of a dimer, which is considered as the building block that assembles via terminal interactions into a polydisperse ensemble under physiological conditions [9][19][20]. N-terminal regions are involved in the formation of large oligomers [18][21][22]. Carver and coworkers proposed, that the mobile C- and N-terminal regions of HSPB5 are involved in regulation of interaction with substrates; they protect ACD domain from amyloid fibril formation [20] and the flexibility of these regions, especially C-terminal regions, provides solubility for sHSPs [18][20]. The importance of the IDRs regions in sHSPs for formation of dynamic macromolecular assemblies and regulation of protein functionality has been discussed in the work [10].

HSPB5 tends to form large polydisperse assemblies ranging from 10-mer to 40-mer and higher, having very dynamic structures [19][23][24][25]. All oligomeric forms possess chaperone-like activity and easily exchange their subunits [20][26][27]. It is well accepted that a flexible dynamic quaternary structure is necessary for sHSPs activity [1][4][10][28]. Changes in the

cellular environment, such as temperature [9][10][27][29], pH [30], the presence of ions [31][32], post-translational modification (phosphorylation) [33][34][35], redox environment [7][30] and crowding [19][25][32][36][37][38] also regulate chaperone activity by affecting the structural and oligomerization dynamics of sHSPs [4][5]. It has been reported that sHSP dynamics is a very complex process that includes five levels of regulation: (1) flexible domains flanking the ACD, (2) polydisperse self-oligomerization, (3) hetero- oligomerization with other sHSPs, (4) subunit exchange, and (5) regulation by the cellular environment [5][39]. As such, multiple oligomeric forms are likely relevant to function, and regulation thereof. The importance of quaternary dynamics for realization of chaperone activity of sHsps was discussed in [5][9][10][19][28][29]. However, the relationship between the dynamic variable structure and functions of sHSPs is far from being clear. The least studied is the effect of crowding on this relationship.

The cellular interior contains large concentrations of macromolecules, reaching up to 400 g/L, including proteins, nucleic acids, lipids, glycans, and solvated ions [40][41]. This means that about 40% of cell volume may be occupied by macromolecules and become physically unavailable for other molecules. A nonspecific influence of such unavailable volume on the specific biochemical reactions [42][43] was termed macromolecular crowding or excluded volume effect [44]. Theory predicts that crowding affects both the kinetics and thermodynamics of interactions between macromolecules, including protein aggregation [38][42][43][45][46][47][48].

It is usual to mimic crowded milieu in vitro by adding high concentration of suitable inert polymer or protein, so-called crowding agent, such as polyethylene glycols (PEG) of different molecular mass, polysaccharides (Ficoll, dextran), polyvinylpyrrolidone (PVP) or proteins (bovine serum albumin, lysozyme) [42]. For a long time, researchers simulated crowding in vitro by adding a single crowding agent at concentrations >100 mg/mL. Considering that the molecules of crowders are completely neutral and the effect of crowding on biochemical reactions is manifested due to steric repulsion of macromolecules, that is, the excluded volume effect (EVE). Currently, it is believed that the influence of crowding can be considered to be a mixture of entropic (excluded volume) and enthalpy based (soft interactions) effects [47][49][50][51][52]. “Soft” interactions include electrostatic, hydrophobic, and van der Waals interactions between the crowding agent and studied protein [53][54]. These interactions can be repulsive or attractive. Therefore, such soft interactions (enthalpy factor) can counteract EVE [50][55][56][57][58].

Given that in a cell crowding is created by the presence of different molecules that differ in size, shape and charge, some groups of researchers came to the conclusion that it is better to imitate physiological conditions in vitro with a mixture of several crowders (mixed crowding) [47][49][50][51][52][59]. It has been shown that two crowders can exhibit a synergistic effect, significantly enhancing the effect of each other, even when using relatively small concentrations (10–20 mg/mL) [49]. It has also been shown that small crowders create more total excluded volume in the vicinity of big crowder than in the bulk [49][58]. Sharp pointed out that when the steric effects of macromolecular crowders and small molecules like water and ions are treated on an equal footing, the effect of the macromolecules are less effective at crowding than water and ions [60]. Shah and coworkers developed a molecular thermodynamic formalism to examine the effects of size-polydispersity of crowders on aggregation reaction equilibrium. They showed that in the case of polydisperse crowders, the crowders with the largest size difference dominate the overall changes in the yield of the reaction [50].

Thus, crowding adds another level of complexity to the relationship between the activity and structural dynamics of HSPB5. Since it has a strong effect on protein–protein interactions, it should affect the conformation and self-association of the chaperone, the interaction of the chaperone with the target protein, and the aggregation of the target protein. Previously, we have shown by the analytical ultracentrifugation (AUC) method that crowding strongly affects the oligomeric state of HSPB5, HSPB6, HSPB1 and α-crystallin [25][32][36][61][62][63][64]. Thus, one might assume that crowding affects the capability of sHSPs to prevent aggregation of target proteins.

The goal of this work was to quantitatively assess the effect of crowding, including mixed crowding, on the chaperone-like activity of HSPB5. As the process of protein aggregation includes the stage of protein unfolding followed by the aggregation of unfolded protein molecules, in a general case, sHSPs can affect the unfolding stages as well as the aggregation stage. Therefore, it is very important to question what stage of the overall aggregation process (unfolding or aggregation) is rate-limiting in the selected test system.

In the present work, two test systems were selected to study the chaperone-like activity of HSPB5, one of which is based on the thermal aggregation of glycogen phosphorylase *b* (Ph*b*) at 48 °C, and the other is based on dithiothreitol-induced aggregation of α-lactalbumin at 37 °C; these test systems were described earlier [65][66][67][68]. Ph*b* exists as a dimer consisting of two identical subunits with molecular mass of 97.4 kDa each [69]. α-Lactalbumin (αLa) is a small Ca^{2+}-binding protein containing four disulfide bridges with molar mass of 14.2 kDa. Under stress conditions, the Ca^{2+}-depleted form of α-lactalbumin attains a classical molten globule state that aggregates amorphously [70][71][72][73]. The molten globule

conformation of α-lactalbumin is thought to be a target for interacting with sHSPs [66][68][70][71][72][73][74][75][76]. The rate-limiting stages were established for the aggregation process for both proteins and the effect of HSPB5 on aggregation of these proteins was quantitatively evaluated.

We compared the effect of crowding by both individual crowders and pairs of crowders on the anti-aggregation activity of HSPB5 using Ph*b* as a target protein. Four pairs of crowders demonstrated a synergistic effect on the activity of HSPB5. This study has provided insights into the mechanism of chaperone function under crowded conditions.

3. Development and Finding

It is well known that the dynamics and polydispersity of oligomers play an important role in the functioning of α-crystallins [10][29][77][78]. It was reported that subunit exchange promotes structural reorganization within the homo-oligomers of αB-crystallin [27] (see review [29]). The equilibrium between oligomeric forms is very sensitive to many factors such as temperature [1][27][79][80][81], post-translational modifications (phosphorylation) [33][35][82], divalent cations [31][32], crowding conditions [25], presence of target protein [25], and many others. Benesh and colleagues [77] suggested that the quaternary structure of α-crystallins is modulated by the assembly of oligomers from monomers or from dimers and there is an exchange between these forms, that have different conformation and chaperone-like activity [77]. Aquilina and coworkers showed that the population of αB-crystallin oligomers from the bovine eye lens contains oligomer consisting of an even and odd number of subunits [83]. They concluded that a monomer is the main building block of this assembly. Considering the monomers as the most active species, one can explain why αB-crystallin can prevent protein aggregation at very low stoichiometric ratio compared to the target protein. Such effective stoichiometry is possible in the cell, since the unfolded target proteins are often present at low concentrations and aggregate slowly (for example, in the lens). Therefore, a high concentration of active monomer species is not required to prevent the unfolding and aggregation of the target protein [29].

In the present work the stoichiometry of the HSPB5–target protein complex (S_0) have been determined for two target proteins using Equation (3). In the case of heat-induced aggregation of Ph*b* we have shown that the dependence of the $K_{agg}/K_{agg,0}$ value on the ratio of molar concentrations of HSPB5 and Ph*b* ([HSPB5]/[target protein]) is complex, and the stoichiometry of the resulting chaperone–target protein complexes is variable (Figure 1). Possibly, the obtained complexity of the above dependence is associated with a change in the quaternary structure of HSPB5 with varying molar ratio [HSPB5]/[Ph*b*]. We suggest that the rather complicated dependence of the $K_{agg}/K_{agg,0}$ value on the molar ratio [HSPB5]/[Ph*b*] (Figure 1) is associated with: (1) the change in the oligomeric state of the chaperone with an increase in its concentration, and (2) possible variations in the affinity of HSPB5 species with respect to the denatured/aggregated protein. We assume that the lower the concentration of HSPB5, the greater is the fraction of “traveling monomers” in the solution at 48 °C [27] and, therefore, the more HSPB5 oligomers consist of monomeric building blocks [77]. With increasing concentration of HSPB5, the proportion of dimers and oligomers constructed from dimeric building blocks grows. The chaperone function of these two states is different: the monomeric substructural state has greater exposed hydrophobic surface area and is consequently more active [77]. All the above mentioned is likely to underlie the obtained value of the stoichiometry of the chaperone–target protein complexes at the initial stages of Ph*b* aggregation at 48 °C at low ratios of molar concentrations [HSPB5]/[Ph*b*] (in the excess of the molar concentration of Ph*b*): when one monomer of HSPB5 (with the molecular mass of 20.2 kDa) is complexed with over three Ph*b* monomers (with the molecular mass of 97.4 kDa each).

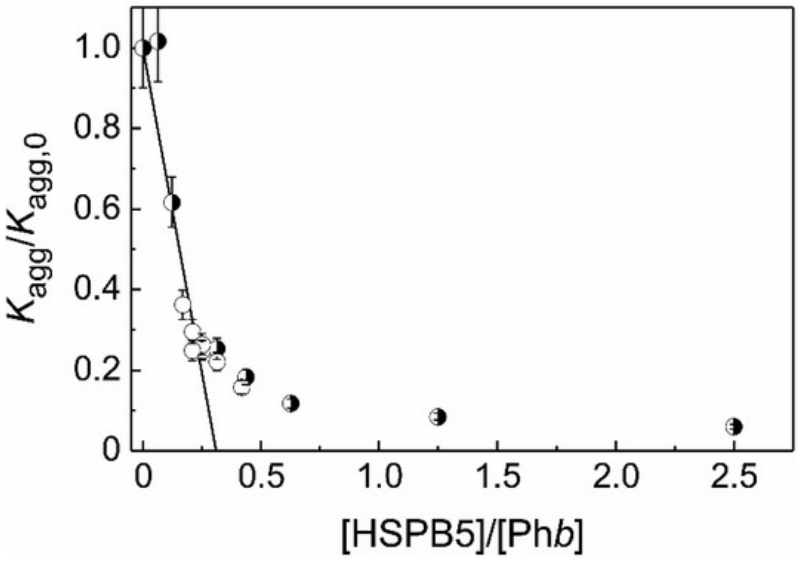

Figure 1. The suppression of Ph*b* aggregation by HSPB5. The dependence of $K_{agg}/K_{agg,0}$ ratio on the ratio of molar concentrations of HSPB5 and Ph*b* (x = [HSPB5]/[Ph*b*]). Open circles correspond to $K_{agg}/K_{agg,0}$ values at constant HSPB5 concentration (0.025 mg/mL). Half-filled circles correspond to $K_{agg}/K_{agg,0}$ values at constant Ph*b* concentration (0.4 mg/mL). The solid line is calculated from the equation $K_{agg}/K_{agg,0} = 1 - x/S_0$ at $S_0 = 0.31$.

When studying the kinetics of DTT-induced aggregation of αLa (a protein with a molecular mass of the monomer of 14.2 kDa, smaller than that of HSPB5 subunit) in the presence of HSPB5 (Figure 2), we obtained an even lower value of the stoichiometry of the chaperone–target protein complexes, namely less than 0.011. According to the sedimentation velocity analysis we conclude that suboligomeric forms of HSPB5 (monomeric or dimeric) may interact with the target protein, DTT-denatured αLa, forming relatively small oligomers (see Table 1). However, the high-order oligomeric HSPB5–αLa complexes may be present. According to Hayashi and Carver the monomeric form of HSPB5 may be its most chaperone-species active [29]. However, the presence of monomers can increase the hydrophobicity of HSPB5 and decrease its solubility. Therefore, for the chaperone-like activity, the balance between monomeric and oligomeric forms is very important.

Table 1. Estimation of the molar mass and $s_{20,w}$ values of the complexes between HSPB5 and αLa denatured by 20 mM DTT (0.1 M Na-phosphate buffer, 0.01 M NaCl, pH 6.8, 37 °C).

[HSPB5] (mg/mL)	$s_{20,w}$ (S)	Friction Ratio, f/f_0	Molecular Mass (kDa)
0	2.9 ± 0.8 6.1 ± 0.4	2.7	
0.00045	1.85 ± 0.13	1.614	25.4
0.0015	2.0 ± 0.1	1.545	26
0.0075	1.9 ± 0.2 2.4 ± 0.3	1.925	33 47
0.03	1.6 ± 0.2 2.0 ± 0.1	1.836	23.9 37.4

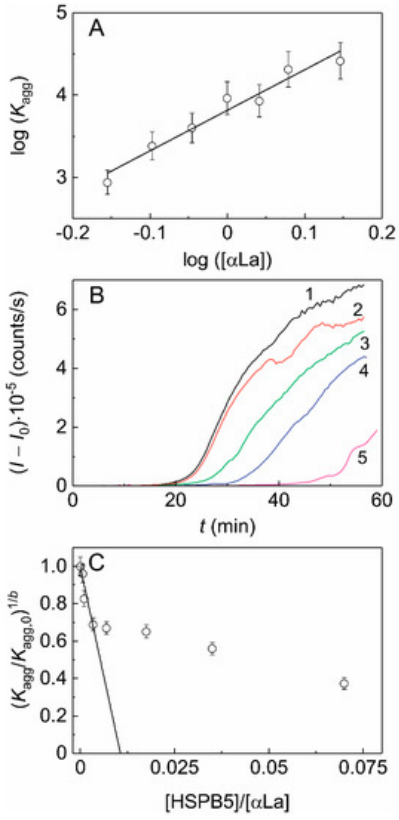

Figure 2. The effect of HSPB5 on DTT-induced aggregation of α-lactalbumin (αLa). (**A**) The relationship between parameter K_{agg} and the initial concentration of αLa in coordinates {log([αLa]); log(K_{agg})}. The slope of the linear fitting is equal to parameter b (b = 4.9 ± 0.5). (**B**) The dependences of the light scattering intensity (I) on time for αLa aggregation ([αLa] = 1 mg/mL, 0.1 M Na-phosphate buffer, pH 6.8, 20 mM DTT, 37 °C) in the absence of HSPB5 (curve1) and in the

presence of the following concentrations of HSPB5: (2) 0.001, (3) 0.0025, (4) 0.0075 and (5) 0.025 mg/mL. (**C**) The dependence of the relative parameter $(K_{agg}/K_{agg,0})^{1/b}$ on the ratio of the molar concentrations of the chaperone and the target protein (x). The linear fitting is drawn according to Equation (3).

Effect of Crowding

In the present work, to quantify the effect of crowding on the chaperone-like activity of HSPB5, we selected parameter K_{agg} characterizing the acceleration of the aggregation process during the nucleation stage. Ph*b* aggregation at 48 °C was chosen as a test system. Table 2 shows the effect of crowding on the kinetic parameters of Ph*b* thermal aggregation. When considering the effect of crowding on Ph*b* aggregation in the presence of HSPB5, it must be emphasized that all crowders used, as well as their pairs, increase the value of parameter K_{agg} as compared to that in the buffer, although to a different extent. That is, the aggregation of the target protein in the presence of HSPB5 under the conditions of crowding created by all used crowders or their mixtures is accelerated compared to the aggregation process in the buffer. Thus, the data obtained by the dynamic light scattering (DLS) method suppose that crowding reduces the chaperone-like activity of HSPB5.

Table 2. Kinetic parameters for aggregation of Ph*b* (0.4 mg/mL) in the presence of HSPB5 at a concentration of 0.025 mg/mL at 48 °C (0.03 M Hepes buffer, 0.1 M NaCl, 0.2 mM EDTA, pH 6.8).

Additions	K_{agg} ((counts/s)/s^2)	t_0 (s)	K_{agg}/K^0_{agg} [1]
Without addition of crowding agents			
–	0.123 ± 0.007	323 ± 9	1.0
Action of individual crowding agents			
PEG_{20kDa} 25 mg/mL	1.85 ± 0.04	287 ± 2	15.0 ± 0.9
PVP_{10kDa} 25 mg/mL	0.28 ± 0.01	266 ± 5	2.3 ± 0.2
PVP_{25kDa} 25 mg/mL	0.52 ± 0.02	232 ± 5	4.2 ± 0.3
$Ficoll_{70kDa}$ 75 mg/mL	0.144 ± 0.015	252 ± 12	1.17 ± 0.14
Combined action of crowding agents			
PEG_{20kDa} 25 mg/mL + $Ficoll_{70kDa}$ 75 mg/mL	1.37 ± 0.05	196 ± 4	11.1 ± 0.8
PVP_{10kDa} 25 mg/mL + $Ficoll_{70kDa}$ 75 mg/mL	1.69 ± 0.05	263 ± 3	13.7 ± 0.9
PVP_{25kDa} 25 mg/mL + $Ficoll_{70kDa}$ 75 mg/mL	3.76 ± 0.21	240 ± 3	30.6 ± 2.4
PVP_{10kDa} 25 mg/mL + PEG_{20kDa} 25 mg/mL	4.00 ± 0.22	227 ± 3	32.5 ± 2.6
PVP_{25kDa} 25 mg/mL + PEG_{20kDa} 25 mg/mL	6.76 ± 0.29	203 ± 2	55 ± 4

[1] K^0_{agg} is the K_{agg} value measured in the presence of HSPB5 and in the absence of any crowders.

It may be assumed that crowding accelerates the aggregation of the chaperone–target protein complexes. However, the data obtained by the sedimentation velocity method indicate the retention of complexes formed by the dissociated forms of HSPB5 and Ph*b* under crowded conditions (Figures 3and 4). This is evidenced by the disappearance of the 23 S peak and a decrease in the 9 S peak in the *c*(*s*) distributions corresponding to the sedimentation of individual HSPB5 and Ph*b*, respectively, and the appearance of peaks with $s_{20,w}$ values of 11, 14, 16 S for HSPB5–Ph*b* mixture under crowded conditions arising from the presence of the pair of crowders, PVP_{25kDa} + PEG_{20kDa} (Figure 4).

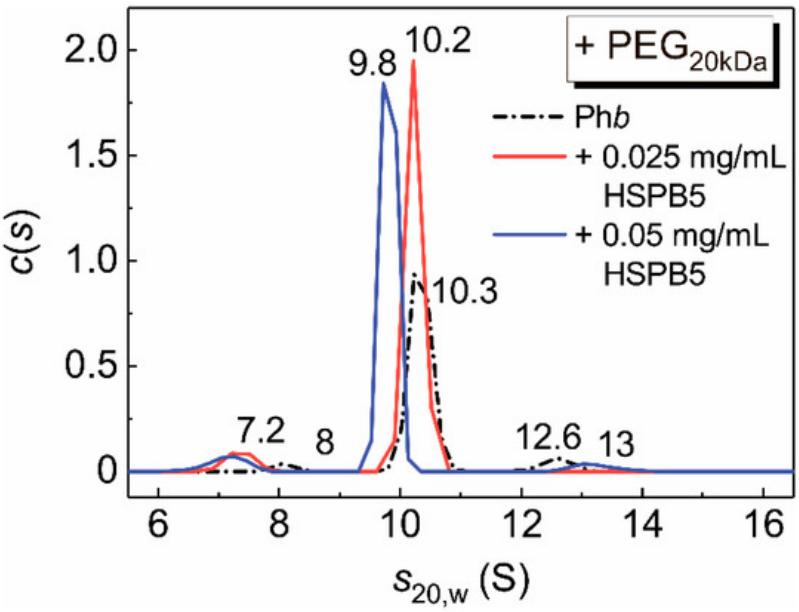

Figure 3. The *c*(*s*) distributions for the mixtures of Ph*b* (0.5 mg/mL) and HSPB5 in the presence of PEG_{20kDa} (25 mg/mL). The following concentrations of HSPB5 were used: 0 (black), 0.025 mg/mL (red) and 0.05 mg/mL (blue). The *c*(*s*) distributions were corrected to the standard conditions. Rotor speed was 48,000 rpm. The total time of the experiment at the elevated temperature was 90 min.

Figure 4. Interaction of HSPB5 with Ph*b* as a target protein at 48 °C under crowded conditions arising from the presence of the mixture of PEG_{20kDa} (25 mg/mL) and PVP_{25kDa} (12.5 mg/mL). The differential sedimentation coefficient distributions, *c*(*s*), for Ph*b* (0.4 mg/mL; dash dot blue line), HSPB5 (0.26 mg/mL; dash red line) and its mixture (solid black line) are presented. The *c*(*s*) distributions were obtained at 48 °C and transformed to standard $s_{20,w}$ distributions. Rotor speed was 48,000 rpm. The total time of the sedimentation experiment at the elevated temperature was about 90 min.

That is, under mixed crowding conditions, although a 55-fold increase in the value of parameter K_{agg} was registered by DLS (compared with the buffer, Table 2), complexes formed by the suboligomeric forms of HSPB5 with the target protein are retained in the solution. This fact is consistent with our earlier data on the interaction of HSPB5 with Phb at 48 °C in the presence of the pair of crowders, PEG_{20kDa} and TMAO [25]. The presence of a target protein may stimulate the

dissociation of large HSPB5 assemblies. However, the existence of high-order oligomeric HSPB5–Phb complexes cannot be ruled out. The obtained results support our previous data on the formation of the complexes between dissociated forms of bovine lens α-crystallin and an apoform of Phb [63], or Phb denatured by ultraviolet radiation [62], apart from the high order complexes. The presence of two types of complexes formed by α-crystallin and target proteins, which differ in their sensitivity to crowding and aggregation, was reported in the work [62]. High molecular mass complexes are aggregation-prone, whereas complexes formed by small suboligomeric forms of chaperone with a target protein are more resistant to aggregation under crowding conditions [36,62]. We suggested that these small complexes are responsible for the realization of the chaperone-like activity of HSPB5 or α-crystallin under crowded conditions [25,36,62]. Our results are consistent with this idea.

4. Conclusions

Thus, we showed that by changing the combination of different crowding agents, almost spherical crowders, like Ficoll, or linear polymers, like PVP, it is possible to regulate (moderate) the activity of the chaperone. Our studies show that adding even one crowder to an existing one dramatically changes the effectiveness of the crowding. Parameter K_{agg} increases in 13.7–55 times in the presence of those pairs of crowders that exhibit synergism (Table 2). On the one hand, this can be explained by the increase (strengthening) of the excluded volume effect, which leads to acceleration of the target protein aggregation. On the other hand, it is currently believed that, in addition to the excluded volume effect (steric repulsion of macromolecules – the entropy factor), the forces of weak interaction between protein molecules and crowder molecules (enthalpy factor) play a significant role in crowded environment [49,50,97–100]. In addition, biopolymers (proteins) capable of reversibly changing the state of association/dissociation or accepting an expanded or compact state of the quaternary structure can change the level of excluded volume in cells [101,102]. It is assumed that proteins with such properties, through their ability to directly influence the degree of excluded volume, will dynamically regulate the functions of proteins in biological media [102]. Small heat shock proteins, such as HSPB5, can take extended or compact states [9,20] and change the state of association, therefore can affect the effective level of excluded volume. Thus, crowding adds another level of complexity to the relationship between the activity and structural dynamics of sHSPs and may subtly modulate sHSPs activity.

References

1. Haslbeck, M.; Vierling, E.A. First line of stress defense: Small heat shock proteins and their function in protein homeostasis. J. Mol. Biol. 2015, 427, 1537–1548.
2. Tyedmers, J.; Mogk, A.; Bukau, B. Cellular strategies for controlling protein aggregation. Nat. Rev. Mol. Cell Biol. 2010, 11, 777–788.
3. Chen, B.; Retzlaff, M.; Roos, T.; Frydman, J. Cellular strategies of protein quality control. Cold Spring Harb. Perspect. Biol. 2011, 3, a004374.
4. Mymrikov, E.V.; Seit-Nebi, A.S.; Gusev, N.B. Large potentials of small heat shock proteins. Physiol. Rev. 2011, 91, 1123–1159.
5. Webster, J.M.; Darling, A.L.; Uversky, V.N.; Blair, L.J. Small heat shock proteins, big impact on protein aggregation in neurodegenerative disease. Front. Pharmacol. 2019, 10, 1047.
6. Landsbury, A.; Perng, M.D.; Pohl, E.; Quinlan, R.A. Functional symbiosis between the intermediate filament cytoskeleton and small heat shock proteins. In Small Stress Proteins and Human Diseases; Arrigo, A.P., Simon, S., Eds.; Nova Science: New York, NY, USA, 2010; pp. 55–87.
7. Arrigo, A.P. HSP27: Novel regulator of intracellular redox state. IUBMB Life 2001, 52, 303–307. [Google Scholar] [CrossRef]
8. Basha, E.; O'Neill, H.; Vierling, E. Small heat shock proteins and α-crystallins: Dynamic proteins with flexible functions. Trends Biochem. Sci. 2012, 37, 106–117.
9. Hilton, G.R.; Lioe, H.; Stengel, F.; Baldwin, A.J.; Benesch, J.L.P. Small heat-shock proteins: Paramedics of the cell. In Molecular Chaperones. Topics in Current Chemistry; Jackson, S., Ed.; Springer: Berlin/Heidelberg, Germany, 2013; Volume 328, pp. 69–98.
10. Carra, S.; Alberti, S.; Benesch, J.L.P.; Boelens, W.; Buchner, J.; Carver, J.A.; Cecconi, C.; Ecroyd, H.; Gusev, N.B.; Hightower, L.E.; et al. Small heat shock proteins: Multifaceted proteins with important implications for life. Cell Stress Chaperones 2019, 24, 295–308.

11. Ciocca, D.R.; Calderwood, S.K. Heat shock proteins in cancer: Diagnostic, prognostic, predictive, and treatment implications. Cell Stress Chaperones 2005, 10, 86–103.
12. Clark, A.R.; Lubsen, N.H.; Slingsby, C. sHSP in the eye lens: Crystallin mutations, cataract and proteostasis. Int. J. Biochem. Cell Biol. 2012, 44, 1687–1697.
13. Makley, L.N.; McMenimen, K.A.; DeVree, B.T.; Goldman, J.W.; McGlasson, B.N.; Rajagopal, P.; Dunyak, B.M.; McQuade, T.J.; Thompson, A.D.; Sunahara, R.; et al. Pharmacological chaperone for α-crystallin partially restores transparency in cataract models. Science 2015, 350, 674–677.
14. Gerasimovich, E.S.; Strelkov, S.V.; Gusev, N.B. Some properties of three αB-crystallin mutants carrying point substitutions in the C-terminal domain and associated with congenital diseases. Biochimie 2017, 142, 168–178.
15. Nefedova, V.V.; Muranova, L.K.; Sudnitsyna, M.V.; Ryzhavskaya, A.S.; Gusev, N.B. Small heat shock proteins and distal hereditary neuropathies. Biochemistry 2015, 80, 1734–1747.
16. Ryazantsev, S.N.; Poliansky, N.B.; Chebotareva, N.A.; Muranov, O.K. 3D structure of the native α-crystallin from bovine eye lens. Int. J. Biol. Macromol. 2018, 117, 1289–1298.
17. Delaye, M.; Tardieu, A. Short-range order of crystallin proteins accounts for eye lens transparency. Nature 1983, 302, 415–417.
18. Sudnitsyna, M.V.; Mymrikov, E.V.; Seit-Nebi, A.S.; Gusev, N.B. The role of intrinsically disordered regions in the structure and functioning of small heat shock proteins. Curr. Protein Pept. Sci. 2012, 13, 76–85.
19. Delbecq, C.P.; Klevit, R.E. One size does not fit all: The oligomeric states of αB crystallin. FEBS Lett. 2013, 587, 1073–1080.
20. Carver, J.A.; Grosas, A.B.; Ecroyd, H.; Quinlan, R.A. The functional roles of the unstructured N- and C-terminal regions in αB-crystallin and other mammalian small heat-shock proteins. Cell Stress Chaperones 2017, 22, 627–638. [Google Scholar] [CrossRef]
21. Peschek, J.; Braun, N.; Rohrberg, J.; Back, K.C.; Kriehuber, T.; Kastenmuller, A.; Weinkauf, S.; Buchner, J. Regulated structural transitions unleash the chaperone activity of αB-crystallin. Proc. Natl. Acad. Sci. USA 2013, 110, E3780–E3789.
22. Hochberg, G.K.A.; Shepherd, D.A.; Marklund, E.G.; Santhanagoplan, I.; Degiacomi, M.T.; Laganowsky, A.; Allison, T.M.; Basha, E.; Marty, M.T.; Galpin, M.R.; et al. Structural principles that enable oligomeric small heat shock protein paralogs to evolve distinct functions. Science 2018, 359, 930–935.
23. Hochberg, G.K.A.; Benesch, J.L.P. Dynamical structure of αB-crystallin. Prog. Biophys. Mol. Biol. 2014, 115, 11–20.
24. Braun, N.; Zacharias, M.; Peschek, J.; Kastenmuller, A.; Zou, J.; Hanzlik, M.; Haslbeck, M.; Rappsilber, J.; Buchner, J.; Weinkauf, S. Multiple molecular architectures of the eye lens chaperone αB-crystallin elucidated by a triple hybrid approach. Proc. Natl. Acad. Sci. USA 2011, 108, 20491–20496.
25. Chebotareva, N.A.; Eronina, T.B.; Roman, S.G.; Mikhaylova, V.V.; Sluchanko, N.N.; Gusev, N.B.; Kurganov, B.I. Oligomeric state of αB-crystallin under crowded conditions. Biochem. Biophys. Res. Commun. 2019, 508, 1101–1105.
26. Mchaourab, H.S.; Godar, J.A.; Stewart, P.L. Structure and mechanism of protein stability sensors: The chaperone activity of small heat-shock proteins. Biochemistry 2009, 48, 3828–3837.
27. Inoue, R.; Takata, T.; Fujii, N.; Ishii, K.; Uchiyama, S.; Sato, N.; Oba, Y.; Wood, K.; Kato, K.; Fujii, N.; et al. New insight into the dynamical system of αB-crystallin oligomers. Sci. Rep. 2016, 6, 29208.
28. Stengel, F.; Baldwin, A.J.; Painter, A.J.; Jaya, N.; Basha, E.; Kay, L.E.; Vierling, E.; Robinson, C.V.; Benesch, J.L.P. Quaternary dynamics and plasticity underlie small heat shock protein chaperone function. Proc. Natl. Acad. Sci. USA 2010, 107, 2007–2012.
29. Hayashi, J.; Carver, J.A. The multifaceted nature of αB-crystallin. Cell Stress Chaperones 2020, 25, 639–654.
30. Alderson, T.R.; Roche, J.; Gastall, H.Y.; Dias, D.M.; Pritišanac, I.; Ying, J.; Bax, A.; Benesch, J.L.P.; Baldwin, A.J. Local unfolding of the HSP27 monomer regulates chaperone activity. Nat. Commun. 2019, 10, 1068. [Google Scholar] [CrossRef]
31. Liu, L.; Ghosh, J.G.; Clark, J.I.; Jiang, S. Studies of αB-crystallin subunit dynamics by surface plasmon resonance. Anal. Biochem. 2006, 350, 186–195.
32. Chebotareva, N.A.; Eronina, T.B.; Sluchanko, N.N.; Kurganov, B.I. Effect of Ca2+ and Mg2+ ions on oligomeric state and chaperone-like activity of αB-crystallin in crowded media. Int. J. Biol. Macromol. 2015, 76, 86–93.
33. Ecroyd, H.; Meehan, S.; Horwitz, J.; Aquilina, J.A.; Benesch, J.L.; Robinson, C.V.; Macphee, C.E.; Carver, J.A. Mimicking phosphorylation of αB-crystallin affects its chaperone activity. Biochem. J. 2007, 401, 129–141.

34. Jovcevski, B.; Kelly, M.A.; Rote, A.P.; Berg, T.; Gastall, H.Y.; Benesch, J.L.P.; Aquilina, J.A.; Ecroyd, H. Phosphomimics destabilize Hsp27 oligomeric assemblies and enhance chaperone activity. Chem. Biol. 2015, 22, 186–195.

35. Muranova, L.K.; Sudnitsyna, M.V.; Gusev, N.B. αB-Crystallin phosphorylation: Advances and problems. Biochemistry 2018, 83, 1196–1206.

36. Roman, S.G.; Chebotareva, N.A.; Kurganov, B.I. Anti-aggregation activity of small heat shock proteins under crowded conditions. Int. J. Biol. Macromol. 2017, 100, 97–103.

37. Fonin, A.V.; Darling, A.L.; Kuznetsova, I.M.; Turoverov, K.K.; Uversky, V.N. Intrinsically disordered proteins in crowded milieu: When chaos prevails within the cellular gumbo. Cell. Mol. Life Sci. 2018, 75, 3907–3929.

38. Kuznetsova, I.M.; Turoverov, K.K.; Uversky, V.N. What macromolecular crowding can do to a protein. Int. J. Mol. Sci. 2014, 15, 23090–23140.

39. Fu, X. Insights into how small heat shock proteins bind a great diversity of substrate proteins: A super-transformer model. In The Big Book on Small Heat Shock Proteins; Tanguay, R.M., Hightower, L.E., Eds.; Springer: Cham, Switzerland, 2015; pp. 101–117.

40. Zimmerman, S.B.; Trach, S.O. Estimation of macromolecule concentrations and excluded volume effects for the cytoplasm of Escherichia coli. J. Mol. Biol. 1991, 222, 599–620.

41. Zimmerman, S.B.; Minton, A.P. Macromolecular crowding: Biochemical, biophysical, and physiological consequences. Annu. Rev. Biophys. Biomol. Struct. 1993, 22, 23–65.

42. Ellis, R.J. Macromolecular crowding: Obvious but underappreciated. Trends Biochem. Sci. 2001, 26, 597–604.

43. Minton, A.P. The influence of macromolecular crowding and macromolecular confinement on biochemical reactions in physiological media. J. Biol. Chem. 2001, 276, 10577–10580.

44. Minton, A.P.; Wilf, J. Effect of macromolecular crowding upon the structure and function of an enzyme: Glyceraldehyde-3-phosphate dehydrogenase. Biochemistry 1981, 20, 4821–4826.

45. Chebotareva, N.A.; Kurganov, B.I.; Livanova, N.B. Biochemical effects of molecular crowding. Biochemistry 2004, 69, 1239–1251.

46. Ellis, R.J.; Minton, A.P. Protein aggregation in crowded environments. Biol. Chem. 2006, 387, 485–497.

47. Zhou, H.X.; Rivas, G.; Minton, A.P. Macromolecular crowding and confinement: Biochemical, biophysical, and potential physiological consequences. Annu. Rev. Biophys. 2008, 37, 375–397.

48. Ellis, R.J. Protein aggregation: Opposing effects of chaperones and crowding. In Folding for the Synapse; Wyttenbach, A., O'Connor, V., Eds.; Springer: Boston, MA, USA, 2011; pp. 9–34.

49. Dewavrin, I.-Y.; Abdurrahiem, M.; Blocki, A.; Musib, M.; Piazza, F.; Raghunath, M. Synergistic rate of collagen fibrillogenesis in heterogeneous mixture of crowding agents. J. Phys. Chem. B 2015, 119, 4350–4358.

50. Shah, D.; Tan, A.L.; Ramakrishnan, V.; Jiang, J.; Rajagopalan, R. Effects of polydisperse crowders on aggregation reactions: A molecular thermodynamic analysis. J. Chem. Phys. 2011, 134, 064704.

51. Du, F.; Zhou, Z.; Mo, Z.-Y.; Shi, J.-Z.; Chen, J.; Liang, Y. Mixed macromolecular crowding accelerates the refolding of rabbit muscle creatine kinase: Implications for protein folding in physiological environments. J. Mol. Biol. 2006, 364, 469–482.

52. Batra, J.; Xu, K.; Zhou, H.-X. Nonadditive effects of mixed crowding on protein stability. Proteins 2009, 77, 133–138.

53. Phillip, Y.; Schreiber, G. Formation of protein complexes in crowded environments—From in vitro to in vivo. FEBS Lett. 2013, 587, 1046–1052.

54. Nakano, S.; Miyoshi, D.; Sugimoto, N. Effects of molecular crowding on the structures, interactions, and functions of nucleic acids. Chem. Rev. 2014, 114, 2733–2758.

55. Sarkar, M.; Smith, A.E.; Pielak, G.J. Impact of reconstituted cytosol on protein stability. Proc. Natl. Acad. Sci. USA 2013, 110, 19342–19347.

56. Mittal, S.; Chowhan, R.K.; Singh, L.R. Macromolecular crowding: Macromolecules friend or foe. Biochim. Biophys. Acta 2015, 1850, 1822–1831.

57. Stepanenko, O.V.; Povarova, O.I.; Sulatskaya, A.I.; Ferreira, L.A.; Zaslavsky, B.Y.; Kuznetsova, I.M.; Turoverov, K.K.; Uversky, V.N. Protein unfolding in crowded milieu: What crowding can do to a protein undergoing unfolding? J. Biomol. Struct. Dyn. 2016, 34, 2155–2170.

58. Shahid, S.; Hassan, M.I.; Islam, A.; Ahmad, F. Size-dependent studies of macromolecular crowding on the thermodynamic stability, structure and functional activity of proteins: In vitro and in silico approaches. Biochim. Biophys. Acta, Gen. Subj. 2017, 1861, 178–197.

59. Shahid, S.; Ahmad, F.; Hassan, M.I.; Islam, A. Relationship between protein stability and functional activity in the prese nce of macromolecular crowding agents alone and in mixture: An insight into stability-activity trade-off. Arch. Biochem. Biophys. 2015, 584, 42–50.

60. Sharp, K.A. Analysis of the size dependence of macromolecular crowding shows that smaller is better. Proc. Natl. Aca d. Sci. USA 2015, 112, 7990–7995.

61. Chebotareva, N.A.; Makeeva, V.F.; Bazhina, S.G.; Eronina, T.B.; Gusev, N.B.; Kurganov, B.I. Interaction of Hsp27 with native phosphorylase kinase under crowding conditions. Macromol. Biosci. 2010, 10, 783–789. [Google Scholar] [Cros sRef]

62. Roman, S.G.; Chebotareva, N.A.; Eronina, T.B.; Kleymenov, S.Y.; Makeeva, V.F.; Poliansky, N.B.; Muranov, K.O.; Kurg anov, B.I. Does the crowded cell-like environment reduce the chaperone-like activity of α-crystallin? Biochemistry 2011, 50, 10607–10623.

63. Chebotareva, N.A.; Eronina, T.B.; Roman, S.G.; Poliansky, N.B.; Muranov, K.O.; Kurganov, B.I. Effect of crowding and chaperones on self-association, aggregation and reconstitution of apophosphorylase b. Int. J. Biol. Macromol. 2013, 6 0, 69–76.

64. Sluchanko, N.N.; Chebotareva, N.A.; Gusev, N.B. Quaternary structure of human small heat shock protein HSPB6 (Hs p20) in crowded media modeled by trimethylamine N-oxide (TMAO): Effect of protein phosphorylation. Biochimie 2015, 108, 68–75.

65. Eronina, T.B.; Mikhaylova, V.V.; Chebotareva, N.A.; Kurganov, B.I. Kinetic regime of thermal aggregation of holo- and a poglycogen phosphorylases b. Int. J. Biol. Macromol. 2016, 92, 1252–1257.

66. Bumagina, Z.M.; Gurvits, B.Y.; Artemova, N.V.; Muranov, K.O.; Yudin, I.K.; Kurganov, B.I. Mechanism of suppression of dithiothreitol-induced aggregation of bovine α-lactalbumin by α-crystallin. Biophys. Chem. 2010, 146, 108–117.

67. Borzova, V.A.; Markossian, K.A.; Kurganov, B.I. Relationship between the initial rate of protein aggregation and the lag period for amorphous aggregation. Int. J. Biol. Macromol. 2014, 68, 144–150.

68. Chebotareva, N.A.; Filippov, D.O.; Kurganov, B.I. Effect of crowding on several stages of protein aggregation in test sys tems in the presence of α-crystallin. Int. J. Biol. Macromol. 2015, 80, 358–365.

69. Barford, D.; Johnson, L.N. The allosteric transition of glycogen phosphorylase. Nature 1989, 340, 609–616.

70. Permyakov, E.A.; Berliner, L.J. α-Lactalbumin: Structure and function. FEBS Lett. 2000, 473, 269–274.

71. Kuwajima, K. The molten globule state of α-lactalbumin. FASEB J. 1996, 10, 102–109.

72. Kulig, M.; Ecroyd, H. The small heat-shock protein αB-crystallin uses different mechanisms of chaperone action to prev ent the amorphous versus fibrillar aggregation of α-lactalbumin. Biochem. J. 2012, 448, 343–352.

73. Carver, J.A.; Lindner, R.A.; Lyon, C.; Canet, D.; Hernandez, H.; Dobson, C.M.; Redfield, C. The interaction of the molec ular chaperone α-crystallin with unfolding α-lactalbumin: A structural and kinetic spectroscopic study. J. Mol. Biol. 2002, 318, 815–827.

74. Lindner, R.A.; Treweek, T.M.; Carver, J.A. The molecular chaperone α-crystallin is in kinetic competition with aggregatio n to stabilize a monomeric molten-globule form of α-lactalbumin. Biochem. J. 2001, 354, 79–87.

75. Ecroyd, H.; Carver, J.A. The effect of small molecules in modulating the chaperone activity of αB-crystallin against orde red and disordered protein aggregation. FEBS J. 2008, 275, 935–947.

76. Sanders, H.M.; Jovcevski, B.; Carver, J.A.; Pukala, T.L. The molecular chaperone β-casein prevents amorphous and fib rillar aggregation of α-lactalbumin by stabilization of dynamic disorder. Biochem. J. 2020, 477, 629–643.

77. Benesch, J.L.P.; Ayoub, M.; Robinson, C.V.; Aquilina, J.A. Small heat shock protein activity is regulated by variable olig omeric substructure. J. Biol. Chem. 2008, 283, 28513–28517.

78. Haslbeck, M.; Peschek, J.; Buchner, J.; Weinkauf, S. Structure and function of α-crystallins: Traversing from in vitro to i n vivo. Biochim. Biophys. Acta 2016, 1860, 159–166.

79. Bova, M.; Ding, L.-L.; Horwitz, J.; Fung, B.K.-K. Subunit exchange of αA-crystallin. J. Biol. Chem. 1997, 272, 29511–29 517.

80. Abgar, S.; Backmann, J.; Aerts, T.; Vanhoudt, J.; Clauwaert, J. The structural differences between bovine lens αA- and αB-crystallin. Eur. J. Biochem. 2000, 267, 5916–5925.

81. Vanhoudt, J.; Abgar, S.; Aerts, T.; Clauwaert, J. Native quaternary structure of bovine α-crystallin. Biochemistry 2000, 3 9, 4483–4492.

82. Bakthisaran, R.; Akula, K.K.; Tangirala, R.; Rao, C.M. Phosphorylation of αB-crystallin: Role in stress, aging and pathop hysiological conditions. Biochim. Biophys. Acta Gen. Subj. 2016, 1860, 167–182.

83. Aquilina, J.A.; Benesch, J.L.P.; Bateman, O.A.; Slingsby, C.; Robinson, C.V. Polydispersity of a mammalian chaperone: Mass spectrometry reveals the population of oligomers in αB-crystallin. Proc. Natl. Acad. Sci. USA 2003, 100, 10611–10616.