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Research Article

Chaperone-like features of bovine serum albumin: a comparison with α-crystallin

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Abstract. The chaperone behaviour of bovine serum albumin was compared with that of α-crystallin. The chaperone activity was assessed by measuring: (i) the ability to antagonize protein aggregation induced by heat; (ii) the capability to protect the activity of thermally stressed enzymes and (iii) the effectiveness in assisting the functional recovery of chemically denatured sorbitol dehydrogenase. Despite the lack of structural analogies, both proteins show several functional similarities in preventing inactivation of thermally stressed enzymes and in reactivating chemically denatured sorbitol dehydrogenase. As with α-crystallin, the chaperone action of bovine serum albumin appears to be ATP independent. Bovine serum albumin appears significantly less effective than α-crystallin only in preventing thermally induced protein aggregation. A possible relationship between chaperone function and structural organization is proposed. Together, our results indicate that bovine serum albumin acts as a molecular chaperone and that, for its particular distribution, can be included in the extracellular chaperone family.

Key words. Bovine serum albumin; α-crystallin; molecular chaperones; extracellular chaperones; sorbitol dehydrogenase; enzyme renaturation; protein refolding.

Bovine serum albumin (BSA) is one of the most studied and well-understood proteins and consists of a single polypeptide chain, containing 580 amino acids with 17 intrachain disulphide bonds [1]. It is involved in the maintenance of pH and in osmotic regulation of blood [2, 3]. Sixty percent of BSA is in the extravascular space of tissues, the remainder being in the circulation. Serum albumin is a thermostable protein; when heat-treated it goes through two structural stages: the first one, up to 65 °C, is reversible, whilst the second, above 65 °C, is irreversible, and appears to be associated with the generation of a secondary structure enriched in beta sheets [4].

An outstanding property of albumin is its strong ability to reversibly bind and transport an enormous variety of substances, generally not well soluble in water, such as fatty acids, hematin, bilirubin and small negatively charged aromatic compounds [3, 5].

Because of its broad ability to interact with various molecular species, albumin regulates the plasma concentration of many xenobiotic and physiological substances [3, 6]. It performs many other functions, such as sequestering oxygen-free radicals [7] and inactivating various toxic lipophilic metabolites such as bilirubin [8] or forming adducts with pyridoxal phosphate, cysteine, glutathione and various metals, such as Cu(II), Ni(II), Hg(II), Ag(II), Au(I) and Au(III) [9, 10]. Moreover, albumin is the key carrier or reservoir of nitric oxide [11].

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BSA is commonly used in many biochemical applications: as a nutrient in mammalian and bacterial cell cultures, as a transport protein for nutrients or as a protein standard in diagnostic and immunochemistry applications [12–15].

Because of its thermostability, BSA has been widely used as a control protein when the protective action of heat shock proteins (Hsps) was investigated [16–18]. Recently, its ineffectiveness in suppressing the thermally induced unfolding and aggregation of citrate synthase and in favouring the refolding of chemically denatured xylanase has been reported [19–21]. In contrast, chaperone-like properties of BSA have been demonstrated by using chemically denatured rhodanese and thermally inactivated β-galactosidase [22, 23].

In this paper, making use of bovine lens sorbitol dehydrogenase (SDH) and other enzymes, a comparison between the chaperoning ability of BSA and α-crystallin was performed by evaluating their features as preservatives of functional structures, as protein antiaggregation agents and as protein refolding assistants.

Materials and methods

Materials. Calf eyes were obtained from a local abattoir soon after slaughtering; the lenses were removed and frozen at –20 °C until used.

Alcohol dehydrogenase (E.C. 1.1.1.1) from yeast, 30.85 U/mg, glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49) from yeast, 528 U/mg, 6-phosphogluconate dehydrogenase (E.C. 1.1.1.44) from yeast, 0.2 U/mg, malic dehydrogenase (E.C. 1.1.1.37) from porcine heart, 248 U/mg, lactic dehydrogenase (E.C. 1.1.1.27) from rabbit muscle, 796 U/mg, isocitric dehydrogenase (E.C. 1.1.1.42) from porcine heart, 44 U/mg, glucose oxidase (E.C. 1.1.3.4), 1 U/mg, molecular weight markers for SDS-PAGE, dithiothreitol (DTT), NAD^+, β-D(-)fructose, glucose-6-phosphate, $NADP^+$, NADPH, oxalacetate, phosphoenolpyruvate, pyruvate, ATP, ADP, AMP, IMP, adenosine (Ado), guanosine (Guo), nicotinamide mononucleotide (NMN), 9,10-phenanthrenequinone, dimethyl sulphoxide (DMSO) and lysozyme were from Sigma-Aldrich. NADH was supplied by Boehringer, EDTA by Serva Feinbiochemica, guanidine hydrochloride (GdnCl) by Fluka and YM30 ultrafiltration membrane by Amicon. The electrophoretic equipment was from Bio-Rad. Sepharose 6B was from Amersham Biosciences. All other chemicals were of reagent grade.

SDH purification and assay. SDH (E.C. 1.1.1.14) was purified to electrophoretic homogeneity as previously described [24]. The final enzyme preparation (approximately 2 mg/ml), with a specific activity of 21 U/mg, was stored at 4 °C in 10 mM sodium phosphate pH 7 (S-buffer), supplemented with 2 mM DTT and 0.1 mM NADH. The purified enzyme was dialysed against S-buffer just before use.

The assay of enzyme activity was performed at 37 °C as previously described [24] by following the decrease in absorbance at 340 nm in a reaction mixture (0.5 ml final volume) containing 0.24 mM NADH and 0.4 M D-fructose in 100 mM Tris/HCl buffer pH 7.4. The rate of NADH oxidation measured in a parallel assay in which the substrate was omitted was subtracted as a blank. One unit of enzyme activity is the amount of SDH that catalyses the oxidation of 1 μmol/min of NADH.

α-Crystallin isolation. All procedures were carried out at 4 °C, unless otherwise stated. Frozen bovine lenses were suspended (1.5 g/10 ml) in S-buffer and homogenized in a Potter-Elvehjem homogenizer. The suspension was then centrifuged at 25,000 *g* for 30 min. An aliquot of the resulting supernatant was applied to a Sepharose 6B column (1.5 x 65 cm), and the elution was performed with S-buffer at a flow rate of 3 ml/h. The eluted fractions (1 ml) containing low molecular-weight α-crystallin were pooled, analysed by SDS-PAGE, concentrated on Amicon YM30 membrane to a final protein concentration of approximately 10 mg/ml and then stored at –20 °C until use.

BSA purification. BSA was purchased from Sigma, dissolved in S-buffer and applied (19 mg) on a Sephacryl S-200 (1.5 x 65 cm) column; the elution was performed with S-buffer at a flow rate of 25 ml/h and fractions of 1.8 ml were collected. The elution profile obtained monitoring the absorbance at 280 nm revealed two peaks. The fractions corresponding to the lower-molecular-weight peak were pooled, analyzed by SDS-PAGE, concentrated on Amicon YM30 membrane to a final protein concentration of approximately 10 mg/ml and then stored at –20 °C until use.

βB2-crystallin isolation. For βB2-crystallin isolation, heat precipitation was used as previously described [25]. Bovine lenses were suspended (1.5 g/10 ml) in S-buffer, homogenized in a Potter-Elvehjem homogenizer and the suspension was centrifuged for 30 min at 25,000 *g*. The surpernatant was heated at 100 °C for 5 min; the mixture was then centrifuged as above. The supernatant was again subjected twice to the above thermal treatment cycle. The final supernatant was then dialysed on YM10 ultrafiltration membrane against S-buffer, concentrated to 5 mg/ml of protein and stored at –20 °C. Purity of βB2-crystallin was assessed by SDS-PAGE.

Enzymatic assays. Aldose reductase (ALR2) (E.C. 1.1.1.21) activity was measured at 37 °C as previously described [26] by using D,L-glyceraldehyde as substrate.

Alcohol dehydrogenase activity was measured at 37 °C by following the increase in absorbance at 340 nm in a reaction mixture containing 0.22 mM NAD^+ and 50 mM ethanol in 100 mM Tris/HCl buffer pH 7.4.

Glucose-6-phosphate dehydrogenase activity was measured at 37 °C by following the increase in absorbance at

340 nm in a reaction mixture containing 0.19 mM $NADP^+$ and 3 mM D-glucose-6-phosphate in 100 mM Tris/HCl buffer pH 7.4.

ζ-Crystallin activity was measured at 37 °C by following the increase in absorbance at 340 nm in a reaction mixture containing 0.19 mM $NADP^+$ and 0.05 mM 9,10-phenanthrenequinone (previously dissolved in DMSO) in 50 mM Tris/HCl buffer pH 7.7.

6-Phosphogluconate dehydrogenase activity was measured at 37 °C by following the increase in absorbance at 340 nm in a reaction mixture containing 0.2 mM $NADP^+$ and 4 mM 6-phospho-D-gluconate in 50 mM Tris/HCl buffer pH 7.4.

Malic dehydrogenase activity was measured at 37 °C by following the decrease in absorbance at 340 nm in a reaction mixture containing 0.26 mM NADH and 0.8 mM oxalacetate in 100 mM Tris/HCl buffer pH 7.4.

Protein aggregation assay. The thermally induced aggregation of target proteins (0.1 mg/ml at 55 °C in 50 mM Tris/HCl buffer pH 7.4) both in the absence and in the presence of the protector protein was followed by monitoring the absorbance at 360 nm in a Beckman DU-6 spectrophotometer.

Preparation of chemically denatured SDH and recovery of enzyme activity. SDH (final concentration 1 mg/ml) was denatured by incubation for 2 h at 25 °C in Tris/HCl 100 mM pH 7.4 in the presence of 6 M GdnCl, 2 mM DTT and 100 µM EDTA.

The recovery of active SDH after the GdnCl treatment was performed by a 100-fold dilution of the denatured enzyme preparation in Tris/HCl 100 mM pH 7.4 and incubation at 25 °C, for appropriate times in the presence of BSA and/or different compounds, as specified. At different times, aliquots were withdrawn and SDH activity measured. The extent of recovery of enzyme activity from denatured SDH is given as the percent of the activity of a control sample of native SDH treated in the same conditions.

Other methods. Protein concentration was determined by the Coomassie Blue binding assay [27] using BSA as a standard. Aldose reductase was purified from bovine lens as previously described [28]. Leucyl amino peptidase was purified from bovine lens as previously described [29]. ζ-crystallin was purified from camel lens essentially according to Garland et al. [30]. SDS-PAGE analyses were performed according to Laemmli [31], using 12% acrylamide slab gels, 0.75 mM thick. The following standards were used for apparent-molecular-weight calibration: bovine serum albumin (M_r 66,000), ovalbumin (M_r 45,000), glyceraldehyde-3-phosphate dehydrogenase (M_r 36,000), human erythrocyte carbonic anhydrase (M_r 29,000), trypsinogen (M_r 24,000) and trypsin inhibitor (M_r 20,000). Gels were stained by the silver stain technique [32].

Results

Prevention of thermally induced inactivation of enzymes by BSA. Similarly to what has been previously reported for α-crystallin [33], thermal inactivation of SDH is prevented by BSA. This is shown in figure 1, where the effectiveness of BSA and α-crystallin in protecting the functional integrity of the enzyme are compared. The stabilization of the enzyme kept at 55 °C for 40 min at different protector/target enzyme ratios ranging from zero to 1, while confirming the well-assessed feature of α-crystallin as a molecular chaperone, also reveals a remarkable stabilization ability for BSA. In fact, even though a ratio BSA/SDH of at least 0.4 (to be compared with the ratio α-crystallin/SDH of 0.1) is required to retain 50% of the enzyme activity, at protector/target ratios higher than 0.7, BSA and α-crystallin behave similarly, almost completely protecting the enzyme. An indication of the specificity of the protective action of BSA and α-crystallin on SDH inactivation comes from the results shown in the inset of figure 1. Here, the residual activity of SDH incubated at 55 °C either alone or with different thermostable proteins (i.e. BSA, α-crystallin, lysozyme and βB2-crystallin), present at a protector/target ratio of 10:1, is reported as a function of time. While βB2-crystallin was only able to protect the enzyme partially (in this case 50% of the ac-

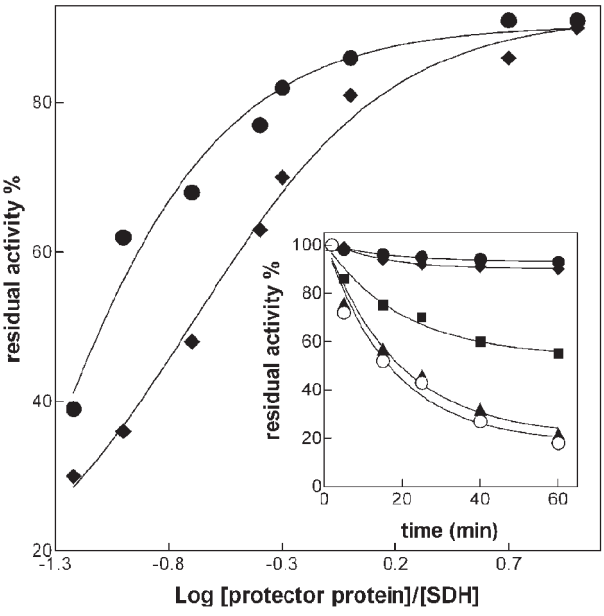

Figure 1. Effect of BSA and α-crystallin on thermal inactivation of SDH. SDH (10 µg/ml) was incubated at 55 °C in 50 mM Tris/HCl pH 7.4 in the presence of either BSA (◆) or α-crystallin (●) at the indicated concentration ratios [protector protein]/[SDH], (w:w). The enzyme activity measured after 40 min of incubation was reported as % residual activity with respect to the activity at zero time. The inset shows the time course of inactivation of bovine lens SDH incubated as above at a ratio protector protein/SDH of 10:1 by weight, either alone (○) or in the presence of α-crystallin (●), BSA (◆), lysozyme (▲) or βB2 crystallin (■).

tivity was lost), lysozyme was completely unable to exert any protective action.

When other enzymes besides SDH were used as target proteins to evaluate the effectiveness of BSA to preserve functional structures of thermally stressed proteins, a comparable, but not identical, pattern of protection for BSA and α-crystallin was observed (see table 1).

Prevention of thermally induced aggregation of enzymes by BSA. When SDH is heated at 55 °C, the solution gets turbid because of the formation of large aggregates; in this condition, BSA present at a ratio of 1:1 by weight with respect to SDH was able to exert an 80% protection against precipitation after 3 h of incubation (fig. 2). An increase in the ratio protector:target to 2:1 or 3:1 gave no further protection. Ratios of BSA:SDH lower than 1 revealed a failure of BSA to prevent protein aggregation. In fact, figure 2 shows that at a BSA:SDH ratio of 0.5, only a delay before aggregation of thermally stressed SDH is observed without any effect on the precipitation phenomenon. Lysozyme, chosen as a control protein, was completely unable to prevent SDH aggregation; actually, lysozyme, present with SDH, co-precipitates with the target enzyme. In contrast, α-crystallin, even at a protector:target ratio of 0.5 was able to completely suppress aggregation of thermally stressed SDH. Neither BSA, lysozyme, nor α-crystallin incubated alone at 55 °C, displayed aggregation phenomena (fig. 2).

The ability of BSA, compared with α-crystallin, to impair thermally induced aggregation of different target proteins, is reported in table 2. The results indicate that, even though far less effective than α-crystallin, BSA displays, in the adopted conditions, a well-detectable antiaggregation effect.

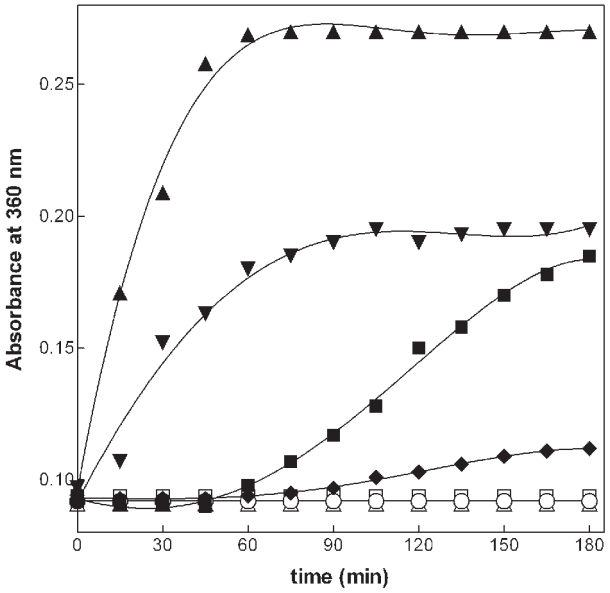

Figure 2. Effect of BSA and α-crystallin on thermal aggregation of SDH. SDH 0.16 mg/ml was heated at 55 °C either alone (▼) or in the presence of BSA (1:0.5 w:w) (■), BSA (1:1 w:w) (◆), lysozyme (1:1 w:w) (▲), α-crystallin (1:0.5 w:w) (△). Symbols (○), (□) and (◇), refer to α-crystallin, lysozyme and BSA, respectively, incubated alone at 0.16 mg/ml. All the open symbols are superimposed.

Assistance of reactivation of chemically denatured SDH by BSA. When SDH inactivated by GdnCl is incubated at 25 °C (see Materials and methods), a spontaneous refold-

Table 1. Effect of BSA and α-crystallin on thermal inactivation of enzymes.

Target enzyme	Temperature (°C)	Percent residual activity BSA	α-crystallin
Alcohol dehydrogenase	45	84	98
Malic dehydrogenase	45	40	50
Glucose-6-phospate dehydrogenase	40	38	20
Aldose reductase	45	50	65
ζ-Crystallin	55	65	50
Sorbitol dehydrogenase	55	93	95
6-Phosphogluconate dehydrogenase	40	35	35

The enzymes were incubated in 50 mM Tris/HCl buffer pH 7.4 at the indicated temperatures with the following concentrations and ratios of enzyme/protector protein by weight (in parentheses): alcohol dehydrogenase 10 μg/ml (1:10); malic dehydrogenase 1 μg/ml (1:100); glucose-6-phosphate dehydrogenase 1 μg/ml (1:50); aldose reductase 25 μg/ml (1:20); ζ-crystallin 18.4 μg/ml (1:10); sorbitol dehydrogenase 10 μg/ml (1:5); 6-phosphogluconate dehydrogenase 0.12 mg/ml (1:10). After 60 min of incubation, enzymatic assays were performed as described in Materials and methods. Percent residual activity refers to the ratio between the activity measured at the end of incubation over the activity measured at zero time x 100.

Table 2. Effect of BSA and α-crystallin on thermally induced aggregation of proteins.

Target protein	Percent protection BSA	α-crystallin
Sorbitol dehydrogenase	80	100
Glucose oxidase	100	100
Malic dehydrogenase	17	100
Aldose reductase	40	100
Alcohol dehydrogenase	42	100
Alcohol dehydrogenase	67[1]	100
ζ-Crystallin	43[1]	100
Glucose-6-phospate dehydrogenase	0	100
Leucyl amino peptidase	50	100

The protein aggregation assay was performed at 55 °C as described in Materials and methods at a protector protein/target ratio of 1:1 by weight. The % protection exerted by BSA (or α-crystallin) was calculated as follows: $[(Abs_{360}\text{-BSA}) - (Abs_{360}\text{+BSA})/(Abs_{360}\text{-BSA})] \times 100$, measured at 60 min of incubation.

[1] BSA/target ratio of 3:1 by weight.

ing is observed giving rise to a recovery of 10–15% of the enzyme activity. When BSA is present during incubation at a BSA:SDH ratio of 20:1 (w:w), the recovery of active SDH was increased up to 35%. No effect on the recovery of the enzyme activity was observed when lysozyme substituted BSA in the incubation mixture (fig. 3). NADH present alone in the reactivation mixture elicited the recovery of SDH activity up to 33%. When NADH and BSA were added together to the denatured SDH, the recovery rose to 76%. Identical results were obtained replacing NADH with NAD^+ (data not shown). When ATP or ADP were present (up to 3.5 mM) in the reactivating mixture instead of NADH, a measurable, but modest, effect of BSA in eliciting the recovery of functional SDH was observed. When chemically denatured SDH was refolded in the presence of BSA and other nucleosides and nucleotides such as Ado, Guo, AMP, IMP or NMN, the recovery of the enzyme activity with time was essentially the same as observed for ATP (data not shown). Such lines of evidence are essentially identical to previously observations when α-crystallin was tested as a molecular chaperone [34].

Discussion

Molecular chaperones prevent irreversible aggregation of temporarily unfolded proteins and assist their folding and assembly by influencing the partitioning between productive and unproductive folding steps without being part of the final structure of the folded protein [35, 36]. Such a functional definition of chaperones groups molecular chaperones irrespectively of the features related to the energy requirement (i. e. ATP hydrolysis) for their action. Even though its mechanism of action is not fully characterized, α-crystallin appears unequivocally to act as an ATP-independent chaperone [34, 37–39]. Thus, by using α-crystallin activity as a reference paradigm for chaperone features, BSA was studied by testing its antiaggregation action towards thermally stressed proteins, its ability to stabilize protein structure and its effectiveness in assisting of denatured-protein refolding.

Making use of different enzymes including SDH, a protein target previously used to test α-crystallin chaperone action, BSA could be closely compared to α-crystallin as a chaperone molecule. BSA seems to share with α-crystallin all the features allowing it to be defined as a molecular chaperone. In particular, BSA and α-crystallin have a comparable effect in protecting enzymes from thermally induced inactivation (table 1). The direct comparison between BSA and α-crystallin in terms of their ability to prevent thermally induced inactivation of SDH at different protector/target ratios (fig. 1) indicates an efficient action of BSA, even though an apparently reduced affinity for the target with respect to α-crystallin is observed. Worth noting here is the failure of other thermostable proteins such as lysozyme and βB2-crystallin to stabilize SDH even at a relatively high protector/target ratio (fig. 1, inset). Concerning the ability to assist protein refolding, BSA, like α-crystallin, is remarkably effective in assisting the recovery of functional SDH from the GdnCl denatured enzyme (fig. 3). The similarity in behaviour between BSA and α-crystallin is emphasized by looking at the remarkable effect exerted by NADH in eliciting the action on the SDH refolding and the rather poor effectiveness of ATP (fig. 2). Even though a specific 1:1 binding of ATP to the BSA monomer was previously demonstrated [40, 41], ATP is unlikely to contribute as an energy donor since ADP, NMN and AMP are as or even slightly more effective than ATP in favouring the recovery of SDH activity. Moreover, no differences were observed upon addition of $MgCl_2$ and KCl, whose presence deeply influences the effectiveness of ATP-dependent molecular chaperones [42, 43] (data not shown). As postulated for the effect of ATP on the α-crystallin-assisted SDH refolding [34], the possibility that ATP or ADP may interact with the target protein, mimicking the enzyme co-factor NADH, cannot be ruled out.

Calcium ion, which like Mg^{2+} [44] was previously shown to impair the protective action of α-crystallin towards thermally induced aggregation of ALR2 [45], as well as inactivation and aggregation of SDH [33], also exerts its effect on the protective action of BSA on thermally

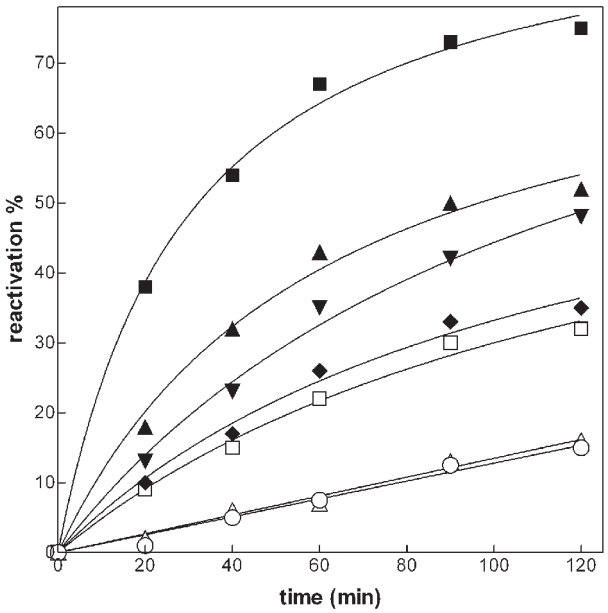

Figure 3. Effect of BSA on the reactivation of chemically denatured SDH. Chemically denatured SDH (10 μg/ml) was reactivated in the presence of 1:10 (w:w) lysozyme (△) or 0.1 mM NADH (□). Closed symbols refer to incubations of SDH in the presence of 1:10 (w:w) BSA either alone (◆) or supplemented with the following: 3.5 mM ATP (▼), 3.5 mM ADP (▲), 0.1 mM NADH (■), SDH (○) . alone. See Materials and methods for details.

stressed SDH. As previously observed for α-crystallin [33, 45], Ca^{2+}, in the concentration range of 0.1–0.5 mM, at 55 °C and at a protector:SDH ratio of 1:1, is able to abolish the protective action of BSA towards the thermally stressed enzyme (data not shown).

The only chaperone feature for which the effectiveness of BSA is rather poor with respect to α-crystallin is the antiaggregation ability towards thermally stressed proteins. Both figure 2 and table 2 clearly show that, despite the intrinsic ability of BSA to interact with proteins undergoing thermal denaturation, its ability to effectively prevent protein precipitation is remarkably reduced with respect to α-crystallin.

While defining the chaperone features of BSA and demonstrating their comparability with those of α-crystallin, the presented results may give insights into the restraints on the functional action of these ATP-independent molecular chaperones. The similarity in the effectiveness and features of BSA and α-crystallin (i. e. enzyme stabilization, SDH renaturation, Ca^{2+} effect), is rather puzzling especially when the multimeric assembly of α-crystallin is invoked as a requirement for its chaperone function [46]. Nevertheless, the remarkable structural differences in terms of quaternary assembly between the two compared chaperones may be key in explaining the observed differences with respect to different stress conditions, in particular, the clearly reduced antagonistic effect of BSA towards the thermally induced protein aggregation. In fact, the refolding and stabilization of an enzyme deal essentially with intramolecular rearrangements and are usually performed and monitored at a rather low level of the target protein. In such a case, the enzyme stabilization or refolding assistance may not require special structural restrictions in the protector or assistant protein, besides its ability to interact with the target. This would simply favour structural modification paths of the target protein with respect to non-productive or irreversible steps either in the denaturation or in the refolding process. On the other hand, the antiaggregation action of chaperone-like proteins is normally tested at rather high absolute concentrations of the target and mainly refers to interchain interactions. In such a case, a structurally organized molecular compartimentalization, as is the case of α-crystallin, would more efficiently, even irreversibly, subtract the target undergoing structural modification from the protein-protein self interaction steps. Such a protective action would be elicited by the increase in surface hydrophobicity observed in native α-crystallin, in the oligomer of recombinant αA and even in a αA-crystallin-derived peptide [47–50]. The dynamic nature of the multimeric assembly of α-crystallin is a well-documented fact [51], and thus it can afford different kinds of interactive structures. Worth noting here is the previous observation that the protective action of α-crystallin towards aggregation and inactivation of thermally stressed SDH was shown to occur through the formation of two kinds of interacting adducts: the first, stable and containing inactive SDH, possibly related to the antiaggregation action of α-crystallin; the second, transient and containing active SDH, appears to be related to the preservation of SDH activity. The first complex has a high molecular weight, suggesting interaction between multimeric 800 kDa α-crystallin with the target protein; the second has a weight comparable to that of SDH, suggesting interaction between enzyme and oligomeric α-crystallin complexes [33]. BSA, which is uncapable of extensive multimeric assembly and has a surface hydrophobicity reduced rather than expanded by a temperature increase [23], may only be able to afford interaction sites for the second type of complex.

While the main families of molecular chaperones and some abundant chaperone-like proteins such as tubulin [52] are intracellular, evidence is emerging for the presence of protein molecules classifiable as extracellular chaperones. This is the case for (i) some proteoglycans involved in the release, transfer and stabilization of lipoprotein lipase from the adipocyte to the luminal endothelial surface [53]; (ii) PrsA, a lipoprotein essential for the viability of B*acillus subtilis*, responsible for the translocation and subsequent folding of secretory proteins [54] and (iii) clusterin, a ubiquitous and conserved protein over-expressed in some pathological states, whose exact function is not yet clear [55]. BSA, with its particular distribution and its multifunctional protein-interactive action may well be a member of this growing class of molecular chaperones.

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