Background: αβ-crystallin and HSP27 are mammalian intracellular small heat shock proteins.

Results: These proteins exchange subunits in a rapid and temperature-dependent manner.

Conclusion: This facile subunit exchange suggests that differential expression could be used by the cell to regulate the response to stress.

Significance: A robust technique defines parameters for the dynamic interaction between the major mammalian small heat shock proteins.

Small heat shock proteins (sHSPs) exist as large polydisperse species in which there is constant dynamic subunit exchange between oligomeric and dissociated forms. Their primary role in vivo is to bind destabilized proteins and prevent their misfolding and aggregation. αβ-crystallin (αβ) and HSP27 are the two most widely distributed and most studied sHSPs in the human body. They are coexpressed in different tissues, where they are known to associate with each other to form hetero-oligomeric complexes. In this study, we aimed to determine how these two sHSPs interact to form hetero-oligomers in vitro and whether, by doing so, there is an increase in their chaperone activity and stability compared with their homo-oligomeric forms. Our results demonstrate that HSP27 and αβ formed polydisperse hetero-oligomers in vitro, which had an average molecular mass that was intermediate of each of the homo-oligomers and which were more thermostable than αβ, but less so than HSP27. The hetero-oligomer chaperone function was found to be equivalent to that of αβ, with each being significantly better in preventing the amorphous aggregation of α-lactalbumin and the amyloid fibril formation of α-synuclein in comparison with HSP27. Using mass spectrometry to monitor subunit exchange over time, we found that HSP27 and αβ exchanged subunits 23% faster than the reported rate for HSP27 and αA and almost twice that for αA and αB. This represents the first quantitative evaluation of αB/HSP27 subunit exchange, and the results are discussed in the broader context of regulation of function and cellular proteostasis.

Incorrect folding (misfolding) of proteins is associated with protein malfunction and aggregation (1). Protein aggregation occurs through either disordered (amorphous) or ordered (amyloid fibril) pathways, both of which are nucleation-dependent and lead to the deposition of protein inclusions in cells and tissues (2). The aggregates formed (in particular, fibrils) can be detrimental to cells and tissues and have been associated with a variety of diseases, some of the most notable being the neurodegenerative disorders Alzheimer disease, Parkinson disease, and Huntington disease (3–5). To prevent protein aggregation, the cell employs quality control machinery that acts to encourage protein folding and to discourage misfolding (3, 4). This quality control machinery therefore acts to maintain protein homeostasis (proteostasis), a process that relies heavily on molecular chaperone and protein degradation systems (6, 7).

Heat shock proteins are abundant intracellular chaperones whose expression is strongly induced in response to physicochemical stresses (7) so that the manifold protein folding events of the cell are maintained. Heat shock proteins are classified into five main families based on the approximate molecular masses of their monomeric forms: the small heat shock proteins (sHSPs),3 HSP60, HSP70, HSP90, and HSP100 (7, 8). The sHSPs are defined by a conserved α-crystallin domain (~90 amino acids in length), which is flanked by a relatively hydrophobic N-terminal domain and a highly polar C-terminal extension (2, 9–11).

The sHSPs act by recognizing partially folded protein intermediates that leave the folding/unfolding pathway and enter one of the two off-folding pathways (2). They act to minimize hydrophobic interactions between these intermediates to prevent aggregation and subsequent precipitation of the protein. The sHSPs are so named due to the relatively modest masses of their constituent subunits (12–42 kDa) (12). Typically, however, sHSPs do not exist as monomers; instead, they form large multisubunit oligomers up to 1 MDa in mass (13, 14), which, in mammals, can also be polydisperse (15). The oligomers formed by sHSPs are dynamic (15–18); they undergo constant subunit exchange, and their average size and substructure can be

*This work was supported in part by National Health and Medical Research Council Grant 514615 (to J. A. A.).
1 To whom correspondence may be addressed. Tel.: 61-2-4221-3340; Fax: 61-2-4221-8130; E-mail: aquilina@uow.edu.au.
2 Recipient of Australian Research Council Future Fellowship FT110100586.
To whom correspondence may be addressed. Tel.: 61-2-4221–3443; Fax: 61-2-4221-8130; E-mail: heathe@uow.edu.au.
3 The abbreviations used are: shSP, small heat shock protein; αβ, αβ-crystallin; αA, αA-crystallin; DLS, dynamic light scattering; ThT, thioflavin T.
were transformed into competent *Escherichia coli* BL21(DE3) cells using standard techniques and expressed and purified as described previously (37). The concentrations of proteins used in this study were determined using the following absorption extinction coefficients (in mM): α-lactalbumin, 2.09 and 14.20 (38); α-synuclein, 0.35 and 14.46 (39); HSP27, 1.65 and 22.78 (40); and αB, 0.83 and 20.15 (38).

**SDS-PAGE and Native PAGE**—SDS-PAGE was performed on discontinuous polyacrylamide gels using a 4% (v/v) stacking gel and a 12% (v/v) resolving gel and standard techniques following the manufacturer’s recommendations (Bio-Rad). Native PAGE was performed on discontinuous polyacrylamide gels using a 4% (v/v) stacking gel and a 6% (v/v) resolving gel as described previously (41).

**Size, Polydispersity, and Thermal Stability of the Oligomeric Complexes**—To investigate the size of homo- and hetero-oligomeric complexes of αB and HSP27, dynamic light scattering (DLS) was performed. Samples of HSP27 and αB and an equimolar mixture of the two were prepared in 50 mM phosphate buffer containing 100 mM NaCl (pH 7.0) at a final concentration of 50 μM and incubated at 37 °C for 1 h to initiate subunit exchange. Samples were then placed on ice, which halts further subunit exchange (15). DLS was performed using a Malvern Zetasizer Auto Plate Sampler (APS) system. Samples (60 μl) were aliquoted into wells of a 96-well microtiter plate, which was held at 4 °C. Measurements were performed at 37 °C following a 30-s equilibration time. Accumulation times for each sample were determined automatically using the inbuilt software, and the correlogram was used to calculate the hydrodynamic diameter (nm) and to estimate the polydispersity of the particles in solution. The thermal stability of the oligomers was measured using the temperature melt trend function of the Malvern APS system. The temperature was increased from 25 to 80 °C (in 1 °C increments), and the melting temperature was taken to be the temperature at which the hydrodynamic diameter of the protein had increased by 2-fold from that measured at 25 °C. All experiments were repeated three times, and results shown are representative of these experiments.

**Aggregation Assays**—Experiments were performed to investigate the relative chaperone activity of homo- and hetero-oligomeric complexes of HSP27 and αB against both amorphous and amyloid fibril type aggregation. At the end of each assay (i.e., 12.5 h for the α-lactalbumin assay and 50 h for the α-synuclein assay), the ability of the sHSPs to inhibit protein aggregation was determined by calculating the percent protection using the following formula: % protection = 100 × (ΔAfinal − ΔAs)/ΔAfinal, where ΔAfinal and ΔAs represent the change in absorbance (A360) or thioflavin T (ThT) fluorescence for the target protein in the absence or presence of chaperones, respectively. All assays were repeated at least three times. Results shown are the changes in light scatter at 360 nm or ThT fluorescence over time for one representative experiment. For percent protection afforded by the chaperones, results are means ± S.E. of three independent experiments. Data were subsequently analyzed using one-way analysis of variance, and differences between treatments were established with Tukey’s post hoc test using GraphPad Prism 5.01. *p* values <0.05 were considered significant.
Hetero-oligomers of Small Heat Shock Proteins

Reduction-induced Amorphous Aggregation of α-Lactalbumin—Bovine milk α-lactalbumin (100 μM) was incubated in 50 mM phosphate buffer containing 100 mM NaCl and 2 mM EDTA (pH 7.0) at 37 °C for 10 h in the absence or presence of sHSPs at molar ratios between 16:1 and 2:1 (α-lactalbumin:sHSPs). In experiments performed with an equimolar mixture of the two sHSPs, the concentration of each individual sHSP was halved so that the final concentration of the hetero-oligomers was the same as that of the homo-oligomers. Samples were prepared in duplicate in Greiner Bio-One 96-well microplates (100 μl/well), and the aggregation of α-lactalbumin was initiated by the addition of 20 mM DTT. The plates were sealed with SealPlate film (Sigma-Aldrich) to prevent evaporation. The precipitation of HSP27, or an equimolar mixture of the two such that the molar ratios between 16:1 and 2:1 (α-lactalbumin:sHSPs) was monitored at intervals of 12 min for 3000 min using a POLARstar OPTIMA (excitation at 440 nm and emission at 490 nm) plate reader (BMG Lab Technologies, Melbourne, Victoria, Australia). Samples were shaken for 30 s prior to the first absorbance reading, and measurements were made every 3 min for 720 min.

Amyloid Fibril Aggregation of α-Synuclein—The formation of amyloid fibrils by α-synuclein was measured using a ThT fluorescence assay adapted from Rekas et al. (42). Briefly, α-synuclein (125 μM) in 50 mM phosphate buffer containing 100 mM NaCl (pH 7.4) was incubated in the absence or presence of αB, HSP27, or an equimolar mixture of the two such that the molar ratios tested ranged from 1:0.05 to 1:0.1 (α-synuclein:sHSPs). Samples were prepared in duplicate and incubated with 10 μM ThT in Greiner Bio-One 384-well microplates to a final volume of 50 μl/well. The plates were sealed with SealPlate film and incubated at 37 °C for 50 h, with 5 min of shaking after every fluorescence reading. The change in ThT fluorescence intensity (excitation at 440 nm and emission at 490 nm) was monitored at intervals of 12 min for 3000 min using a POLARstar OPTIMA plate reader (BMG Lab Technologies).

Subunit Exchange and Mass Spectrometry—Approximately 2 mg of the purified and concentrated proteins (25 μg/ml) were buffer-exchanged by loading onto a Superdex™ 200 10/300 GL column and eluting at 0.3 ml/min with 200 mM NH₄OAc. A 0.3-ml fraction corresponding to the apex of the A₂₈₀ chromatogram for each sHSP was collected for use in all subunit exchange experiments. Subunit exchange reactions were performed in Eppendorf tubes in a temperature-controlled water bath at 23, 30, and 37 °C. Protein concentrations were 20 μM HSP27 and 10 μM αB in 50 μl of 200 mM NH₄OAc and 1 mM DTT at time zero. 5-μl volumes of sample were taken at regular time intervals during the exchange and immediately put on ice prior to analysis. Nanoelectrospray mass spectrometry was performed using a SYNAPT™ G2 HDMS spectrometer (Waters) in positive ion mode with the following instrument conditions: capillary, 1.6 kV; sample cone, 200 V; extractor, 10 V; trap collision energy, 80 V; and transfer collision energy, 180 V. Argon gas was introduced into the collision cell at 9.0 ml/min to promote the dissociation of monomers from the hetero-oligomeric assemblies. The pressures in the various stages of the instrument were as follows: backing, 7.3 mbar; trap, 3.8 × 10⁻² mbar; ion mobility, 2.66 × 10⁻² mbar; and TOF, 2.2 × 10⁻⁶ mbar. All spectra were externally calibrated using a solution of cesium iodide (10 mg/ml in water) and processed post-acquisition using MassLynx 4.1 software (Waters). Kinetic data were extracted from the mass spectra by summing the homo-oligomeric signature peak intensities and plotting these values on a log e scale versus time. The intensity data (I) were fitted to the exponential function I(t) = Iₒe⁻kt, where k is the subunit exchange rate constant.

RESULTS

Native gel electrophoresis confirmed that HSP27 and αB formed hetero-oligomeric complexes in vitro (Fig. 1A). The absence of individual bands corresponding to homo-oligomeric αB or HSP27 in the lane containing an equimolar mixture of the two indicated that significant subunit exchange had occurred. DLS measurements demonstrated that, when mixed, αB (blue) and HSP27 (red) formed a polydisperse population (gold) with a size distribution intermediate between the two homo-oligomers. C, thermal denaturation curves for αB (blue), HSP27 (red), and an equimolar mixture of the two (gold). The change in average particle size (Z-average) as measured by DLS was used to estimate protein aggregation as the temperature was increased by 1 °C/min from 25 to 95 °C. Results shown are representative of three independent experiments.
Reduction-induced amorphous aggregation of α-lactalbumin was used to assess the chaperone efficiency of the sHSPs against amorphously aggregating target proteins, as this is an established model and is conducted under conditions of physiological temperature and pH. In the absence of sHSPs, aggregation of α-lactalbumin commenced after 15 min and rapidly increased until it reached a plateau at ~250 min (Fig. 2A). In the presence of sHSPs, there was an increase in the time before aggregation began (i.e. lag phase) and an overall reduction in the light scattering associated with α-lactalbumin aggregation. There was no change in light scattering when the buffer and sHSPs were incubated in the absence of α-lactalbumin, indicating that the increase in light scatter was associated with the aggregation of α-lactalbumin. Overall, there was a concentration-dependent increase in the protection afforded by the homo- and hetero-oligomeric sHSPs, demonstrating that these related sHSP species comingly rather than formed self-chaperoning complexes. The αB homo-oligomers and αB/HSP27 hetero-oligomers were significantly more effective in inhibiting α-lactalbumin aggregation than the HSP27 homo-oligomers at the molar ratios of 4:1, 8:1, and 16:1 (α-lactalbumin:sHSPs) (Fig. 2B). However, there was no significant difference in chaperone activity between the αB homo-oligomers or hetero-oligomers at these molar ratios.

A ThT binding assay was used to assess the chaperone efficacy of the sHSPs against α-synuclein amyloid fibril-type aggregation. This is a disease-based model that is physiologically relevant, as the in vivo aggregation occurs inside cells. In the absence of sHSP, ThT fluorescence associated with α-synuclein fibril formation was observed to increase from 15 h and to continue rising over the remaining 35 h of the assay (Fig. 2C). The inclusion of HSP27 at a 10:1 molar ratio (α-synuclein:sHSPs) resulted in a modest reduction in fluorescence; however, the onset of fibril formation and the kinetics of propagation were similar to the control. The αB homo-oligomers and αB/HSP27 hetero-oligomers had a much more pronounced effect on the kinetics of α-synuclein fibril formation. First, there was a significant increase in the lag phase of the aggregation, extending to 36 h compared with the 15-h lag phase for HSP27. Second, there was an 80% reduction in ThT fluorescence after 50 h compared with the control, demonstrating superior efficacy over HSP27 homo-oligomers (which reduced ThT fluorescence by only 32%). There was no change in ThT fluorescence when the buffer and sHSPs were incubated in the absence of α-synuclein.

At molar ratios of 10:1 and 20:1 (α-synuclein:sHSPs), homo-oligomeric αB and αB/HSP27 hetero-oligomers were significantly more effective in inhibiting α-synuclein fibril formation compared with HSP27 (Fig. 2D). There was no difference in the chaperone activity between the αB homo-oligomers and αB/HSP27 hetero-oligomers at these molar ratios; both suppressed the increase in ThT fluorescence associated with α-synuclein fibril formation by ~85%.

Although native PAGE of the αB/HSP27 mixed oligomers provided evidence that these sHSPs were able to exchange subunits (Fig. 1A), it revealed little about the kinetics or the extent of hetero-oligomerization. To address these parameters in some detail, we employed nanoelectrospray ionization mass spectrometry, a technique that is well established for the analysis of polydisperse protein assemblies and their subunit exchange (43). The method developed to monitor subunit exchange exploits the phenomena of asymmetric dissociation and charge stripping, which take place in the trap region of the spectrometer. For the purposes of this study, neutral gas pressure and accelerating potential across the trap were adjusted to promote the removal of up to three monomers from the sHSP assemblies prior to their detection in the TOF tube.

Dissociation spectra for HSP27 and αB homo-oligomers were acquired (Fig. 3A), and the m/z regions corresponding to oligomers from which monomers had been stripped are labeled. A notable difference between the spectra of HSP27 and αB was the more facile removal of monomers from αB under identical instrument conditions. The most abundant signal in the HSP27 spectrum arose from oligomers stripped of one monomer (n−1), with smaller populations of non-stripped oligomers (n) and oligomers stripped of two monomers (n−2) also present. However, αB had no intact oligomers remaining, and the majority of signal was centered around the n−2 oligomers. Therefore, under the same conditions, it was possible to remove, on average, one additional αB subunit from its homo-oligomers compared with HSP27.

The n−2 region is particularly useful in the analysis of polydisperse proteins, as it includes a “signature” peak at an m/z value equal to the mass of a single subunit. This unique feature arises from the fact that, for all oligomers in the polydisperse assembly, when the number of subunits is equal to the number of charges, i.e. n+x−, the resulting ions overlap at the signature peak m/z value (43). This singularity has the effect of greatly
simplifying the \( n - 2 \) region, as shown for \( \alpha B \) and HSP27 (Fig. 3, B and C). Consequently, it is relatively straightforward to assign this region of the spectrum for a polydisperse protein, as indicated by the labeled ions in these spectra. For \( \alpha B \), the major oligomers were found to range in size from 18 to 30 subunits \((n - 2)\), with those containing an even number of subunits dominating the distribution (Fig. 3B). HSP27 had the same range of oligomers as \( \alpha B \); however, the distribution was significantly skewed due to a high proportion of signal arising from 20- and 22-mers.

This clear separation of \( \alpha B \) and HSP27 signature peaks on the \( m/z \) scale allowed us to examine the solution-phase subunit exchange reaction between these two sHSPs. Mixtures were incubated at 23, 30, or 37 °C, and spectra were acquired at regular time intervals to monitor the extent of hetero-oligomerization. Data for the 30 °C reaction mixture, acquired over a period of 30 min, are presented in Fig. 4A. As subunit exchange proceeded, a peak corresponding to the formation of a hetero-oligomeric product was observed to emerge at \( m/z \sim 22,400 \). This was accompanied by a minor decay in the relative intensities of the signature peaks; however, unlike exchange reactions between \( \alpha A \) and \( \alpha B \) (43), there was a negligible shift in the \( m/z \) values of these peaks. This implies that the subunit exchange between \( \alpha B \) and HSP27 does not proceed uniformly throughout the system, but rather, a subpopulation of oligomers participates preferentially to the remaining non-exchanged homo-oligomers.

As noted above, the hetero-oligomeric signature peak emerged at \( m/z \sim 22,400 \), a value much higher than that expected for a fully exchanged \( \alpha B \)/HSP27 species. The homo-oligomer concentrations used in these experiments were 10 \( \mu M \) (\( \alpha B \)) and 20 \( \mu M \) (HSP27), which, if fully and freely exchanged, would generate hetero-oligomers with a signature peak at \( m/z \sim 21,850 \); i.e. 550 \( m/z \) units less than the species observed (Fig. 4A). We investigated this disparity by examining the mass spectra acquired after greater periods of subunit exchange. Although the signal-to-noise ratio of the data decreased significantly with time, we were in fact able to observe a broad peak centered at \( m/z \sim 21,900 \) after 75 min (Fig. 4B), indicating that complete exchange did eventuate. For each of the reaction temperatures, the subunit exchange rate constant \( k \) was obtained by plotting the homo-oligomeric intensities versus time (Fig. 4C) and was determined to be as follows: \( k_{23} = 2.0 \times 10^{-3} \text{ min}^{-1} \), \( k_{30} = 1.4 \times 10^{-2} \text{ min}^{-1} \), and \( k_{37} = 7.1 \times 10^{-2} \text{ min}^{-1} \).
Hetero-oligomers of Small Heat Shock Proteins

The hetero-oligomeric α-crystallin of the eye lens is massively abundant at a ratio of approximately three αA subunits to one αB subunit. Although αB is also widely distributed throughout the body, αA is virtually confined to lenticular fiber cells. Considering that HSP27 and αB are known to co-localize in many tissues, including skeletal muscle, this points to HSP27 being the extralenticular compere of αB. However, although the interactions between αA and αB have been exhaustively examined, the literature regarding mixed assemblies of HSP27 and αB is scant. In this study, we endeavored to address this deficiency by characterizing some key functional and structural aspects of HSP27 and αB hetero-oligomerization.

In agreement with previous work (36, 44), HSP27 and αB were able to form hetero-oligomeric complexes in vitro. Thus, native PAGE showed a single diffuse band when HSP27 and αB were mixed, indicative of the comprehensive dynamic subunit exchange that is typical of mammalian sHSPs (17, 45), along with the well known polydispersity of mammalian sHSPs. Furthermore, these complexes exhibited an amalgamation of structural and functional characteristics derived from the homo-oligomeric populations. For example, the hetero-oligomers had a size and thermal stability midway between the homo-oligomeric forms. The functional efficiencies of the hetero-oligomeric complexes were assessed using target proteins that either aggregate amorphously (α-lactalbumin) or form disease-associated amyloid fibrils (α-synuclein). In both cases, the hetero-oligomers were equivalent in their chaperone capacity to αB homo-oligomers and superior to HSP27 homo-oligomers. Thus, our data suggest that it is advantageous for HSP27 and αB to form hetero-oligomeric complexes: HSP27 would stabilize αB in tissues in which they are coexpressed (as αA does in the eye lens), and the chaperone activity is as good as (or, in the case of HSP27, better than) the homo-oligomeric forms.

Although it is well established that HSP27 and αB can form mixed assemblies when incubated in vitro, it is important to understand the kinetics of the subunit exchange process to determine whether the time scale is relevant to their in vivo expression. Subunit exchange studies using FRET at 37 °C have been reported for HSP27/αA and αA/αB (17), with rate constants of 5.76 × 10^{-2} and 3.78 × 10^{-2} min^{-1}, respectively. These FRET studies depend upon the attachment of a fluorescent probe to a free cysteine under basic conditions for several hours, which, although not structurally destructive, has the potential to alter the dynamics of subunit exchange. For example, we have observed relatively high levels of disulfide-linked dimers in the mass spectrum of HSP27 expressed in E. coli, whereas in vivo, these dimers have been observed to account for >50% of total HSP27 in SH-SY5Y cells expressing the protein (46). These researchers suggested that the ratio between monomeric and dimeric HSP27 is a key determinant for the activity of the protein. In this context, FRET experiments, which rely on the attachment of fluorophores to Cys residues, preclude the formation of HSP27 (and αA) dimers, thereby excluding this major parameter from the system. As noted above, our recombinant HSP27 was a mixture of disulfide-linked dimers and monomers after purification; however, for this initial work and to facilitate comparison with previously published studies, we reduced all disulfides in HSP27 prior to use.

Using nanoelectrospray mass spectrometry, we were able to monitor (in real time) the subunit exchange between native label-free HSP27 and αB. Both proteins are broadly distributed in mammalian tissue and overexpressed under stress conditions; thus, a description of the process(es) leading to heterocomplex formation is germane. At 37 °C, the exchange rate constant was found to be 7.1 × 10^{-2} min^{-1}, which is 23% faster than the HSP27/αA rate and almost twice that of αA/αB exchange at the same temperature reported previously (17). Considering the relatively static environment of the lens, it is not surprising that α-crystallin subunit exchange is less vigorous; however, it should be emphasized that modulation of sHSP interactions via HSP27 phosphorylation and disulfide redox may provide considerable dynamic range to the system. For example, the triple serine-to-aspartate phosphomimic of HSP27 is a discrete dimer (40, 47, 48), which, in vitro at least, is a superior chaperone to the larger oligomers (18). Therefore, in vivo, one may envisage a complex matrix of hetero-oligomeric permutations, which, coupled with highly dynamic subunit exchange, perform multifarious roles in the cell. Conversely, it
Hetero-oligomers of Small Heat Shock Proteins

may be that the overproduction of sHSP species with enhanced or inappropriate chaperone activity leads to co-localized deposition of sHSPs in some pathologies. Currently, many of the characteristics of polydisperse and hetero-oligomeric sHSPs are unknown, which presents an exciting area of protein homeostasis to explore.

REFERENCES

1. Dobson, C. M. (2003) Protein folding and misfolding. Nature 426, 884–890
2. Ecroyd, H., and Carver, J. A. (2009) Crystallin proteins and amyloid fibrils. Cell. Mol. Life Sci. 66, 62–81
3. Chiti, F., and Dobson, C. M. (2006) Protein misfolding, functional amyloid, and human disease. Annu. Rev. Biochem. 75, 333–366
4. Dobson, C. M. (2001) The structural basis of protein folding and its links with human disease. Philos. Trans. R. Soc. Lond. B Biol. Sci. 356, 133–145
5. Muchowski, P. J. (2002) Protein misfolding, amyloid formation, and neurodegeneration: a critical role for molecular chaperones? Neuron 35, 9–12
6. Muchowski, P. J., and Wacker, J. L. (2005) Modulation of neurodegeneration by molecular chaperones. Nat. Rev. Neurosci. 6, 11–22
7. Hartl, F. U., Bracher, A., and Hayer-Hartl, M. (2011) Molecular chaperones in protein folding and proteostasis. Nature 475, 324–332
8. Voisine, C., Pedersen, J. S., and Morimoto, R. I. (2010) Chaperone networks: tipping the balance in protein folding diseases. Neurobiol. Dis. 40, 12–20
9. Stanler, R., Kappé, G., Boelens, W., and Slingsby, C. (2005) Wrapping the α-crystallin domain fold in a chaperone assembly. J. Mol. Biol. 353, 68–79
10. Sun, Y., and MacRae, T. H. (2005) Small heat shock proteins: molecular characteristics of polydisperse and hetero-oligomeric sHSPs or inappropriate chaperone activity leads to co-localized deposition of sHSPs in some pathologies. Currently, many of the characteristics of polydisperse and hetero-oligomeric sHSPs are unknown, which presents an exciting area of protein homeostasis to explore.

REFERENCES

1. Dobson, C. M. (2003) Protein folding and misfolding. Nature 426, 884–890
2. Ecroyd, H., and Carver, J. A. (2009) Crystallin proteins and amyloid fibrils. Cell. Mol. Life Sci. 66, 62–81
3. Chiti, F., and Dobson, C. M. (2006) Protein misfolding, functional amyloid, and human disease. Annu. Rev. Biochem. 75, 333–366
4. Dobson, C. M. (2001) The structural basis of protein folding and its links with human disease. Philos. Trans. R. Soc. Lond. B Biol. Sci. 356, 133–145
5. Muchowski, P. J. (2002) Protein misfolding, amyloid formation, and neurodegeneration: a critical role for molecular chaperones? Neuron 35, 9–12
6. Muchowski, P. J., and Wacker, J. L. (2005) Modulation of neurodegeneration by molecular chaperones. Nat. Rev. Neurosci. 6, 11–22
7. Hartl, F. U., Bracher, A., and Hayer-Hartl, M. (2011) Molecular chaperones in protein folding and proteostasis. Nature 475, 324–332
8. Voisine, C., Pedersen, J. S., and Morimoto, R. I. (2010) Chaperone networks: tipping the balance in protein folding diseases. Neurobiol. Dis. 40, 12–20
9. Stanler, R., Kappé, G., Boelens, W., and Slingsby, C. (2005) Wrapping the α-crystallin domain fold in a chaperone assembly. J. Mol. Biol. 353, 68–79
10. Sun, Y., and MacRae, T. H. (2005) Small heat shock proteins: molecular characteristics of polydisperse and hetero-oligomeric sHSPs or inappropriate chaperone activity leads to co-localized deposition of sHSPs in some pathologies. Currently, many of the characteristics of polydisperse and hetero-oligomeric sHSPs are unknown, which presents an exciting area of protein homeostasis to explore.

REFERENCES

1. Dobson, C. M. (2003) Protein folding and misfolding. Nature 426, 884–890
2. Ecroyd, H., and Carver, J. A. (2009) Crystallin proteins and amyloid fibrils. Cell. Mol. Life Sci. 66, 62–81
3. Chiti, F., and Dobson, C. M. (2006) Protein misfolding, functional amyloid, and human disease. Annu. Rev. Biochem. 75, 333–366
4. Dobson, C. M. (2001) The structural basis of protein folding and its links with human disease. Philos. Trans. R. Soc. Lond. B Biol. Sci. 356, 133–145
5. Muchowski, P. J. (2002) Protein misfolding, amyloid formation, and neurodegeneration: a critical role for molecular chaperones? Neuron 35, 9–12
6. Muchowski, P. J., and Wacker, J. L. (2005) Modulation of neurodegeneration by molecular chaperones. Nat. Rev. Neurosci. 6, 11–22
7. Hartl, F. U., Bracher, A., and Hayer-Hartl, M. (2011) Molecular chaperones in protein folding and proteostasis. Nature 475, 324–332
8. Voisine, C., Pedersen, J. S., and Morimoto, R. I. (2010) Chaperone networks: tipping the balance in protein folding diseases. Neurobiol. Dis. 40, 12–20
9. Stanler, R., Kappé, G., Boelens, W., and Slingsby, C. (2005) Wrapping the α-crystallin domain fold in a chaperone assembly. J. Mol. Biol. 353, 68–79
10. Sun, Y., and MacRae, T. H. (2005) Small heat shock proteins: molecular characteristics of polydisperse and hetero-oligomeric sHSPs or inappropriate chaperone activity leads to co-localized deposition of sHSPs in some pathologies. Currently, many of the characteristics of polydisperse and hetero-oligomeric sHSPs are unknown, which presents an exciting area of protein homeostasis to explore.

REFERENCES

1. Dobson, C. M. (2003) Protein folding and misfolding. Nature 426, 884–890
2. Ecroyd, H., and Carver, J. A. (2009) Crystallin proteins and amyloid fibrils. Cell. Mol. Life Sci. 66, 62–81
3. Chiti, F., and Dobson, C. M. (2006) Protein misfolding, functional amyloid, and human disease. Annu. Rev. Biochem. 75, 333–366
4. Dobson, C. M. (2001) The structural basis of protein folding and its links with human disease. Philos. Trans. R. Soc. Lond. B Biol. Sci. 356, 133–145
5. Muchowski, P. J. (2002) Protein misfolding, amyloid formation, and neurodegeneration: a critical role for molecular chaperones? Neuron 35, 9–12
6. Muchowski, P. J., and Wacker, J. L. (2005) Modulation of neurodegeneration by molecular chaperones. Nat. Rev. Neurosci. 6, 11–22
7. Hartl, F. U., Bracher, A., and Hayer-Hartl, M. (2011) Molecular chaperones in protein folding and proteostasis. Nature 475, 324–332
8. Voisine, C., Pedersen, J. S., and Morimoto, R. I. (2010) Chaperone networks: tipping the balance in protein folding diseases. Neurobiol. Dis. 40, 12–20
9. Stanler, R., Kappé, G., Boelens, W., and Slingsby, C. (2005) Wrapping the α-crystallin domain fold in a chaperone assembly. J. Mol. Biol. 353, 68–79
10. Sun, Y., and MacRae, T. H. (2005) Small heat shock proteins: molecular characteristics of polydisperse and hetero-oligomeric sHSPs or inappropriate chaperone activity leads to co-localized deposition of sHSPs in some pathologies. Currently, many of the characteristics of polydisperse and hetero-oligomeric sHSPs are unknown, which presents an exciting area of protein homeostasis to explore.
αB-crystallin with α-synuclein: effects on amyloid fibril formation and chaperone activity. J. Mol. Biol. 340, 1167–1183

43. Aquilina, J. A., Benesch, J. L., Ding, L. L., Yaron, O., Horwitz, J., and Robinson, C. V. (2005) Subunit exchange of polydisperse proteins: mass spectrometry reveals consequences of αA-crystallin truncation. J. Biol. Chem. 280, 14485–14491

44. Fu, L., and Liang, J. J. (2003) Enhanced stability of αB-crystallin in the presence of small heat shock protein Hsp27. Biochem. Biophys. Res. Commun. 302, 710–714

45. Sobott, F., Benesch, J. L., Vierling, E., and Robinson, C. V. (2002) Subunit exchange of multimeric protein complexes. Real-time monitoring of subunit exchange between small heat shock proteins by using electrospray mass spectrometry. J. Biol. Chem. 277, 38921–38929

46. Almeida-Souza, L., Goethals, S., de Winter, V., Dierick, I., Gallardo, R., Van Durme, J., Irobi, J., Gettemans, J., Rousseau, F., Schymkowitz, J., Timmerman, V., and Janssens, S. (2010) Increased monomerization of mutant HSPB1 leads to protein hyperactivity in Charcot-Marie-Tooth neuropathy. J. Biol. Chem. 285, 12778–12786

47. Lambert, H., Charette, S. J., Bernier, A. F., Guimond, A., and Landry, J. (1999) HSP27 multimerization mediated by phosphorylation-sensitive intermolecular interactions at the amino terminus. J. Biol. Chem. 274, 9378–9385

48. McDonald, E. T., Bortolus, M., Koteiche, H. A., and Mchaourab, H. S. (2012) Sequence, structure, and dynamic determinants of Hsp27 (HspB1) equilibrium dissociation are encoded by the N-terminal domain. Biochemistry 51, 1257–1268