Nature's molecular sponges: Small heat shock proteins grow into their chaperone roles

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Although their better-known cousins, the Hsp70, Hsp90, and Cpn60 molecular chaperone families, have been the subjects of ever more detailed mechanistic scrutiny (1, 2), the small heat shock proteins (sHSPs) have remained mysterious and relatively understudied, even though they are ubiquitous and intimately linked to protein homeostasis and survival under stress conditions (3). There are several reasons for this. First, sHSPs form large and dynamic oligomers with a technically daunting proclivity to heterogeneity in their stoichiometry of oligomerization and client binding. Second, this family of molecular chaperones does not use ATP to kick off their substrates for a new start in folding and assembly. Third, the best studied sHSPs in vertebrates, the α-crystallins, are associated with a very specialized task: maintenance of eye lens proteins, which may have led to a misguided oversimplification of sHSP cellular roles. Finally, structural and biophysical data describing sHSPs and their interactions with clients are relatively sparse (4).

Like other chaperones, sHSPs bind partially unfolded polypeptides so that they may retain the capacity to refold. As "holdase" chaperones, they protect cells from protein losses or toxicity caused by aggregation, but they necessarily must release their still aggregation-prone clients to other downstream chaperones that facilitate folding. Mounting evidence points to extremely important roles for sHSPs in chaperone networks in organisms from bacteria to higher eukaryotes, and sHSPs have been implicated in several misfolding and aggregation diseases (5).

In a recent study of a eukaryotic sHSP, HSP18.1 from pea, reported in this issue of PNAS (6), powerful mass-spectrometry experiments have yielded a quantitative description of the dynamic oligomerization and client-binding reactions of sHSPs, and how these reactions are modulated by temperature. This work has shed light on fundamental behaviors of sHSPs, and the findings will help researchers understand their elusive mechanisms of action. Excitingly, sHSPs appear to use a unique paradigm for chaperone action that takes advantage of the temperature- and client-induced remodeling of an oligomerization landscape.

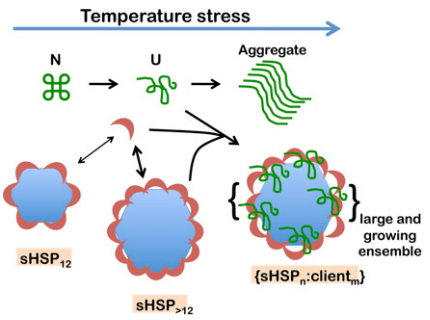

Fig. 1. When the native states of proteins (N) become unstable under heat stress, sSHPs passively protect cells against potentially deleterious aggregates by remodeling their oligomeric landscapes. Both increased dissociation of dimeric subunits and a shift to higher oligomeric structures are accompanied by higher affinity for unfolded client molecules (U). The resulting hetero-oligomeric sSHP:client complexes agglomerate into an array of coaggregates that can be recovered by disaggregating Hsp100/Hsp70 chaperone teams.

Small HSPs have in common a 100-amino acid core α-crystallin domain, to which are appended N- and C-terminal extensions that are variable in length and sequence. These extensions participate in the association of small HSPs into large dynamic oligomers and mediate recognition of client proteins (7). Many sHSPs stably assume defined oligomeric states, such as a dodecamer or 24-mer under a given set of conditions, but others display polydispersity in their oligomeric states at all times. Even when there is a predominance of oligomers of a specific size, dimeric subunits can escape and rejoin in a dynamic fashion (8). Electron microscopy and x-ray crystallography have shed light on the structures of the oligomers, which are ordered spherical or ring-like structures with internal cavities comprised of symmetrically packed dimeric subunits (4).

In a tantalizing link between physiological need and biophysical properties, temperature influences the sHSP oligomeric landscape, enhancing dynamics and shifting the distribution to larger oligomers. Indeed, this direct temperature modulation of sHSPs is at the heart of the response to heat stress: Increased temperature exposes hydrophobic surfaces, such that the species populated at high temperatures bind client proteins better. To decipher the complex array of sHSP dynamic assembly/disassembly and client-binding events and the role of temperature, Stengel et al. (6) applied a quantitative mass spectrometry method that enables rigorous analysis of oligomeric species distribution as a function of temperature, even for high molecular-mass multimers.

Crucial to these experiments is the method of ionizing the sample for mass spectrometric analysis: nano-electrospray (nanoESI). Conventional ESI techniques require the heating of the nebulizer gas or the analyte solution to attain sufficient desolvation for high-quality mass spectra, but this can alter distributions and stabilities of macromolecular complexes, particularly for a temperature-dependent system like the sHSPs. The narrower capillary orifice of a nanoESI tip leads to more facile ionization and desolvation, allowing the transfer of intact complexes into the gas phase for analysis by mass spectrometry, while remaining compatible with more physiologically relevant aqueous buffered solutions (9). Robinson and colleagues developed a method to heat the protein solution in the nanoESI capillary in a controlled manner (10), which opens the way to equilibrium temperature studies of high-mass protein complexes.

At ambient temperature, the predominant HSP18.1 species is a dodecamer. Increasing temperature led to a shift to higher oligomers from 13 to 20 subunits each (6). The rate-limiting step in oligomer rearrangement is subunit exchange, which is enhanced at higher temperatures. All of these processes are reversible. Thermodynamic analysis of these association/dissociation equilibria show that their temperature dependence can be explained without invoking a thermally induced conformational change within the subunit, as had previously been reported for a yeast sHSP (11). Notably, the domain found to undergo a conformational change with temperature in the yeast HSP26 is unique to that sHSP family, suggesting that the thermal modulation of an oligomerization landscape is

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the more general mechanism by which sHSPs alter their functions in response to heat shock.

How does all this relate to client-protein interactions? First, it seems that the species highly populated at ambient temperature, the dodecamer, does not bind client, and thus serves as a reservoir for subunits. Once temperature is increased and subunit exchange is enhanced, client-molecule association with the sHSPs is favored, and oligomeric sHSP:client complexes are populated. Brief incubation of the sHSP with the model client-protein luciferase at 42 °C led to a rapid disappearance of the free client pool accompanied by a shift to larger oligomeric sHSP/luciferase complexes (6). The kinetics of these processes point to a mechanism (Fig. 1) with rapid binding of client either to released sHSP dimers or to thermally rearranged oligomers, yielding an array of hetero-oligomers, followed by a slower phase in which the sHSP:client complexes grow more massive by incorporating more sHSP and client molecules, not unlike a sponge becoming moist and swelling so that it accommodates yet more water. One of the greatest strengths of modern mass spectrometry is the ability to select a specific mass species and, using controlled collisional dissociation, to probe the nature and subunit stoichiometry of complex mixtures by determining the masses of the resulting components (12). Strikingly, the authors found over 300 different sHSP:client combinations populated in samples created from 1:1 mixtures of client and dodecamer, demonstrating vividly why it has proved so difficult to characterize the bound species.

Many questions lack detailed mechanistic answers: How does the sHSP family provide chaperone activity? How is the binding of unfolded protein

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accomplished? And how does the release of substrate take place without the usual nucleotide signal? sHSPs are passive, functioning without use of ATP energy to mediate cycles of substrate release/binding. Synthesis of many chaperones is induced under stress conditions, with the heat-shock promoter being a common regulator. The temperature dependence of the sHSP oligomeric landscape joins the production of increased amounts of sHSP and other chaperones via heat shock to prepare cells to absorb large quantities of partially folded client proteins. Decreasing temperature leads sHSPs to hand off their aggregation-prone unfolded passenger proteins to a waiting "foldase" chaperone (or potentially a protease) (13, 14). Hence, temperature cycling of the sHSP oligomeric landscape takes the place of nucleotide binding and hydrolysis in driving cycles of client binding and release.

The association of incompletely folded clients with sHSPs into an array of ever larger hetero-oligomers is a kind of coaggregation, which also plays a significant cellular role in keeping cellular protein aggregates in a state from which they may be readily recovered by Hsp100/Hsp70 chaperone teams (15–17). One of the familiar systems illustrating this panoply of events is the way *Escherichia coli* handles burdens of high amounts of recombinant protein. Excesses are shunted into the familiar fate of inclusion body formation. The orchestrated binding of the sHSP family members, IbpA and IbpB (inclusion body-binding proteins A and B), enables disaggregation and resolubilization via the ClpB/DnaK disaggregation machinery.

Focused mechanistic studies of individual chaperones are important starting points to understanding cellular handling of incompletely folded proteins, but both addressing protein-folding diseases and gaining control over recombinant protein production will require elucidation of the mechanistic interplay among different chaperones and the impact on client-energy landscapes. The sHSPs illustrate not only new ways proteins may respond to physiological stresses like heat, but also how crucial interactions among chaperones are for cellular stress responses.

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1. Hartl FU, Hayer-Hartl M (2009) Converging concepts of protein folding in vitro and in vivo. *Nat Struct Mol Biol* 16:574–581.
2. Saibil HR (2008) Chaperone machines in action. *Curr Opin Struct Biol* 18:35–42.
3. Nakamoto H, Vígh L (2007) The small heat shock proteins and their clients. *Cell Mol Life Sci* 64:294–306.
4. Mchaourab HS, Godar JA, Stewart PL (2009) Structure and mechanism of protein stability sensors: Chaperone activity of small heat shock proteins. *Biochemistry* 48: 3828–3837.
5. Sun Y, MacRae TH (2005) The small heat shock proteins and their role in human disease. *FEBS J* 272:2613–2627.
6. Stengel F, et al. (2010) Quaternary dynamics and plasticity underlie small heat-shock protein chaperone function. *Proc Natl Acad Sci USA* 107:2007–2012.
7. Jaya N, Garcia V, Vierling E (2009) Substrate binding site flexibility of the small heat shock protein molecular chaperones. *Proc Natl Acad Sci USA* 106:15604–15609.
8. Sobott F, Benesch JLP, Vierling E, Robinson CV (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.
9. Sobott F, Robinson CV (2002) Protein complexes gain momentum. *Curr Opin Struct Biol* 12:729–734.
10. Benesch JL, Sobott F, Robinson CV (2003) Thermal dissociation of multimeric protein complexes by using nanoelectrospray mass spectrometry. *Anal Chem* 75: 2208–2214.
11. Franzmann TM, Menhorn P, Walter S, Buchner J (2008) Activation of the chaperone Hsp26 is controlled by the rearrangement of its thermosensor domain. *Mol Cell* 29:207–216.
12. Benesch JLP, Ruotolo BT, Simmons DA, Robinson CV (2007) Protein complexes in the gas phase: Technology for structural genomics and proteomics. *Chem Rev* 107: 3544–3567.
13. Veinger L, Diamant S, Buchner J, Goloubinoff P (1998) The small heat-shock protein IbpB from *Escherichia coli* stabilizes stress-denatured proteins for subsequent refolding by a multichaperone network. *J Biol Chem* 273: 11032–11037.
14. Mogk A, et al. (2003) Refolding of substrates bound to small Hsps relies on a disaggregation reaction mediated most efficiently by ClpB/DnaK. *J Biol Chem* 278: 31033–31042.
15. Mogk A, Deuerling E, Vorderwülbecke S, Vierling E, Bukau B (2003) Small heat shock proteins, ClpB and the DnaK system form a functional triade in reversing protein aggregation. *Mol Microbiol* 50:585–595.
16. Liberek K, Lewandowska A, Zietkiewicz S (2008) Chaperones in control of protein disaggregation. *EMBO J* 27:328–335.
17. Ratajczak E, Zietkiewicz S, Liberek K (2009) Distinct activities of *Escherichia coli* small heat shock proteins IbpA and IbpB promote efficient protein disaggregation. *J Mol Biol* 386:178–189.