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Breaking Down Order to Keep Cells Tidy

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DOI 10.1016/j.chembiol.2012.05.003

Small heat shock proteins form large assemblies that protect cytoplasmic components when stressed. In this issue of *Chemistry & Biology*, Stengel et al. show that disturbing oligomer symmetry allows weak interfaces to catch intact substrate dimers.

Heat shock proteins provide quality control by recognizing and dealing with errors in the expressed proteome. This vital function contributes to cellular robustness conferring tolerance to the stresses of life by preventing cells, organelles, and tissues from becoming clogged with aggregated protein junk (Tyedmers et al., 2010). Heat shock proteins usually perform this function by acting as molecular chaperones, binding to nonnative polypeptide chains and unfolding/refolding the chain using energy stored in ATP. Members of the "small heat shock proteins" family have a short polypeptide sequence (molecular mass ~20 kDa), but they assemble into very large but polydisperse oligomers that undergo rapid subunit exchange and equilibrium dissociation. Although polydispersity has hindered the structural biology of small heat shock proteins, it is likely key to their function. Stengel et al. (2010) have previously employed a two-stage nanoelectrospray mass spectrometry technique (nanoES MS) whereby a spectrum of distributions of macromolecular assemblies within a polydisperse ensemble is measured first, followed by precise but arduous stoichiometric measurements of components of individual assemblies. In the new work published here they replace the second experimental step with modeling from the one dimensional data using newly developed algorithms, rendering the methodology suitable for high throughput applications.

NanoES MS can measure the mass of single assemblies in the gas phase under conditions that simulate stress, such as heat. Using this new technique, the small heat shock protein under study by Stengel et al. (2012) in this issue of *Chemistry & Biology* is from peas. Plants must withstand a wide range of temperatures, and it has been shown that at laboratory ambient temperature, the pea small heat shock protein is a dodecamer. The crystal structure of a closely related dodecameric small heat shock protein from wheat showed it was symmetrically built from six dimers (van Montfort et al., 2001). Previously, nanoES MS techniques revealed that the pea dodecamer rearranged to a polydisperse distribution of higher-order oligomers, with a preference for an even number of monomers, coincident with it becoming an active chaperone binding thermally unstable protein substrate (Stengel et al., 2010). In agreement with the two-dimensional experimental approach, the new method described here (Stengel et al., 2012) showed that for a given ratio of chaperone to a monomeric substrate (luciferase), the most highly populated complex assembly comprised 18 chains of small heat shock protein to 1 chain of luciferase.

Symmetric protein assemblies require strict geometric constraints between complementary, often interwoven, interfaces, and they can be visualized using PDBePISA (http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html) in all their variety (Krissinel and Henrick, 2007). Changing from a regular oligomer into a polydisperse ensemble is expected to lead to an increase in exposure of interface regions, and these rearrangements likely result in the presentation of protein surfaces ready to engage with destabilized substrates. However, most small heat shock proteins across all kingdoms of life are innately polydisperse. In fact, for the first described member, α-crystallin from the eye lens, assembly polydispersity confers solubility and transparency (Clark et al., 2012) and requires some level of activation to increase its affinity for denatured substrates. It is likely, then, that regulated exposure of chaperone binding regions for misfolded protein substrates is linked to conformational change in both substrate and chaperone. In the ATP-driven machines such as HSP60 chaperone, conformational change is driven by ATP-binding and hydrolysis, leading to substrate binding and release (Clare et al., 2012). The ability of small heat shock proteins to manage without ATP binding is in keeping with the energetically cheap exposure of binding sites in these flexible oligomeric assemblies. The new nanoES MS work now embellishes this view with the finding

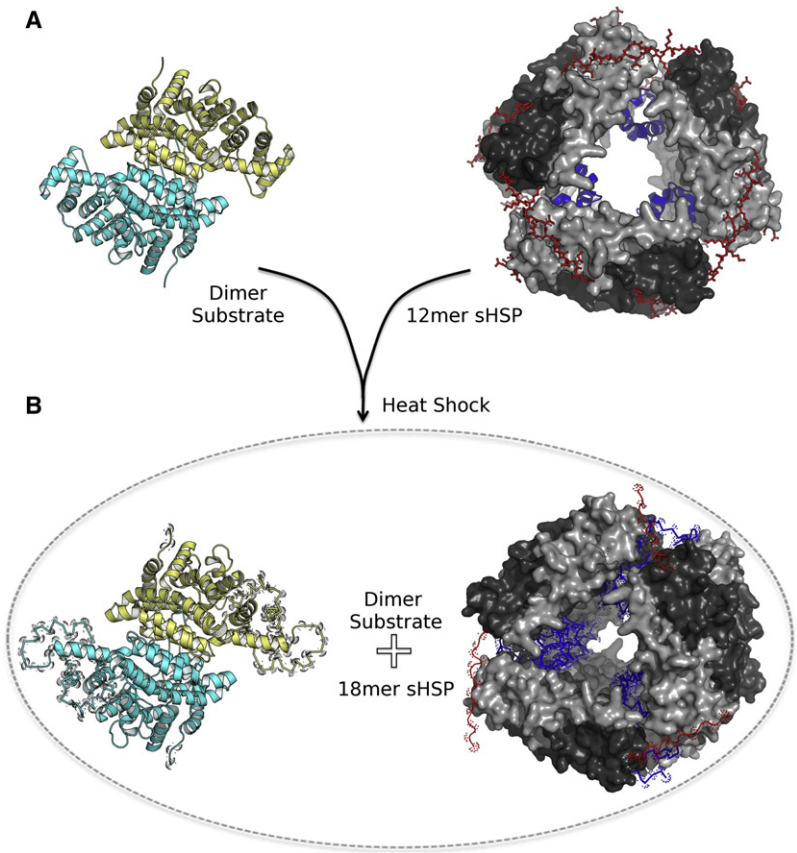

Figure 1. Putative Mechanism of Formation of a Small Heat Shock Protein (sHSP):Target Complex Whereby during Heat Shock Stress, Disorder in both Substrate and Chaperone are Considered to Drive Complex Formation

(A) The native crystal structures of a substrate dimer (blue and yellow) of the open form of citrate synthase (Protein Data Bank [PDB] 1CTS) is shown, and a 12-mer of wheat sHSP (PDB 3GME) is depicted as gray domains with blue and red flanking extensions.

(B) A region of the citrate synthase that changes conformation in the closed conformation (PDB 2CTS) is modeled with disorder, and the plant sHSP is rendered as an 18-mer with the extensions disordered.

that when challenged with two destabilized substrate dimers, the higher-order small heat shock oligomers preferentially bind them as dimers.

The crystal structure of the wheat small heat shock protein dodecamer showed how edge β strands in the α-crystallin β-sandwich domain, common to all small heat shock proteins, act as surfaces for building the assembly by interacting with hydrophobic flanking sequence extensions coming from partner subunits (Figure 1A). One view is that these extensions mimic the binding of unfolded substrate to binding sites formed by the α-crystallin domain, and that by disturbing the symmetry of the assembly (by heat, for example), binding sites are unmasked. Alternatively, the released hydrophobic extensions themselves may be the substrate binding sites. In terms of the conformational state of the substrate, it is unclear whether the parts that interact with the chaperone are partially unfolded protein regions representing kinetically stable (un)folding intermediates or are highly hydrophobic segments buried deep within the native protein fold, hence requiring major unfolding of the substrate protein before being bound to the chaperone. The new work described by Stengel et al. (2012) favors an early unfolding state for two dimeric substrates tested. For example, the citrate synthase dimer, the first enzyme of the Krebs cycle, forms a closed state when two molecules of acetyl coenzyme A bind in two symmetry-related clefts near the dimer interface, relaxing to an open state on coenzyme release (Figure 1A) (Remington et al., 1982). The open state is likely to be the starting model for unfolding, with the part of the chain that changes on binding coenzyme a hot candidate for early melting (Figure 1B). The idea is that under heat stress, the partially unfolded yet largely dimeric enzyme would bind to disordered interface regions of the higher-order plant chaperone (Figure 1B).

These new results are important, because details of how a chaperone assembly interacts with a misfolded substrate at an atomic level are difficult to determine but are central to our understanding of the protective mechanism. In terms of human health, the family of small heat shock proteins appears to have acquired broad protective roles, particularly in long-lived cells such as eye lens, myofibrils, and neuroglia. Members that are upregulated during stress are biomarkers for a range of muscular, vascular, and neurodegenerative diseases. The challenge is to boost levels and activity of small heat shock proteins in long-lived cells and tissues stressed by disease, but to block performance in cancer cells, especially after toxic therapy.

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