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COMMENTARY

Dynamism in Molecular Chaperones

Molecular chaperone proteins are involved in a wide range of tasks ranging from facilitating protein folding to general protein stabilisation. A major family of chaperones is the small heat-shock proteins (sHsps).[1] sHsps have attracted less research interest than the other chaperones despite their ubiquitous presence in all organisms and their significantly enhanced expression under conditions of cellular stress, for example, elevated temperature. A variety of factors have contributed to this sidelining. Firstly, sHsps are not involved in the highly topical task of protein folding. Rather, they have a primary role in preventing protein aggregation whereby, under stress conditions, they interact with partially folded states of target proteins.[1,2] Secondly, there is a paucity of structural data relating to sHsps, particularly the mammalian forms, because of their inherent polydispersity (which leads to a wide range of oligomer stoichiometries) and their dynamic nature coupled with large segments of their polypeptide chain being unstructured and flexible. As a result, there is no X-ray crystal structure of any mammalian sHsp, although recently, crystal structures have become available for the central, conserved, dimeric and mostly β-sheet "α-crystallin" domain.[3,4] In contrast to the α-crystallin domain, the N- and C-terminal flanking regions are mostly unstructured with the last 12 amino acids in the archetypical sHsp, αB-crystallin, for example, being highly mobile.[5] Recent complementary NMR, electron microscopy and small-angle X-ray scattering studies have produced a quaternary structural model for αB-crystallin.[6,7] However, none of these studies have provided a definitive rationale as to how sHsps achieve their polydispersity. Indeed, the functional role of this property, along with the associated subunit exchange,[8] in sHsp chaperone action is not clear. Since sHsps are associated with many diseases involving protein misfolding and aggregation (e.g., cataract, Parkinson's disease, Alzheimer's disease, Huntington's disease and Creutzfeldt–Jakob disease),[9] understanding such processes may have direct applications in, for example, the development of therapeutics for their treatment.

Two papers in this issue, both by Baldwin *et al.*,[10,11] provide significant insight into the mechanism of αB-crystallin polydispersity and subunit exchange. Via the use of state-of-the-art mass spectrometry (MS) and NMR spectroscopy, the physical basis for the polydispersity of αB-crystallin has been explained along with the role of the C-terminal region in this process.

In the first paper,[10] MS was used to determine the oligomeric distribution and subunit exchange rate of αB-crystallin over a range of pH and temperature values. As a result, the thermodynamic and kinetic parameters that regulate such processes were elucidated. Importantly, the polydispersity of αB-crystallin can be explained simply by the association and dissociation of monomers, a process that is independent of the size of the oligomer. The oligomeric distribution of αB-crystallin is an interplay between intra-dimer and inter-dimer interactions. Destabilisation of the dimer interface occurs at low pH and upon phosphorylation of αB-crystallin at various sites in its N-terminal domain. This is compensated for by stabilisation of inter-dimer interactions, which leads to overall conservation of the free energy of the system. The functional role of the extensive phosphorylation of αB-crystallin (and other sHsps) *in vivo* has provided much conjecture, the effects of which modify αB-crystallin chaperone ability.[12] Thus, the interplay between intra-dimer and inter-dimer interactions may be the regulator of chaperone action rather than dissociation of the dimer from the oligomer, as had been implied previously.[2,8]

The second paper[11] describes the use of a variety of solution-state NMR spectroscopic techniques (e.g., methyl-transverse relaxation optimised spectroscopy, pulsed field gradient NMR, covalent addition of paramagnetic probes and relaxation dispersion measurements) to examine the role of the C-terminal region of αB-crystallin in regulating inter-subunit interactions. It was concluded that each αB-crystallin monomer donates and receives one extended C-terminus during oligomeric formation with C-terminal fluctuations on a millisecond timescale regulating subunit exchange by monomers. During chaperone action, the fluctuations may

also regulate access to the target protein binding sites. The conserved IXI/V sequence (I159 to I161 in αB-crystallin) in the unstructured C-terminal region is dynamic and is not responsible for regulating inter-dimer interactions in contrast to the conclusions from solid-state NMR[6] and crystallographic[4] studies in which this region is immobilised by lying within a hydrophobic groove in an adjacent dimer. Thus, inter-dimer interactions primarily arise from other regions in αB-crystallin.

Finally, from the MS and NMR data, an energy profile was generated for αB-crystallin monomer dissociation from the oligomer. In a broader context, Baldwin *et al.*'s novel approach of using a combination of MS and NMR to generate detailed structural, thermodynamic and kinetic information about the αB-crystallin oligomer potentially can be applied to other heterogeneous and dynamic protein systems.

References

1. Van de Schootbrugge, C. & Boelens, W. C. (2010). Introduction to small heat shock proteins. In *Small Stress Proteins and Human Diseases* (Simon, S. & Arrigo, A. P., eds), Nova Science Publishers Inc., New York, NY.
2. Ecroyd, H. & Carver, J. A. (2009). Crystallin proteins and amyloid fibrils. *Cell. Mol. Life Sci.* **66**, 62–81.
3. Bagnéris, C., Bateman, O. A., Naylor, C. E., Cronin, N., Boelens, W. C., Keep, N. H. & Slingsby, C. (2009). Crystal structures of α-crystallin domain dimers of αB-crystallin and Hsp20. *J. Mol. Biol.* **392**, 1242–1252.
4. Laganowsky, A., Benesch, J. L., Landau, M., Ding, L., Sawaya, M. R., Cascio, D. *et al.* (2010). Crystal structures of truncated alphaA and alphaB crystallins reveal structural mechanisms of polydispersity important for eye lens function. *Protein Sci.* **19**, 1031–1043.
5. Carver, J. A., Aquilina, J. A., Truscott, R. J. W. & Ralston, G. B. (1992). Identification by ^{1}H NMR spectroscopy of flexible C-terminal extensions in bovine lens α-crystallin. *FEBS Lett.* **311**, 143–149.
6. Jehle, S., Rajagopal, P., Bardiaux, B., Makovic, S., Kuhne, R., Stout, J. R. *et al.* (2010). Solid-state NMR and SAXS studies provide a structural basis for the activation of αB-crystallin oligomers. *Nat. Struct. Mol. Biol.* **17**, 1037–1042.
7. Jehle, S., Vollmar, B. S., Bardiaux, B., Dove, K. K., Rajagopal, P., Gonen, T. *et al.* (2011). N-terminal domain of αB-crystallin provides a conformational switch for multimerization and structural heterogeneity. *Proc. Natl Acad. Sci. USA*, **108**, 6409–6414.
8. Bova, M. P., Ding, L. L., Horwitz, J. & Fung, B. K. K. (1997). Subunit exchange of αA-crystallin. *J. Biol. Chem.* **272**, 29511–29517.
9. Simon, S. & Arrigo, A. P. (Eds.). (2010). *Small Stress Proteins and Human Diseases*. Nova Science Publishers Inc., New York, NY.
10. Baldwin, A. J., Lioe, H., Robinson, C. V., Kay, L. E. & Benesch, J. L. P. (2011). αB-crystallin polydispersity is a consequence of unbiased quaternary dynamics. *J. Mol. Biol.* this issue. doi:10.1016/j.jmb.2011.07.016.
11. Baldwin, A. J., Hilton, G. R., Lioe, H., Bagnéris, C., Benesch, J. L. P. & Kay, L. E. (2011). Quaternary dynamics of αB-crystallin as a direct consequence of localised tertiary fluctuations in the C-terminus. *J. Mol. Biol.* this issue. doi:10.1016/j.jmb.2011.07.017.
12. Ecroyd, H., Meehan, S., Horwitz, J., Aquilina, J. A., Benesch, J. L. P., Robinson, C. V. *et al.* (2007). Mimicking phosphorylation of αB-crystallin affects its chaperone activity. *Biochem. J.* **401**, 129–141.

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