m06.p04

New insights into the quaternary structure of small Heat Shock Proteins under stress investigated by Small Angle X-ray Scattering

S. Finet[1], F. Skouri-Panet[2], S. Quévillon-Chéruel[3], M. Michiel[4] and A. Tardieu[4]

[1]ESRF-ID2, 6 rue Horowitz, 38043 Grenoble-France [2]IMPMC, 140 rue de Lourmel, 75015 Paris-France [3]IBBMC, 430 Université Paris sud, 91405 Orsay-France [4]PBSF, 7 Quai Saint Bernard, 75252 Paris-France

Keywords: SAXS, structure-function relationships, alpha-crystallins, HSP26

Small heat shock proteins (sHSPs) are found in most organisms (archea, bacteria, plants, animals) where they are induced upon stress. They function as molecular chaperones since they are able to prevent thermal and chemical-induced aggregation of a variety of substrates both in vitro and in vivo. A typical feature of sHSPs is the formation of large oligomeric complexes: 12 - 40 subunits of 12 - 43kDa. The sHSPs possess the original property to rapidly exchange their subunits while keeping the same average number of subunits. Moreover, if the environment is modified in vitro (pH, temperature...), they can change their size and oligomeric state through subunit exchange. In the chaperone-like function, sHSPs have been shown to incorporate the non-native proteins into large sHSP-substrate complexes, thus preventing their non-specific aggregation. In vivo, subunit exchange controls the chaperone properties, often associated with changes of oligomeric state. Despite a number of efforts, little is known on the structural features that regulate the sHSP oligomeric size and chaperone properties. So far, only three 3D structures are known [1,2,3]. In that context, Small Angle X-ray Scattering (SAXS) was used to characterize the temperature and pressure induced structural transitions of native calf lens alpha-crystallins, recombinant human alphaB-crystallins, and of monodisperse yeast HSP26 [4]. The alphaN-crystallins and the alphaB-crystallins were known to increase in size (doubling in size) with increasing temperature whereas HSP26 partially dissociates into dimers. These temperature-induced transitions were irreversible. Similar transitions, yet reversible, could be recently induced with pressure up to 300 MPa for the crystallins and 160 MPa for the HSP26. The diamond windows absorption of the pressure cell was overcame by the high brilliance and the energy tenability of ID2 beamline. SAXS curves recorded during pressure scans with monodisperse 24-mer HSP26 revealed a reversible dissociation of the 24-mer into dimers. For example, the recent analysis of the SAXS data provides new insights into the low-resolution structure of *Yeast* HSP26.

[1] R. Kim, K.K. Kim, H. Yokota, S.H. Kim, Small heat shock protein of *Methanococcus jannaschii*, a hyperthermophile, Proc Natl Acad Sci U S A (1998) 95, 9129-33.

[2] R.L. van Montfort, E. Basha, K.L. Friedrich, C. Slingsby, E. Vierling, Crystal structure and assembly of an eukaryotic small heat shock protein, Nat Struct Biol (2001) 8, 1025-30.

[3] R. Stamler, G. Kappe, W. Boelens, C. Slingsby, Wrapping the alpha-Crystallin Domain Fold in a Chaperone Assembly, J Mol Biol (2005) 353, 68-79.

[4] F. Skouri-Panet, S. Quevillon-Cheruel, M. Michiel, A. Tardieu & S. Finet, sHSPs under temperature and pressure: the opposite behaviour of lens alpha-cristallins and *Yeast* HSP26, Biochim. Biophys. Acta (In press).

m06.p05

High-salt Peridinin-Chlorophyll-Protein from *A.carterae*: The Structure of the Monomeric Antenna Protein Complex

E. Hofmann[a], T. Schulte[a], S.P. Sharples[b], R.G. Hiller[b]

[a] Proteinkristallographie, LS Biophysik, Ruhr-Universität Bochum, ND04/316, 44780 Bochum, Germany [b] School of Biochemical Sciences, Maquarie University, NSW 2109, Australia

Keywords: protein X-ray crystallography, protein complex, photosynthetic proteins

Peridinin-chlorophyll *a*-proteins (PCPs) allow dinoflagellates (a form of eucaryotic algae) to efficiently use carotenoids for light harvesting [1,2]. Up to 25% of the molecular mass of these complexes is contributed by bound pigments. So far the structure of the main form of PCP (MFPCP) from *A.carterae* has been solved at a resolution of 2Å [3]. The clustering of the pigment molecules inside the protein cage is the basis of the very efficient energy transfer between the carotenoids and chlorophyll. To further test and refine the resulting theoretical models for the underlying processes we have to compare the pigment arrangement within different types of PCPs with the concurrent changes in spectroscopic. Therefore we try to solve the structures of several different forms of PCPs. Here we present the 2.1Å X-ray structure of the high-salt peridinin-chlorophyll a-protein (HSPCP) from *A.carterae*, solved by MR using a model based on the M-monomer of MFPCP (PDB: 2C9E, structure on hold). The protein structure of HSPCP shows high homology (SSM superimpose: RMSD Ca-Ca of 1,89 Å) to the main form. Major conformational differences between both are in regions involved in trimerization in the MFPCP structure. In the main form two additional peridinins are probably not directly involved in energy transfer to chlorophyll [4], but play a structural role in trimerization. Therefore we propose that HSPCP is a monomeric protein. The pigment arrangement will be discussed with respect to spectroscopic results.

[1] Sharples FP, Wrench PM, Ou K, Hiller RG. (1996). Two distinct forms of the peridinin-chlorophyll a-protein from Amphidinium carterae; Biochim Biophys Acta 1276, 117-23.

[2] R.G.Hiller, L.G.Crossley, P.M.Wrench, N.Santucci, E.Hofmann (2001). The 15kDa forms of the apo-peridinin-chlorophyll a protein (PCP) show high identity with the apo-32kDa PCP forms, and have similar N-terminal leaders and gene arrangements; Mol.Genet.Genomics 266, 254-259.

[3] E.Hofmann, P.M.Wrench, F.P.Sharples, R.G.Hiller, W.Welte, K.Diederichs (1996). Structural Basis of Light Harvesting by Carotenoids: Peridinin-Chlorophyll-Protein from Amphidinium carterae; Science 272, 1788-1791.

[4] Damjanovic, A.; Ritz, T. & Schulten, K. (2000). Excitation transfer in the peridinin-chlorophyll-protein of Amphidinium carterae; Biophys J,, 79, 1695-705.