Abstract

Heat shock cognate protein 70 (HSC70) is a 71 kDa chaperone protein belonging to the ubiquitous family of heat shock proteins 70 (Hsp70). The representatives of this protein family are considered as molecular machines with ATP-hydrolase activity facilitating correct folding of spatial protein structure, both in normal and stressful conditions (hypoxia, heat shock, pH fluctuations etc.) In addition, HSC70 was identified as an uncoating enzyme for triskelion meshwork on the surface of clathrin-coated vesicles. Among other roles, HSC70 prevents protein aggregation and assists the polypeptide maturation, it facilitates the protein transport into organelles, such as endoplasmic reticulum and mitochondria. It is involved in targeting proteins for lysosomal degradation and in many other dramatically important cellular processes related to protein homeostasis. Therefore, the regulation of HSC70 and other HSP70 proteins is believed to be dramatically important, especially in a context of cellular stress. Based on the experimental observation, the mechanism of inactivation through oligomerization was hypothesized. The dimer and trimer species of Hsp70 proteins were identified both in case of prokaryotic and eukaryotic homologs. It was also speculated that Hsp40 cofactors promote oligomerization to even higher-order oligomers. This and other possible oligomerization models of wild type HSC70 and the certain subset of HSC70 mutants were investigated by cross-linking mass spectrometry. The distance constraints between certain amino acid residues imposed by the cross-linker length allowed us to build structural models of Hsc70 oligomeric species. To decipher inter/intra molecular restraints and allow the precise mapping of identified cross-links, the studied proteins were produced and analyzed in a mixture containing ^{14}N- and ^{15}N-labeled form.

Keywords: Structural biology, Mass spectrometry, Protein structure, Protein-protein interaction, Allostery, Chaperones, Heat shock proteins, HSC70