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Streszczenie rozprawy doktorskie w języku angielskim: **The mechanism of chaperone-dependent disaggregation of complexes comprising small heat shock proteins and their substrates**

Small heat shock proteins (sHsps) are an evolutionary conserved class of ATP-independent molecular chaperones. sHsps efficiently prevent protein aggregation in the cell. Several modes of their chaperone action have been elucidated that contribute to maintaining protein homeostasis both under permissive and stress conditions. Under heat stress conditions sHsps form complexes with misfolded proteins, preventing them from further aggregation and rendering them in a refoldable state. Subsequent solubilisation and refolding of substrates from these complexes by ATP-dependent Hsp70 and Hsp100 chaperones is much more efficient compared to aggregates formed in the absence of sHsps. To extract and refold substrates from a sHsp-substrate complex the initially beneficial sHsp-substrate interaction has to be disrupted. How this Janus-faced sHsp feature is circumvented has not been addressed so far.

This doctoral dissertation provides insights into the role of Hsp70/Hsp100 bichaperone system in solubilisation and refolding of substrates from the sHsp-substrate complexes. Collectively, experiments presented here unravel a novel, conserved function of Hsp70, which displaces surface-bound sHsp molecules during the initial phase of the reactivation process. Hsp70 acts in a passive manner by outcompeting sHsp molecules that dynamically interact with the surface of sHsp-substrate complexes. Hsp70 binding preserves the architecture of complexes following dissociation of sHsps, prevents further association and aggregation, and allows for superior substrate solubilisation upon Hsp100 recruitment.

The process of solubilisation and refolding of substrates from the complexes with sHsps has been studied here in detail. A complete set of experiments was performed using *Escherichia coli* chaperones; Luciferase was used as the main substrate for sHsps and/or Hsp70/Hsp100. To generalize the findings the crucial experiments were repeated using Malate Dehydrogenase (MDH) or Green Fluorescent Protein (GFP) as alternative substrates; to show that the phenomenon described here is evolutionary conserved

Saccharomyces cerevisiae sHsp, Hsp70 and Hsp100 molecular chaperones were used. Additionally, *in vivo* experiments fully support the described *in vitro* mechanism. Not only mechanistic insights into the efficient disaggregation and refolding of substrates complexed to sHsps are revealed, a novel and conserved Hsp70 activity in displacing surface-exposed sHsps from the complex is unravelled, but also information on the structure and organization of the sHsp-substrate complex is provided. The above findings expand the knowledge of sHsp chaperone action and place them in a broader context of the sHsp/Hsp70/Hsp100 chaperone triad, the most efficient system in preventing aggregation and disaggregating proteins that exists.