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Tytuł rozprawy: Analiza struktury kompleksów tworzonych przez małe białka szoku cieplnego IbpA i IbpB oraz kompleksów tych białek z substratami

Tytuł w języku angielskim: Structural analysis of complexes formed by small heat shock proteins IbpA and IbpB and complexes formed by these proteins with substrates

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ABSTRACT

Maintaining protein homeostasis is crucial for cell survival. Chaperone proteins are the main players in this process. One of the families of these proteins are small heat shock proteins (sHSPs), which are rather atypical chaperones, as they act strictly passively. sHSPs are believed to bind to the unfolded substrate polypeptides, inhibit aggregation or change aggregate properties, thus stimulating further substrate refolding. They have very remarkable quaternary structure. The low molecular weight monomers (usually of mass between 12 and 25 kDa) assemble in a large oligomers, up to MDa size range, often possessing multi-level internal architecture with sHSP dimer as the most basic subunit. Monomer structure is highly conserved, all sHSPs contains an α-crystallin domain, named from archetypical protein of this family - eye lens crystallin and two less structured N- and C-terminal tails of variable length. Almost all chaperones from sHSP family form oligomers, but the exact oligomerization pattern is highly variable. The relationship between oligomerization and chaperone activity is still unknown.

In this work I have focused on the analysis of two interacting small heat shock proteins from *Escherichia coli* - IbpA and IbpB. My goal was to analyze the morphology and structure of their oligomers, define the building block of the oligomer, and try to elucidate the architecture of sHSP-substrate complexes. I have analyzed the morphology of these proteins using dynamic light scattering and atomic force microscopy. All possible oligomers, IbpA, IbpB, and IbpA/IbpB mixed oligomer are large, probably fibrillar structures. To define the building block of the oligomer I have used isolated α-crystallin domains to reduce the complexity arising from oligomerization. Once I defined the preferred building block as IbpA/IbpB heterodimer, I analyzed IbpA/IbpB oligomer formed by these heterodimers as a form which is closest to native protein structure. I have used a genetically-encoded crosslinker and mass spectrometry to analyze the oligomer structure in more detail. Obtained data allowed to propose the structure of tetramer formed by two IbpA/IbpB dimers. Finally, I have used these crosslinking variants to analyze the sHSP-substrare coaggregate structure. Results of these analyses suggest that IbpA/IbpB complex de-oligomerize and interacts as free dimers with their substrate proteins, however, this scenario requires further investigations.

In conclusion, the most important result of this work is defining the building block of IbpA/IbpB oligomer as heterodimer. Furthermore, I have managed to propose structural model of higher order oligomer subunit and the overall oligomer morphology. These results can be further applied to perform more directed experiments, which enable to elucidate the *in vivo* structure of IbpA and IbpB and structures formed by these proteins inside sHSP-substrate coaggregates.