Cryo-EM Studies of the Chloroplast Small Heat Shock Protein Hsp21

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Environmental stresses often lead to protein unfolding and the formation of cytotoxic aggregates that compromise cell survival. In the cell, proper folding of proteins is constantly monitored and maintained by the protein quality control system. One essential component of protein quality control is molecular chaperones that stabilize non-native protein conformations and facilitate the refolding of proteins. The small heat shock proteins (sHsps) are ubiquitous and diverse family of molecular chaperones present in all living organisms. While they bind to and sequester misfolding proteins to prevent their aggregation, they themselves do not promote substrate refolding, distinguishing them from other major chaperone families.

How do sHsps recognize a wide variety of unfolding substrate proteins? Currently, little is known about the structural organization of the sHsp-substrate complexes due to their inherent heterogeneity and lack of high-resolution information. This proposed work focuses on the chloroplastic Hsp21, a member of the organelle-localized sHsps unique to plants. In preliminary analysis we identified a natural substrate of Hsp21, deoxyxylulose 5-phosphate synthase, which catalyzes the synthesis of precursors to metabolites that function in photosynthesis, growth regulation and plant-environment interaction. To obtain insights into the regulation and determinants of substrate binding and specificity of Hsp21, we will use cryo-EM combined with mutagenesis to study the Hsp21 structure and its interaction with deoxyxylulose 5-phosphate synthase. The proposed study herein will improve our understanding of the molecular mechanism of protein aggregation suppression by sHsps. Because overexpression of Hsp21 in transgenic plants enhances tolerance towards heat stress, the outcome of this research will offer potential applications for agricultural biotechnology to develop stress-resistant crops.

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