Title

Properties of Potential Substrates of a Cyanobacterial Small Heat Shock Protein

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Abstract

Most proteins must fold into native three-dimensional structures to be functional. But, newly synthesized proteins are at high risk of misfolding and aggregating in the cell. Stress, disease or mutations can also cause protein aggregation. A cyanobacterial small heat shock protein, Hsp16.6, can act as a chaperone to prevent irreversible protein aggregation during heat stress. This thesis is focused on the properties of proteins that were associated with Hsp16.6 during heat stress, and which therefore may be “substrates” of Hsp16.6. Bioinformatics were used to determine if Hsp16.6 preferentially binds to proteins with certain properties, and biochemical studies were performed to investigate how the substrates actually behave with Hsp16.6 during heat stress. It was found that Hsp16.6 preferentially binds to proteins with higher molecular weight, higher acidity, higher percentage of charged residues (especially negatively charged residues), and a lower percentage of hydrophobic residues compared to all proteins encoded by the *Synechocystis* genome. Proteins bound to Hsp16.6 were also slightly enriched in VQL motifs. The potential substrate fructose bisphosphate aldolase class II (FBA) was expressed in *E.coli* and purified. FBA could be protected by Hsp16.6 from aggregation through forming a complex with Hsp16.6 during heat stress in vitro, consistent with it being a substrate of Hsp16.6. Another potential substrate, elongation factor G1 (EF-G1) was also expressed in *E.coli* and purified. EF-G1 did not form insoluble aggregates even at 47°C, but circular dichroism spectroscopy revealed the secondary structure has melted at this temperature, and the protein eluted earlier than unheated protein on size exclusion chromatography. Thus, EF-G1 appears heat sensitive, and may also be an in vivo substrate of Hsp16.6. Lastly, in vivo study studies were performed to determine the amount of FBA and EF-G1 in *Synechocystis* cells. Both proteins are abundant, with FBA levels (around 2% of total cell protein) being about twice that of EF-G1. Further in vivo experiments will be needed to confirm that FBA and EF-G1 are substrates of Hsp16.6.

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