Biochemical insights into the Cyanobacterial Clp protein family of chaperones and proteases

Abstract

Proteins are needed for all forms of life and obviously perform a range of essential biochemical tasks within each organism. Living cells are, hence, under constant pressure to maintain an optimal protein environment, assuring functionally important polypeptides are correctly folded. This is accentuated during adverse growth conditions such as heat- or cold stress. During such conditions, proteins become destabilized and unfolded. Unfolded proteins are "sticky" and have a propensity for binding to each other and forming cytotoxic aggregates. To better cope with this, cells have evolved a quality control system of molecular chaperones and proteases. Molecular chaperones bind to non-native proteins and aid in their folding/refolding as well as prevent their aggregation. Proteases, on the other hand, bind to and degrade polypeptides that are beyond repair. One important group of molecular chaperones and proteases is the Clp protein family present throughout nature. The model Clp protease from the Gram-negative bacterium *Escherichia coli* consists of a double heptameric ring of the ClpP peptidase, harboring the proteolytic chamber, flanked on either end by a hexamer of the Hsp100 proteins ClpA or ClpX. The Hsp100 proteins bind and unfold the globular substrate protein and then translocate it into the ClpP complex for degradation. In comparison, the complexity of Clp proteins in photosynthetic organisms, such as plants and cyanobacteria, is far greater. This is highlighted in the cyanobacterium *Synechococcus elongatus* that encodes 10 different Clp proteins. These include several Hsp100 proteins (ClpC, ClpX, and ClpB1-B2), three ClpP paralogs (ClpP1-P3), one ClpP-like protein named ClpR and two small adaptor proteins (ClpS1-S2). To better understand the reason why many Clp paralogs exist within *Synechococcus* and determine how these distinct proteins interact and function, we purified recombinant cyanobacterial Clp proteins using an *E. coli* expression system. **Paper I** describes the first *in vitro* biochemical characterization of the two ClpB proteins in *Synechococcus*. Besides differing in their intracellular location, ClpB1 and –B2 also varied in their ATPase activity and refolding activity with a cognate Hsp70/40-system, likely mirroring their different biological roles *in vivo*. Yet another cyanobacterial Hsp100 protein, ClpC, was analyzed in **Paper II**. ClpC is an essential protein in *Synechococcus* and is homologous to *E. coli* ClpA. Cyanobacterial ClpC was proven a bonafide molecular chaperone, displaying both refolding activity and the ability to prevent unfolded proteins from aggregating. Additionally, ClpC was shown to interact with the adaptor protein ClpS1. **Paper III** revealed the existence of at least two distinct ATP-dependent Clp proteases in *Synechococcus*, both with heterogeneous proteolytic cores: ClpCP3/R and ClpXP1/P2. In **Paper IV** the ClpCP3/R protease was reconstituted *in vitro* and analyzed in detail. The ClpP3/R proteins form a stable tetradecamer proteolytic core, with each heptameric ring consisting of three ClpP3 and four ClpR subunits in a specific arrangement. Furthermore, the ClpR subunits were shown to be proteolytically inactive, a factor that likely contributed to the relatively slow activity of the ClpCP3/R protease compared to that of *E. coli* ClpAP. Overall, this thesis presents several intriguing revelations about photosynthetic Clp proteins - opening up many future research possibilities!