GÖTEBORGS UNIVERSITET

Structural and Functional Studies of the ATP-dependent Clp Proteases in Cyanobacteria

Frida M Ståhlberg

Institutionen för biologi och miljövetenskap
Naturvetenskapliga fakulteten

Akademisk avhandling för filosofie doktorsexamen i Naturvetenskap med inriktning Biologi, som med tillstånd från Naturvetenskapliga fakulteten kommer att offentligt försvaras fredag den 26 September 2013 kl. 10.00 i Hörsalen, Institutionen för biologi och miljövetenskap, Carl Skottsbergs gata 22B, Göteborg.

ISBN 978-91-85529-68-1

Structural and Functional Studies of the

ATP-dependent Clp Proteases in Cyanobacteria

Frida M Ståhlberg

Göteborg University, Department of Biological and Environmental Sciences
Box 461, SE-405 30 Göteborg, Sweden

ABSTRACT

Proteins are essential in all living organisms and they are involved in a myriad of biological functions. It is vital for cells to have efficient surveillance and quality control systems that ensure damaged proteins are either repaired to their functional state or quickly removed by degradation. Two crucial components of these protein quality systems are molecular chaperones and proteases, of which one major contributor is the AAA+ (ATPases Associated with diverse cellular Activities) family that includes the Clp proteases.

The Clp protease exists in many forms of life, ranging from eubacteria to mammals. In the bacterium *E. coli*, the hexameric ATPases ClpX and ClpA recognize the substrate and once unfolded translocate it into the proteolytic core, which is formed by two heptameric rings of ClpP. The complexity of Clp proteases in terms of both composition and functionality is far greater in photosynthetic organisms compared with their bacterial counterparts. This is highlighted in the cyanobacterium *Synechococcus elongatus* (*Synechococcus*), which has at least two Clp proteases, the essential ClpCP3/R and the non-essential ClpXP1/P2. Of these various Clp proteins, the ClpR subunit is unique to photosynthetic organisms and is proteolytically inactive, while the existence of two ClpS adaptors (ClpS1 and ClpS2) is also unique for cyanobacteria. This thesis focuses on the continued characterization of these Clp proteins in *Synechococcus*.

In paper I, two conserved motifs in the ClpR and one motif in the ClpP3 N-terminus were identified as being crucial for association to ClpC. It was also shown that these motifs were important for the stability of the ClpP3/R complex. A C-terminal motif in ClpC (the R-domain) was also demonstrated as conferring the specificity for ClpP3/R association. In paper II, the subunit stoichiometry of the ClpP1/P2 proteolytic core was determined by non-denaturing mass spectrometry. The proteolytic core was composed of an equal amount of ClpP1 and ClpP2 subunits arranged in an alternating pattern within each heptameric ring. The two double heptameric rings had dual stoichiometry, where two different proteolytic cores could be formed, (4ClpP1+3ClpP2) + (3ClpP1+4ClpP2) and 2×(3ClpP1+4ClpP2). In paper III, the functionality of the ClpP1/P2 protease was further characterized *in vitro*. ClpP1/P2 displayed the expected proteolytic activity with ClpX, but no activity was observed with ClpC. Both types of ClpP subunit appear to contribute to the proteolytic activity of the ClpP1/P2 core, but the arrangement of these two ClpP paralogs somehow limits the overall degradation rate. It was also revealed that ClpP2 alone could not assemble into higher molecular mass complexes, whereas ClpP1 readily formed a homo-tetradecamer that was proteolytically active with both ClpC and ClpX but whose activity was dependent on increased Mg^{2+} concentrations. In paper IV, the cyanobacterial-specific ClpS2 adaptor was shown to be a relatively low-abundant, soluble protein that is essential for phototrophic growth. Like ClpS1, ClpS2 redirects the general substrate specificity of ClpC to N-end rule substrates, but the two adaptors differ in exactly which N-end rule substrates they target. ClpS1 recognizes Phe and Tyr as destabilizing amino acids, while ClpS2 recognizes Leu. In the final paper (paper V), the Δ*clpS*1 and Δ*clpP2* mutants are shown to have greater resistance to exogenously added H_2O_2, while Δ*clpP1* was extremely sensitive. The different phenotypes of these mutants were dependent on the level of the catalase peroxidase KatG, where elevated basal expression of the *katG* gene was responsible for the resistance observed in Δ*clpS1* and Δ*clpP2*. In contrast, increased proteolysis of the KatG protein in Δ*clpP1* caused the extreme sensitivity of this mutant to the oxidative stress.

ISBN 978-91-85529-68-1