GÖTEBORGS UNIVERSITET

Biochemical Studies of the Essential Clp protease in Cyanobacteria and its Associated Adaptor Proteins

Anders Tryggvesson

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 24:e oktober 2014 kl. 10.00 i Hörsalen, Institutionen för biologi och miljövetenskap, Carl Skottbergs gata 22B, Göteborg.

ISBN: 978-91-85529-70-4

Biochemical Studies of the Essential Clp protease in Cyanobacteria and its Associated Adaptor Proteins

Anders Tryggvesson

Göteborgs universitet. Institutionen för Biologi och Miljövetenskap.

Box 461, SE-405 30 Göteborg

Abstract

Proteins are an essential part of all organisms and are involved in many cellular processes. To regulate the function of proteins and facilitate their removal when damaged or otherwise compromised, sophisticated control systems have evolved that include molecular chaperones and proteases. These regulatory proteins attempt to repair damaged polypeptides and if necessary degrade them before they can interfere with cellular activities. Clp/Hsp100 proteins are a family of chaperones that belong to the broader family of AAA+ proteins (ATPases associated with diverse cellular activities) that are present in a wide range of organisms. Many of these AAA+ Clp proteins function as the chaperone partner within Clp proteases, conferring substrate specificity and transferring the unfolded protein substrate to the proteolytic component for degradation. The Clp chaperones form single hexameric rings that associate to a proteolytic complex consisting of two opposing heptameric rings comprised typically of a single type of subunit, ClpP. The catalytic sites of these ClpP subunits are sequestered within the tetradecamer to avoid inadvertent protein degradation.

The Clp protease in *E. coli* is the best studied Clp proteases to date, with two distinct types depending on if the chaperone partner is ClpA or ClpX. Also present are adaptor proteins that modify the substrate specificity of the chaperone component, such as ClpS that redirects the ClpAP protease to degrade N-end rule substrates. Although Clp proteins are found in a wide range of organisms, those in photosynthetic organisms such as cyanobacteria and vascular plants are by far the most numerous and diverse.

In the cyanobacterium *Synechococcus elongatus* (*Synechococcus*) two types of mixed Clp proteolytic cores exist; ClpP3/R and ClpP1/P2. The ClpP3/R core associates to the chaperone ClpC to produce a protease that is essential for phototrophic growth, whereas ClpP1/P2 binds to ClpX to form a second Clp protease whose activity is non-essential. This thesis work has examined the structure and function of the mixed proteolytic core within the essential ClpCP3/R protease and its associated adapter proteins ClpS1 and ClpS2. This has been done using molecular and biochemical methods to purify recombinant versions of each Clp protein or complex and analyzing them *in vitro*. In **Paper I**, the ClpP3/R complex was over-expressed in *E. coli* and purified by column chromatography. The proteolytic core was shown to consist of two identical heptameric rings, each with three ClpP3 and four ClpR subunits in an alternating configuration. The ClpR subunit is catalytically inactive but its inclusion within the ClpP3/R core did not appear rate-limiting for the activity of the ClpCP3/R protease. The general architecture of ClpP3/R mirrored that of the proteolytic core within the eukaryotic 26S proteasome, with three active and four inactive subunits in the central heptameric rings. A model of ClpP3/R was also presented in this paper, along with the finding that the ClpS1 adaptor protein binds to ClpC and modifies its substrate specificity.

In **Paper II**, two N-terminal regions in ClpR (the Tyr- and Pro motifs) and one in ClpP3 (the MPIG motif) were shown to be important for the interaction with ClpC and correct assembly of the ClpP3/R tetradecamer. We also identified a motif in the C-terminal region of ClpC (the R-motif) that confers the specific association to the ClpP3/R core.

In **Paper III**, we investigated the essential adaptor protein ClpS2 that is so far unique to cyanobacteria. A recombinant version of ClpS2 was purified and its activity compared to that of ClpS1. Like ClpS1, ClpS2 binds to ClpC and alters its substrate specificity. However, ClpS1 and ClpS2 recognize different destabilizing residues and thus target different N-end rule substrates for degradation by the ClpCP3/R protease. Overall, this thesis provides new insights into the structure and function of the essential ClpCP3/R protease in cyanobacteria and how its substrate specificity is modified by the ClpS adaptor proteins.

ISBN: 978-91-85529-70-4