Structural and functional characteristics of the cyanobacterial and chloroplastic Clp proteins

Tara M. Stanne

Department of Plant and Environmental Sciences
Gothenburg University
2007

Abstract

The protein environment within cells is very dynamic. Proteins are continually being synthesized and degraded in response to a variety of developmental and physiological needs. Molecular chaperones and proteases are essential components of this process. Chaperones are involved in helping to stabilize newly synthesized proteins, in protein folding/unfolding and in the assembly/disassembly of complexes. Proteases, on the other hand, degrade proteins that are no longer necessary or are damaged beyond repair. A well defined energy-dependent protease is the Clp protease from *E. coli*. It is a two-component enzyme that relies on the unfolding activity of a hexameric ATP-dependent Hsp100 molecular chaperone ring, either ClpA or ClpX, to 'feed' substrate proteins into a central proteolytic chamber, composed of two face-to-face heptameric rings of ClpP for degradation. This family of proteins is not limited to eubacteria and Clp proteins are also found in mammals, plants and certain protista.

Members of the Clp protein family are by far more diverse and numerous in photosynthetic organisms. Cyanobacteria have three ClpP paralogs as well as a ClpP-variant (ClpR) which is catalytically inactive. By studying the Clp complexes formed *in vivo* we have revealed the presence of two distinct soluble Clp proteases, each containing a unique core comprised of two separate Clp subunits: one with ClpP1 and ClpP2, the other with ClpP3 and ClpR. Each core also associates to a hexamer of a particular Hsp100 chaperone partner- ClpX for the ClpP1/P2 core and ClpC in the case of the ClpP3/R core. In addition we provide evidence suggesting the existence of a third Clp protease, which is associated to the internal membrane network (**Paper I**). Altogether, we show the presence of several distinctive Clp proteases in cyanobacteria, a feature which contrasts from that in most other organisms.

In chloroplasts of higher plants the Clp protein family is even more complex, with five ClpP (ClpP1, ClpP3-6) and four ClpR (ClpR1-4) paralogs, in addition to three Hsp100 molecular chaperone partners (ClpC1, -C2, -D) as putative regulatory subunits of an active chloroplast Clp protease, and two plant-specific Clp proteins renamed here as ClpT1 and ClpT2. Unlike cyanobacteria, only one Clp proteolytic core exists despite the numerous paralogs present, and is composed of all nine ClpP/R subunits in addition to ClpT1-2. We have used genetically modified *Arabidopsis thaliana* plants with reduced levels of, or totally lacking specific Clp proteins to study the structure and function of Clp chaperones and proteases in chloroplasts. The transgenic lines we characterized include a mutant for the Hsp100 ClpC1 protein (**Paper II**), antisense repression lines for ClpP4 and ClpP6 (**Paper III** and **V**, respectively), and a mutant lacking all ClpR1 protein (**Paper IV** and **VI**). Each of these transgenic plants exhibit a slow-growing, chlorotic phenotype with impaired photosynthesis. These studies have revealed that the Clp protease is required for early chloroplast development and differentitation. Additionally we have identified two distinct sub-complexes of the Clp proteolytic core, presumably corresponding to single heptameric rings: one composed of ClpP1 and ClpR1-4, the other of ClpP3-6, with ClpT1 associating to the latter subcomplex (**Paper V**). A specific stoichiometry exists and altering this by even one subunit is sufficient to destabilize the entire complex (**Paper V** and **VI**). Additionally we have identified 25 putative native substrates for the chloroplastic Clp protease in higher plants (**Paper V** and **VI**) - a first!

Key words: *Arabidopsis*, chloroplast, Clp protease, cyanobacteria, Hsp100, molecular chaperones

Gothenburg, April 2007