Dissertation Abstract

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Dissertation title	**pH-regulated chaperone function of cyanobacterial Hsp90, Hsp70, and Hsp60: Implications for light/dark regulation** (シアノバクテリアの Hsp90，Hsp70 及び Hsp60 のシャペロン機能に及ぼす pH の影響－細胞における明暗調節との関連－)		

Maintenance of a constant internal environment is necessary and a fundamental feature of all living systems. Molecular chaperones which are highly conserved proteins play a key role in the maintenance of (protein) homeostasis in cells.

Cyanobacteria are oxygenic photosynthetic bacteria. Proton pumping into the thylakoid lumen which is coupled to photosynthetic electron transport results in alkalization of cytosol in cyanobacteria. Because of the pumping, pH in the cyanobacterial cytosol and chloroplast stroma increases by one pH unit following a shift from darkness to light. Genes associated with photosynthetic CO_2 fixation and various molecular chaperones are upregulated upon the shift. We postulated that expression and function of molecular chaperones are regulated in a synchronized manner with those of proteins/enzymes involved in photosynthetic CO_2 fixation. In the present study, the effect of pH on the chaperone function of major molecular chaperones such as Hsp90 (HtpG), Hsp70 (DnaK2), Hsp60 (GroEL1 and GroEL2) from the cyanobacterium *Synechococcus elongatus* PCC7942 are examined.

HtpG suppressed aggregation of various heat-denatured proteins, especially lactate dehydrogenase, at an equimolar ratio of HtpG to protein substrate in a pH-dependent manner. HtpG showed the highest activity at pH 8.5 over the examined pH range from 7.0 to 8.5. pH affected the anti-aggregation activity of DnaK2 in a similar manner to that of HtpG in the presence of half equimolar DnaK2 to protein substrate. The ATPase activity of HtpG and DnaK2 was pH-dependent, with a three- to four-fold increase in activity when the pH was raised from 7.0 to 8.5. An increase in pH from 7.0 to 8.5 enhanced activities of both HtpG and DnaK2 in protein-folding assistance by two- to three-fold.

In contrast to *E. coli*, most cyanobacterial species have two *groEL* genes (paralogs). Our group showed that cyanobacterial GroEL1 and GroEL2 are mutually distinct and different from *E. coli* GroEL. GroEL1 and GroEL2 also showed pH dependency in suppression of aggregation of heat-denatured substrates. They exhibited higher activity at more alkaline pHs (varied from 7.0 to 8.5). There was no significant influence of pH on the chaperone activity of GroEL1, GroEL2, and *E. coli* GroEL to promote refolding of heat-denatured malate dehydrogenase.

The present study suggests that the pH-change upon a shift from darkness to light modulates activity of major molecular chaperones in cyanobacteria. As described above, light induces expression of proteins involved in photosynthetic CO_2 fixation. In addition, it is known that light causes stresses, e.g. oxidative stress to photosynthetic organisms. Increase in the cellular level of molecular chaperones as well as activation of their chaperone activities by an increase in pH may be required to ‘chaperone’ newly synthesized proteins and also develop the tolerance and protection from cellular damage associated with the light stress.