The chloroplast lumen

- New insights into thiol redox regulation and functions of lumenal proteins

Michael Hall

Akademisk avhandling

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Fakultetsopponent: Professor Bob B. Buchanan,
Department of Plant and Microbial Biology, University of California, Berkeley, USA.

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Author
Michael Hall

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Abstract
In higher plants oxygenic photosynthesis primarily takes place in the chloroplasts of leaves. Within the chloroplasts is an intricate membrane system, the thylakoid membrane, which is the site of light harvesting and photosynthetic electron transport. Enclosed by this membrane is the lumen space, which initially was believed to only contain a few proteins, but now is known to house a distinct set of >50 proteins, many for which there is still no proposed function. The work presented in this thesis is focused on understanding the functions of the proteins in the lumen space. Using proteomic methods, we investigated first the regulation of lumenal proteins by light and secondly by dithiol-disulphide exchange, mediated by the disulphide reductase protein thioredoxin. We furthermore performed structural and functional studies of the lumenal pentapeptide repeat proteins and of the PsbP-domain protein PPD6. When studying the diurnal expression pattern of the lumen proteins, using difference gel electrophoresis, we observed an increased abundance of fifteen lumen protein in light-adapted *Arabidopsis thaliana* plants. Among these proteins were subunits of the oxygen evolving complex, plastocyanin and proteins of unknown function. In our analysis of putative lumenal targets of thioredoxin, we identified nineteen proteins, constituting more than 40 % of the lumen proteins observable by our methods. A subset of these putative target proteins were selected for further studies, including structure determination by x-ray crystallography. The crystal structure of the pentapeptide repeat protein TL15 was solved to 1.3 Å resolution and further biochemical characterization suggested that it may function as a novel type of redox regulated molecular chaperone in the lumen. PPD6, a member of the PsbP-family of proteins, which is unique in that it possesses a conserved disulphide bond not found in any other PsbP-family protein, was also expressed, purified and crystallized. A preliminary x-ray analysis suggests that PPD6 exists as a dimer in the crystalline state and binds zinc ions. The high representation of targets of thioredoxin among the lumen proteins, along with the characterization of the pentapeptide repeat protein family, implies that dithiol-disulphide exchange reactions play an important role in the thylakoid lumen of higher plants, regulating processes such as photoprotection, protein turnover and protein folding.

Keywords
photosynthesis, chloroplast, thylakoid lumen, proteomics, DIGE, thioredoxin, pentapeptide repeat protein, PsbP domain protein

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