

# Regulation of Electron Donation to Photosystem 1

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## Abstract

Photosynthesis is the process where energy from light is used to catalyze the formation of energy-rich molecules such as NADPH and ATP. These reactions are in fact the result of a long series of electron-transfer reactions where water molecules are the source of electrons and NADP<sup>+</sup> is the terminal electron acceptor. A consequence of the electron-transfer reactions is the translocation of protons across a phospholipid membrane. This proton gradient in turn drives ATP synthase to catalyze the formation of ATP from ADP. From this chain of events, molecular oxygen is a byproduct, leading to the oxygen-rich atmosphere we live in today.

In this thesis, the objective has been to study the specific protein-protein interaction between the electron-donor protein plastocyanin (Pc) and the photosystem 1 (PSI) subunit PsaF. The physiological regulation of this interaction has been a major subject and hence should be kept in mind to understand the purpose of the different papers and studies reported here.

In paper I we report on the cloning, expression and characterization of the luminal domain of spinach PsaF. Characterization by several different biophysical techniques revealed a protein domain which is very dynamic and consistent with molten-globule like structural features. A disulphide bridge formed between cysteine residues 8 and 63 appeared to have a major role in stabilizing the tertiary fold of this domain. Site-directed mutagenesis and zero-length cross-linking revealed a native-like interaction with Pc, strongly dependent on the electrostatic character of the two proteins.

The findings in paper I led us to investigate whether light-induced changes in the Mg(II) content in the chloroplast lumen can modulate the electron donation to PSI, in particular the electrostatic interaction between Pc and PsaF. Using NMR and EPR spectroscopy to characterize the Pc-PsaF complex and the one formed between Pc and Mg(II) we could observe similar binding constants. A competitive effect could be observed for the binding of Mg(II) to the Pc-PsaF complex, hence suggesting that the two interact with the same region of Pc. By studying the paramagnetic relaxation enhancement of the Mg(II) analogue, Mn(II), and its effect on Pc's <sup>15</sup>N-HSQC spectra, we could calculate the structure of the transient Pc-Mn(II) complex. The results presented suggest a specific binding site for Mg(II) that may regulate the binding of Pc to PSI *in vivo*.

In paper III, we show that the luminal domain of PsaF is a target for thioredoxin-mediated reduction of the disulphide bridge formed between cysteines 8 and 63. Furthermore, we show that the thiolated form of PsaF has a lower affinity towards reduced Pc than when the disulfide bridge is intact. Time-resolved absorbance measurements and fluorescence electrophoresis show that oxidized Pc can re-oxidize PsaF and thus restore the active form of this domain.

The PSI subunit PsaN is a weak modulator of the electron donation from Pc to PSI. In paper IV we present a methodology for the recombinant production of this protein subunit. Problems with unspecific proteolysis and degradation by *Escherichia coli* proteases are tackled.

KEYWORDS: EPR, NMR, photosystem 1, plastocyanin, protein-protein interaction, PsaF

## List of original publications

- I. **Cloning, expression and purification of the luminal domain of spinach photosystem 1 subunit PsaF functional in binding to plastocyanin and with a disulfide bridge required for folding**  
Daniel Farkas, Lars-Gunnar Franzén, Örjan Hansson  
*Protein Expression and Purification* (2011) In press
- II. **A paramagnetic NMR study elucidating the binding of Mg(II) and Mn(II) to spinach plastocyanin. Regulation of the binding of plastocyanin to subunit PsaF of photosystem I**  
Daniel Farkas and Örjan Hansson  
Submitted (2011)
- III. **Thioredoxin-mediated reduction of the photosystem I subunit PsaF and activation through oxidation by the interaction partner plastocyanin**  
Daniel Farkas and Örjan Hansson  
*FEBS Letters* (2011) In press
- IV. **Expression of Photosystem I subunit PsaN from *Arabidopsis thaliana* in *E. coli*.**  
Daniel Farkas, Örjan Hansson, Lars-Gunnar Franzén  
Manuscript (2011)

## Related articles

- V. **A comparative study of Cu(II) binding to angiogenin and a peptide fragment encompassing the first  $\alpha$ -helix of the protein**  
Diego La Mendola, Daniel Farkas, Francesco Bellia, Antonio Magri, Örjan Hansson, Raffaele P. Bonomo, Enrico Rizzarelli  
Manuscript (2011)
- VI. **pH dependence of copper geometry, reduction potential and nitrite affinity in nitrite reductase**  
Frida Jacobson, Arthur Pistorius, Daniel Farkas, Willem De Grip, Örjan Hansson, Lennart Sjölin, Richard Neutze  
*The Journal of Biological Chemistry*, 282 (2006) 6347-6355

## Contribution report

- Paper I** I conducted all experimental work except for the MS experiments and took a major part in writing the manuscript.
- Paper II** I conducted all experimental work and a significant part of the data analysis. I wrote a large part of the manuscript.
- Paper III** I conducted all experimental work and took a major part in writing the manuscript.
- Paper IV** I took a large part in planning the project and was partially involved in the experimental work. I supervised one diploma worker and took a large part in writing the manuscript.
- Paper V** I contributed with a significant part in the production of recombinant hAng. Furthermore, all activity measurements and some optical spectroscopy of the protein were conducted by me. I wrote a minor part of the manuscript.
- Paper VI** I performed the anaerobic redox titrations, EPR measurements and the following data analysis.

## Abbreviations

CD	circular dichroism
EDAC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
EPR	electron paramagnetic resonance
hAng	human angiogenin
HSQC	heteronuclear single quantum coherence
IMAC	immobilized metal affinity chromatography
MIANS	2-(4'-maleimidylanilino) naphthalene-6-sulfonic acid
NMR	nuclear magnetic resonance
Pc	plastocyanin
PRE	paramagnetic resonance enhancement
PSI	photosystem I
PSII	photosystem II
SEC	size exclusion chromatography
TF	thermofluorescence
Trx	thioredoxin
Wt	wild type