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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+ 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 15N-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

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