## S6-020

# Dissociation of the PSI-N subunit from photosystem I results in decreased electron transport

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### Introduction

PSI from plants consists of 13 subunits of which three are plant specific: PSI-G, PSI-H, and PSI-N. The remaining ten subunits are shared between cyanobacteria and plants. In addition to the 13 subunits of the PSI core complex, plant PSI also binds light harvesting complex I (LHCI) consisting of four different types of protein. For recent reviews on PSI see Scheller et al. (2001).

The PSI-N subunit and the corresponding cDNA were isolated first from barley (Knoetzel and Simpson 1993). PSI-N, encoded by the nuclear gene *psa*N, has an approximate mass of 9-10 kDa. An extensive cross-linking study indicated an interaction between PSI-N and PSI-F although this could not be unambiguously concluded (Jansson et al. 1996). No other cross-linking products between PSI-N and other small PSI subunits were observed. If the PSI-N subunit is situated close to the docking site for plastocyanin, it could have a function in electron flow. This assumption was confirmed in transgenic *Arabidopsis* plants without detectable levels of PSI-N (Haldrup et al. 1999). In plants lacking PSI-N the second-order rate constant for electron transfer from plastocyanin to $P700^+$ was only 55 % of the wild-type value and steady-state $NADP^+$ reduction was decreased to a similar extent. He and Malkin (1992) developed a method to specifically dissociate PSI-N from the PSI complex of spinach; however with the methods they used, no effect on electron transport was detected. In order to clarify this discrepancy in results with spinach and *Arabidopsis* we undertook a reinvestigation of the role of PSI-N in spinach with the use of different and more specific methods. The results reported here show that PSI-N is required for maximal rate of plastocyanin oxidation also in spinach.

### Materials and methods

PSI-200 complexes were prepared with Triton X-100 according to Tjus et al. (1995) from fresh spinach (*Spinacia oleracea* L.). The PSI preparations had a Chl/P700 ratio of about 250. PSI-200 at a final concentration of 0.25 mg Chl $ml^{-1}$ was incubated with $CaCl_2$ (0.4 or 0.5 M) in 50 mM Tris (pH 8.0), 0.05 % Triton X-100. After incubation on ice for 45 min (shaked every 10 min) the PSI complex was recovered by overnight centrifugation at 100,000 x **g**. The pellet was resuspended in incubation buffer without $CaCl_2$. A control sample was treated in the same way using incubation in the absence of $CaCl_2$.

Steady state electron flow was measured in isolated PSI-200 complexes as rates of $O_2$ consumption, using a Clark-type oxygen electrode. The reaction mixture of 1 ml contained PSI-200 particles (20 nM P700 which equals approx. 5 µg $ml^{-1}$ chlorophyll), 20 mM Tricine

(pH 7.5), 40 mM NaCl, 8 mM $MgCl_2$, 0.3 mM $NaN_3$, 7 mM $NH_4Cl$, 3 mM sodium ascorbate, 0.1 mM 2,6-dichlorphenol-indophenol (DCPIP), 5 µM plastocyanin, and 0.1 mM methyl viologen (MeV). Saturating light was provided with a Schott 1500 KL light source. A background oxygen consumption in the absence of MeV was subtracted from the rate of consumption in the presence of MeV. The measurements were also performed without plastocyanin.

Flash-induced P700 absorption decay was measured at 834 nm, essentially as previously described (Haldrup et al. 1999). The sample (250 µl) contained 20 mM Tricine (pH 7.5), 40 mM NaCl, 8 mM $MgCl_2$, 0.325 % *n*-decyl-β-D-maltopyranoside, 2 mM sodium ascorbate, 6 µM DCPIP, 5 µM plastocyanin, and PSI-200 corresponding to 100 µg Chl $ml^{-1}$. A total of 16-32 absorbance transients were collected with 4 s interval and averaged for each decay curve. The recorded absorbance changes were resolved into exponential decays by a Levenberg-Marquardt non-linear regression procedure (Press et al. 1989). The dissociation and rate constants were calculated using a simple model described by Nordling et al. (1991).

## Results

The PSI-N subunit is removed with 0.4 or 0.5 M $CaCl_2$ treatment.

PSI-N was totally removed by washing with 0.4 as well as with 0.5 M $CaCl_2$ (Fig. 1). Because PSI-F is known to be important for electron transport it was essential to ensure that the $CaCl_2$-treatment did not dissociate PSI-F. No loss of PSI-F was detected (Fig. 1). The polypeptide composition of the PSI preparations was analysed by SDS-PAGE showing that all known PSI subunits are present in the preparations and confirms that only PSI-N is dissociated by the $CaCl_2$ treatment (results not shown).

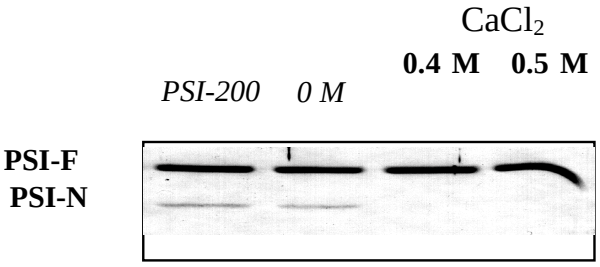


**Fig. 1.** Immunoblot analysis of PSI-200 from spinach with and without dissociation of PSI-N by washing with 0, 0.4 and 0.5 M $CaCl_2$. The PSI-200 preparation before $CaCl_2$ treatment is included. The blots were developed with polyclonal antibodies raised against the PSI-N and PSI-F subunits from barley. 10 pmol P700 were loaded in each lane.

The absence of PSI-N decreases steady state electron transport and affects the rate of plastocyanin oxidation

In the presence of 5 µM plastocyanin, the PSI lacking PSI-N showed only 50% oxygen consumption compared to the control sample (Table 1).

**Table 1.** Activity and kinetic constants for control and $CaCl_2$ treated PSI-200 complexes. $k_{et}$ is the rate constant for intracomplex electron transfer from plastocyanin to $P700^+$ while $k_{on}$ reflects the second order rate constant for complex formation between plastocyanin and oxidized PSI. $K_D$ is the dissociation constant for the plastocyanin-PSI complex prior to illumination (Nordling et al. 1991). The values are the means ± S.E. based on independent measurements of two untreated preparations and two $CaCl_2$ treated preparations.

| | Untreated | $CaCl_2$ treated |
|---|---|---|
| Oxygen consumption (± SD) | | |
| (µmol e-/(µmol P700 x s) | | |
| without plastocyanin | 19.2 ± 2.0 | 15.2 ± 1.1 |
| with plastocyanin | 90.9 ± 0.1 | 44.8 ± 1.6 |
| $k_{et}$ ($10^4$ $s^{-1}$) | 9.5 | 9.7 ± 0.1 |
| $k_{on}$ ± SD ($10^{-8}$ $M^{-1}s^{-1}$) | 2.00 ± 0.17 | 1.65 ± 0.04 |
| $K_D$ (µM) | 23 | 21 ± 4 |

In the absence of plastocyanin, electron transfer was much lower and there was only a small difference between the PSI-samples lacking or containing PSI-N.

The steady state electron transport measurements showed an involvement of PSI-N in the interaction with plastocyanin. In order to analyse the interaction with plastocyanin in more details we observed P700-absorption transients following short flashes (Fig. 2, Table 1). The results show that the second order rate constant, $k_{on}$, for plastocyanin docking onto photooxidised PSI is significantly lowered in the absence of PSI-N. In contrast, neither the intracomplex electron transfer rate constant, $k_{et}$, nor the dissociation constant, $K_D$ is affected by the absence of PSI-N (Table 1). The 20 % decrease in $k_{on}$ is smaller than the decrease observed with the oxygen electrode.

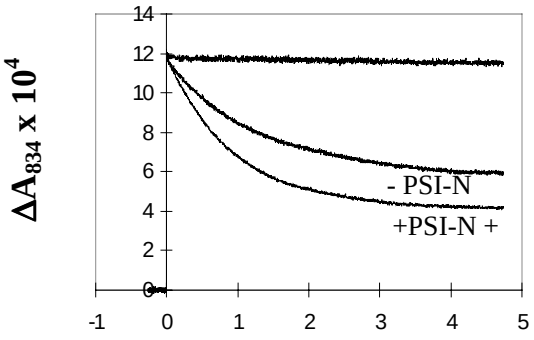


**Fig. 2.** Reduction of $P700^+$ by plastocyanin. Flash-induced absorption transients were recorded at 834 nm in samples of solubilised thylakoids. The transients observed with PSI containing PSI-N and lacking PSI-N (washed with 0.5 M $CaCl_2$) in the presence of 5 µM plastocyanin are shown. The traces shown are averages of 16-32 recordings.

## Discussion

We found that the absence of PSI-N decreases the rate of electron transfer from plastocyanin to $P700^+$. The decrease indicates that the PSI-N subunit plays a role in the docking of plastocyanin to the PSI complex. The involvement of PSI-N in electron transport is confirmed by steady state electron transport measurements. Plastocyanin may interact directly with PSI-N or the effect of PSI-N could be indirect through an interaction with PSI-F. The salt washing is a harsh treatment that could have different effects. The treatment did cause some inactivation in addition to the dissociation of PSI-N. However PSI-F was clearly not dissociated by the salt treatment.

The results reported here contrast with those obtained by He and Malkin (1992) who found no difference in electron transport after physical dissociation of PSI-N from spinach judged on the rates of oxygen uptake and $NADP^+$ photoreduction. A possible explanation for this discrepancy in conclusion may be that the second order reaction between plastocyanin and PSI has not been the limiting factor in the $NADP^+$ assay used by He and Malkin. Ferredoxin may instead have been the limiting factor because of a relative low amount of ferredoxin used in the assay. He and Malkin (1992) performed their oxygen consumption measurements without plastocyanin whereas we have performed the experiment both with and without plastocyanin. In agreement with He and Malkin (1992) we only found a small effect of PSI-N on oxygen consumption when no plastocyanin was present. The present investigation supports fully the results obtained in transgenic *Arabidopsis* plants without PSI-N which were generated using antisense and cosuppression strategies (Haldrup et al. 1999). Interestingly, although the basic function of PSI-N was found to be the same in our studies of *Arabidopsis* and spinach, the plastocyanin oxidation rate was affected more by the absence of PSI-N in *Arabidopsis* than in spinach.

PSI-N and the important N-terminal domain of PSI-F (Hippler et al. 1996) are both found only in eukaryotes. We suggest that PSI-N further enhance the function of PSI-F in guiding of plastocyanin during complex formation. The tertiary structure of PSI-N is unknown but the protein has many positively charged amino acid residues and it is conceivable that these could augment the electrostatic interactions with the acidic plastocyanin. Quantum yield of oxygen evolution and PSII photochemistry at leaf level were about 10 % lower than in the wild-type (Haldrup et al. 1999). Photochemical fluorescence quenching was lowered to a similar extent. Thus, the 40-50 % lower activity of PSI at the molecular level was much less significant at the whole-plant level. This compensation can partly be explained by a 17 % increase in PSI content in the plants without PSI-N (Haldrup et al. 1999).

## Acknowledgements

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