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# The PsbW-protein; its location and involvement in photoinhibition

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### Introduction

The low molecular weight photosystem two (PSII) protein PsbW has recently been shown to stabilise the dimeric form of PSII in *Arabidopsis thaliana* [Shi et al. 2000]. This was revealed by sucrose gradient centrifugation of briefly solubilised thylakoid membranes of transgenic plants, lacking the PsbW-protein up to 96%. The 6.1 kDa PsbW-protein has a high turnover rate in high light, similar to the D1 polypeptide [Hagman et al. 1997]. It is imported from the cytosol with a biparthite transit peptide [Lorkovik et al. 1995]. The mature protein crosses the thylakiod membrane with one α-helix and has the N-terminal located in the lumen and the C-terminal on the stromal side [Shi and Schröder 1997]. In this study we show by analysis of a blue native gel system, that resolves PSII dimercomplexes from PSII in monomeric form, that the PsbW-protein is exclusively located in the PSII dimer and that it's assembly kinetic is very fast (< 3min). When plants are exposed to light intensities that exceed their photosynthetic capacity they suffer photoinhibition, which can be observed as a lowering of maximal PSII quantum yield detected as reduced ratio of $F_v/F_{max}$. Our light stress experiments reveal that the anti-sense plants lacking the PsbW-protein are more vulnerable to photoinhibition than wild type *Arabidopsis thaliana* and that their recovery is slower.

### Materials and methods

*Chloroplast isolation:* Intact chloroplasts from pea (*Pisum sativum*) were prepared according to [Brock et al. 1993]. *Generation and import of precursor proteins:* The precursor of the PsbW-protein was prepared *in vitro* by transcription of cDNA and import was performed with intact pea chloroplasts essentially as in [Robinson et al. 1996, Mant and Robinson 1998]. *Sample preparation and BN-PAGE* was carried out according to [Schägger and von Jagov 1991, Schägger et al. 1994] with modifications [Thidholm & Lindström et al. 2001]. The gel was dried and exposed to biomax ms Kodak film and the film was developed after three weeks. *Generation of A. thaliana – PsbW anti-sense plants* is described in [Shi et al. 2000]. *Chlorophyll fluorescence measurements* were carried out using *A. thaliana* wild type and PsbW anti-sense plants, grown hydroponically with an 8 hour light period (100 $\mu E\ m^{-2}\ s^{-1}$) for 8 weeks. Leaves were cut off and placed in the dark between moisted filterpapers for 20 minutes followed by $F_v/F_{max}$ measurements using a PAM-fluorimeter. The

leaves were then transferred to a cooling waterbath (10°C) and exposed to light (3000 μE $m^{-2}$ $s^{-1}$) for 18 minutes. After light exposure, the leaves were transferred to the dark and the $F_v/F_{max}$ was measured after 2, 5, 10, 20, 40, and 80 minutes recovery.

## Results and Discussion

*Localisation of the PsbW-protein*

As seen in Fig. 1 A, our blue native polyacrylamide gel electrophoresis (BN-PAGE) system is able to resolve briefly solubilsed thyalkoid membranes into at least 9 distinct bands. The identity off the different bands has been analysed by western blotting with antisera directed against D1, PsbS, CP43, LHCII and PsaH [Thidholm & Lindström et al. 2001]. The identity of the dimeric PSII complexes was further confirmed by comigration of PSII supercomplexes isolated via sucrose density centrifugation according to [Eshagi et al. 1999]. The western blotting of the BN-gel with antisera raised against the PsbW-protein (fig1C) shows that the PsbW-protein is exclusively located in the PSII dimer complexes. No anti-PsbW immunostain could be detected in the PSII subcomplex bands, corresponding to the monomeric form of PSII. Meanwhile, the D1 and PsbS proteins were detected in the PSII monomer bands as well as in the PSII dimer [Thidholm & Lindström et al. 2001]. These results clearly show that the PsbW protein is only found in the dimeric form of PSII. The variations of PsbW protein content reported for various PSII reaction centre preparations [Shi and Schröder, 1997] could thus be due to different amounts of dimers in these preparations.

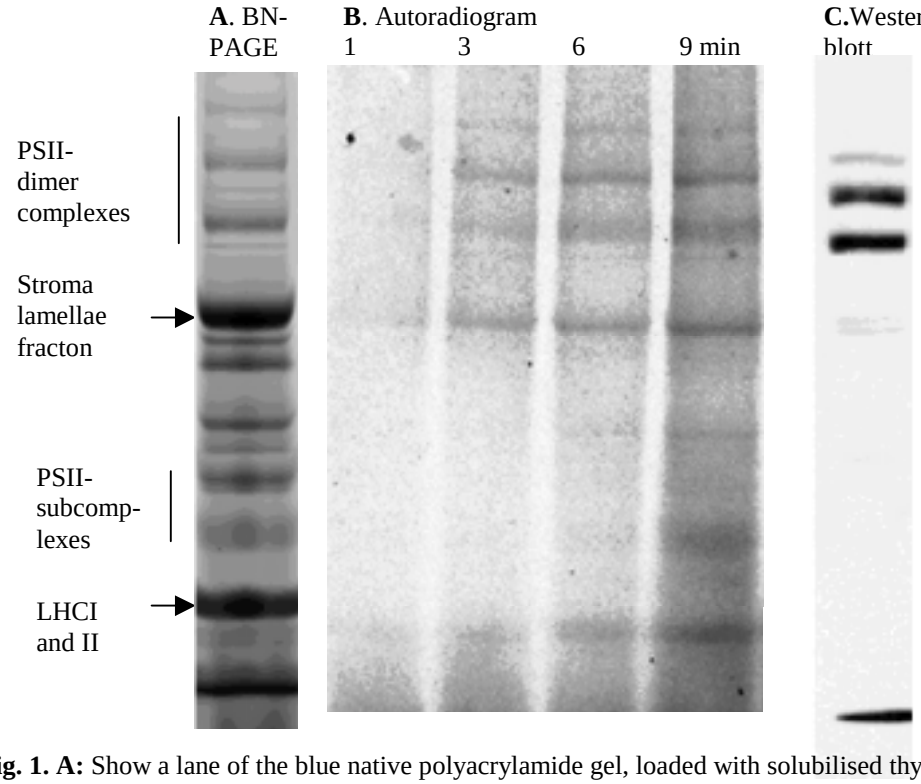


**Fig. 1. A:** Show a lane of the blue native polyacrylamide gel, loaded with solubilised thylakoid membranes corresponding to 15 μg chlorophyll. B: Picture of an autoradiogram of radiolabelled PsbW-protein imported into intact chloroplasts analysed on BN-page. The import was terminated after 1, 3, 6 and 9 minutes. C: Show immunoblot of thylakoid membranes resolved on the BN-gel with antisera raised against the PsbW-protein.

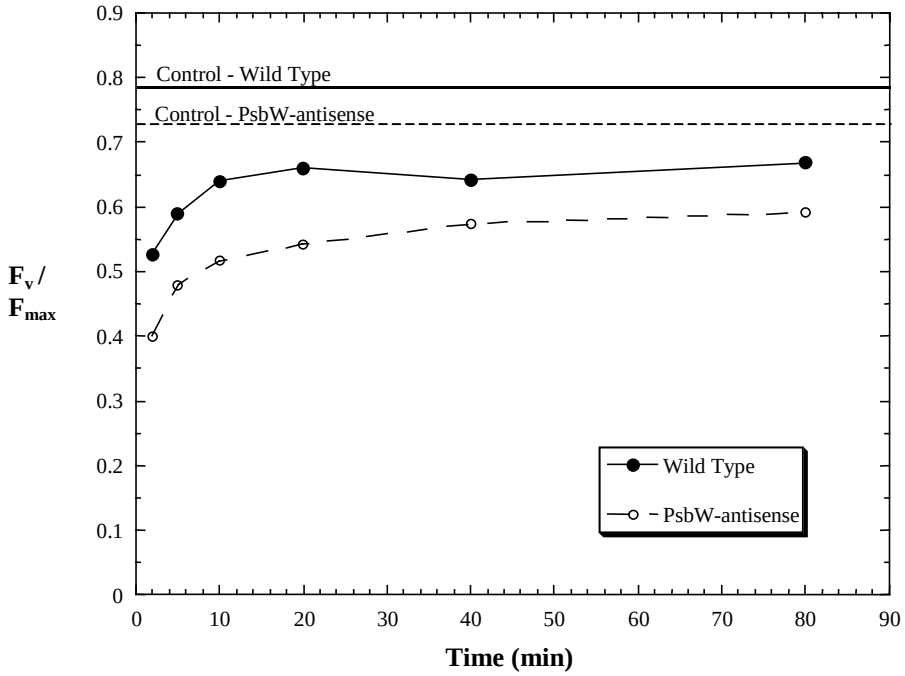


**Fig. 2.** $F_v$ / $F_{max}$ measured on dark adapted leaves = control. $F_v$ / $F_{max}$ plotted against recovery time after 18 minutes light stress (3000 µE $m^{-2}$ $s^{-1}$). Measurement on *Arabidopsis thaliana* antisense plants with a strongly reduced PsbW-protein content and wild type *Arabidopsis thaliana* plants was carried out under controlled and equal conditions.

*Kinetic analyses of the PSII dimer assembly*

The next point to investigate is how fast the PsbW protein is assembled into the PSII dimeric complex. To do this, radiolabelled PsbW-precursor was incubated with intact chloroplasts for different time periods (1, 3,6 and 9 min). In this timeperiods the labelled PsbW-protein was imported through the chloroplast envelope, processed in the stroma, inserted into the thylakoid membrane, processed on the lumenal side of the thylakoid membrane and assembled into the PSII dimer. The autoradiograph (Fig 1B) show that imported PsbW-protein first is visible in the stroma lamellae band (1 min of import) but already after three minutes import it starts to be visible in the PSII dimer bands. In less than three minutes the PsbW protein had found its functional place and assembled into PSII.

*Functional reasons for the dimerisation of PSII*

Using anti-sense plants of *A. thaliana* that have a strongly reduced PsbW-protein content and thus contains mainly monomeric PSII, enables us to address this question. Leaves of transgenic-PsbW and wild type (WT) plants were exposed to the same high light intensity and the maximum quantum yield of PSII was determined after various recovering times in the dark. Dark adapted, unstressed leaves generally show a $F_v/F_{max}$ ratio of 0.78-0.83, representing a high quantum yield of PSII. However, the control value of the transgenic-PsbW plant exhibits already a decreased PSII quantum yield in a dark adapted state (Fig 2). After being exposed to light stress, $F_v/F_{max}$ was measured after 2, 5, 10,

20 and 80 minutes of recovery in darkness (Fig. 2). After 2 minutes recovery, the transgenic-PsbW plant PSII quantum yield had dropped 46%, while the WT only dropped 31% compared to the control values. As seen in figure 2 the PSII quantum yield is lower in the plants lacking the PsbW-protein throughout the measured timespan. After 10 minutes the WT has recovered to almost the same levels as after 80 minutes, meanwhile the transgenic plants only has reached 50% of the final level. These results clearly show that monomeric PSII lacking the PsbW-protein are more susceptible to photoinhibition. The recover after a high light treatment is slower in the transgenic plants, which could be explained by assuming that the lack of the PsbW-protein hampers the assembly of new stable PSII dimers.

## Conclusion

The PsbW protein is exclusively located in the PSII dimer complex. The PsbW protein is imported into the chloroplast and assembled into the dimer in less than 3 minutes. Arabidopsis plants that lack the PsbW protein, can not assemble dimers and are therefore more sensitive to high light stress.


## Acknowledgements

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