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The role of PSI-G and PSI-K of higher plants in the interaction between light harvesting complex I and the photosystem I reaction center core.

PE Jensen, J Knoetzel, H V Scheller

Plant Biochemistry Laboratory, Department of Plant Biology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark.
peje@kvl.dk.

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Introduction

Photosystem I (PSI) of higher plants consists of at least 17 different polypeptides and is composed of a chlorophyll *a* binding core complex and a chlorophyll *a* and *b* binding peripheral antenna called LHCI (Scheller et al., 2001). The two homologous PSI-A and PSI-B subunits together with PSI-C binds all the co-factors involved in electron transport (Jordan et al., 2001). Almost all of the Chl *a* and β-carotene molecules are bound to the PSI-A/B dimer. However, recent data have shown that some of the pigment molecules are bound to some of the smaller subunits (Jordan et al., 2001). Higher plants and green algae contain the additional subunits PSI-G, PSI-H, and PSI-N, which have not been identified in the PSI complexes from cyanobacteria.

The LHCI is arranged around the core and is composed of four different proteins, Lhca1-4. LHCI binds about 100 chlorophyll *a* and *b* molecules and approximately 20 xanthophylls molecules per P700 (Bassi and Simpson, 1987). Lhca1 and Lhca4 form heterodimers whereas Lhca3 and Lhca2 form homodimers (Knoetzel et al., 1992). The dimer complexes associate independently with the reaction center (Jansson et al., 1996) and the complexes only binds to the core complex at the side of the PSI-F/J subunits (Boekema et al., 2001).

There is significant sequence similarity between PSI-G and PSI-K from eukaryotic phototrophs. A comparison of PSI-G and PSI-K from *Arabidopsis* displays approximately 30% amino acid identity. Chemical cross-linking of plant PSI showed that PSI-G and PSI-K differed from all the other small PSI subunits by not forming cross-linking products with other small core subunits. This suggested that both subunits should be located away from the two-fold symmetry axis. The involvement of PSI-K in interaction with Lhca2 and Lhca3 has recently been shown using plants in which the *psaK* gene was suppressed using antisense technology (Jensen et al., 2000). A putative cross-linking product between PSI-G and Lhca2 has been reported (Jansson et al., 1996). Thus, due to the homology between PSI-G and PSI-K and since light-harvesting chlorophyll *a/b* binding proteins are only present in plants and algae, a function of PSI-G in the interaction with LHCI is likely.

The function of PSI-G and PSI-K was characterized in *Arabidopsis* plants transformed with a *psaG* or *psaK* cDNA in antisense orientation under the control of a constitutive promoter. Transformants with either undetectable or very low levels of PSI-G or PSI-K protein were obtained and the plants were analyzed both at the biochemical and leaf level.

Materials and methods

DNA fragments containing the entire coding regions of PSI-G or PSI-K were cloned in antisense orientation between the CaMV 35S promoter and 35S terminator and transformed into *Arabidopsis* plants. Seeds harvested from transformed plants were germinated on MS medium containing kanamycin and green seedlings were transplanted to peat. Fully expanded rosette leaves from 8-10 weeks old plants were used for isolation of thylakoids. Total Chl and Chl *a*/*b* ratio were determined in 80% acetone according to Lichtenthaler (1987). For immunoblotting either crude leaf extracts or thylakoids were used and immunoblotting was carried out as described in Jensen et al. (2000). State 1 – state 2 transitions were measured with a pulse amplitude (PAM) 101-103 fluorometer (Walz, Effeltrich, Germany) as outlined in Lunde et al., (2000). The relative change in fluorescence was calculated as Fr = ((Fi'-Fi)-(Fii'-Fii)/(Fi'-Fi). The fluorescence spectrum at 77K was recorded for thylakoids purified from dark adapted plants using a bifurcated light guide connected to a spectrofluorometer. The excitation light had a wavelength of 435 nm and emission was detected from 650 to 800 nm.

Results and discussion

The transformed plants were self-fertilised and the seeds produced were germinated and selected on kanamycin-containing plants. Several plants were analysed by protein extraction and immunoblotting using antibodies raised against PSI-G, PSI-K and PSI-F. As seen in Fig. 1, several plants transformed with the antisense *psa-g* gene was found to have no or low levels of PSI-G protein. We have previously reported similar finding for plants transformed with an antisense *psa-k* gene (Jensen et al., 2000). There was no obvious visible difference between wild-type plants and plants lacking PSI-G or PSI-K under the growth conditions used (100-120 µmol photons m^{-2} s^{-1}, 20 °C and 70% relative humidity).

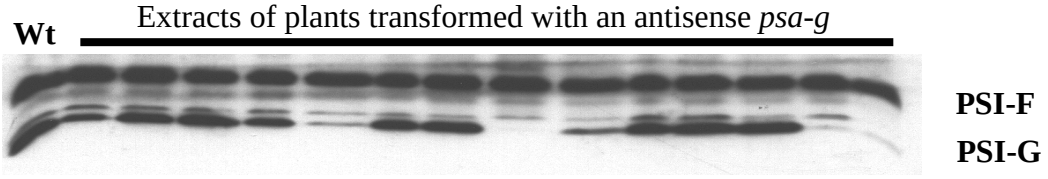

Figure 1. **Immunoblotting of protein extracts prepared from plants transformed with the *psa-g* gene in antisense orientation.** Total leaf extracts corresponding to 2 µg Chl was separated in each lane. The blotted proteins were developed with antibodies directed against PSI-G and PSI-F.

The chlorophyll *a/b* ratio was clearly increased in thylakoids from plants devoid of PSI-K whereas it was slightly decreased in plants devoid of PSI-G (Fig. 2). An increase in the Chl a/b ratio was also observed in studies with plants devoid of PSI-N and PSI-H, and was caused by an increase in PSI (Haldrup et al. 1999, and Naver et al 1999). In the absence of PSI-K, the increase in Chl a/b ratio is caused, in part, by an increase in PSI and, in part, a decrease in the PSI antenna (Jensen et al., 2000). It therefore seems that, in the absence of specific PSI subunits, the plants compensate for a less efficient PSI by increasing the amount of PSI. The decreased Chl a/b ratio in thylakoids devoid of PSI-G therefore indicate that there is a decreased amount of PSI, which suggests that PSI-G is important for stability of PSI.

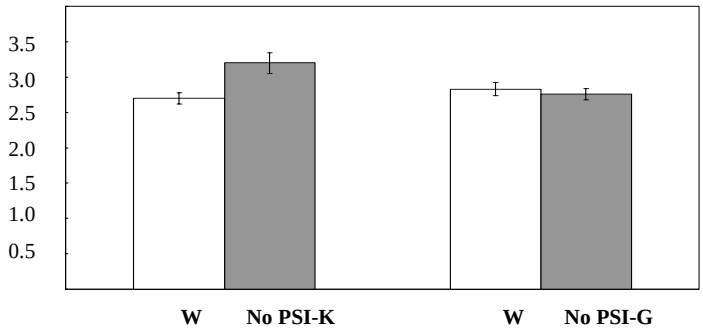

Figure 2. Chlorophyll *a/b* ratio in thylakoids from plants devoid of PSI-K or PSI-G compared to their respective wild-types. Shown are the average of 5-6 samples of each line ± S.D.

Because both PSI-G and PSI-K have been proposed to interact with LHCI the low temperature fluorescence properties were analysed. Fluorescence emission spectra obtained at 77K revealed a 2-nm blue-shift in the long wavelength emission in plants lacking PSI-K and a 1-nm blue-shift in plants devoid of PSI-G suggesting a perturbation of the LHCI antenna in the absence of PSI-G or PSI-K (Fig. 3). Immunoblot analysis of thylakoids and isolated PSI without PSI-K revealed a 20-30% reduction in the amount of Lhca2 and a 30-40% reduction in the amount of Lhca3 while Lhca1 and Lhca4 were unaffected (Jensen et al., 2000). However, the absence of PSI-G did not have any significant effect on the amount of LHCI proteins (Results not shown).

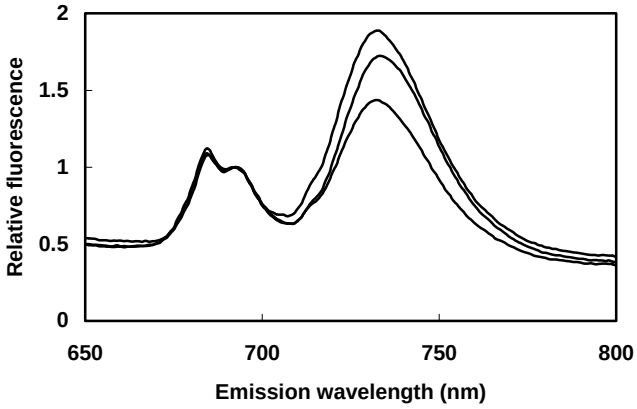

Figure 3. Fluorescence emission spectra at 77K of wild-type (middle) and antisense plants devoid of PSI-G (bottom) or PSI-K (top). The excitation wavelength used was 435 nm.

State 1 – state 2 transition is a dynamic mechanism that enables plants to respond rapidly to changes in illumination and involves the dissociation of a mobile pool of the light-harvesting chlorophyll *a*/*b* protein complex (LHCII) from PSII and concomitant association of this LHCII with PSI. Expressed as relative fluorescence changes (Fr), plants devoid of PSI-G have only 50 % of the state transitions observed in the wild-type and plants devoid of PSI-K have 85% of the state transitions observed in wild-type (Fig. 4). This suggests that the redistribution of absorbed excitation energy between the two photosystems is significantly reduced especially in plants without PSI-G and to a lesser extent in plants devoid of PSI-K. We have recently shown that in plants without PSI-H there are almost no state transitions suggesting that PSI-H is involved in interaction with the mobile LHCII, probably as the docking site (Lunde et al., 2000). Work is in progress to find out whether the absence of PSI-G also affects the amount of PSI-H.

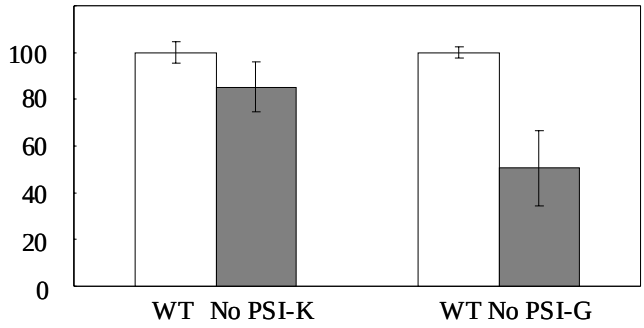

Figure 4. State 1-state 2 transitions in plants devoid of PSI-G or PSI-K. Shown is the average ± S.D.

In conclusion: The observed effects demonstrate that PSI-G and PSI-K are not strictly necessary for attachment of the light harvesting complexes to the core but are important for their stable interaction with the core. PSI-K is directly involved in interaction with Lhca2 and Lhca3, whereas it is not clear whether PSI-G is in direct contact with any of the LHCI complexes. Instead, PSI-G seems to have an additional role in the overall stability of the PSI complex and also affects the interaction between PSI and LHCII during state transitions. A spectroscopic characterization of PSI complexes from plants devoid of PSI-G or PSI-K has recently been initiated and results of this are presented by Ihalainen et al. (Symposium number S6-013).

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