S6-013

Spectroscopic characterization of isolated PSI-200 complexes from plants devoid of the PSI-G, PSI-K, PSI-L and PSI-N subunits

JA Ihalainen[1], PE Jensen[2], A. Haldrup[2], IHM van Stokkum[3], R van Grondelle[3], HV Scheller[2], JP Dekker[3]

[1]*Department of Chemistry, University of Jyväskylä, P.O. Box 35, FIN-40351 Jyväskylä, Finland. E-mail:* janihal@st.jyu.fi

[2]*Plant Biochemistry Laboratory, Department of Plant Biology, The Royal Veterinary and Agricultural University, 40 Thorvaldensvej, DK-1871 Frederiksberg C, Copenhagen, Denmark.*

[3]*Faculty of Sciences, Division of Physics and Astronomy, Vrije Universiteit, De Boelelaan 1081, 1081 HV Amsterdam, The Netherlands.*

Keywords: photosystem I, energy transfer, subunit function

Introduction

Green plant photosystem I (PSI) uses light to catalyze the oxidation of plastocyanin and the reduction of $NADP^+$ in the thylakoid membranes of chloroplasts. The complex consists of at least 17 different protein subunits (Scheller et al., 2001), 13 of which form the so-called PSI core complex. This complex binds about 100 chlorophyll *a* (Chl *a*) molecules and is responsible for the first electron transfer reactions. The structure of the PSI core complex from the cyanobacterium *Synechococcus elongatus* has been resolved at 2.5 Å resolution (Jordan et al., 2001). The remaining four proteins form the peripheral antenna (LHCI), which binds another 80-100 Chls, from which small part are Chl *b* molecules. In this paper, we report a spectroscopic analysis of PSI-200 particles isolated from *Arabidopsis thaliana* and from its mutants lacking the PSI-G, PSI-K, PSI-L and PSI-N subunits by low-temperature steady-state and time-resolved fluorescence spectroscopy, to get more insight in the functioning of these small PSI core subunits.

Materials and methods

PSI-200 particles were prepared from wild-type *Arabidopsis thaliana* and its mutants lacking PSI-G, PSI-K, PSI-L or PSI-N subunits as described by Jensen et al. (2000) for the minus-K mutant. Pigment analysis suggested that PSI-200 complexes with very comparable pigment compositions could be isolated from all mutants. For the spectroscopic measurements, the isolated complexes were diluted in 20 mM Bis-Tris (pH 6.5), 20 mM NaCl, and 0.06 % β-DM to an optical density of 0.6 cm^{-1} at 679 nm. For the time-resolved fluorescence measurements, 10 mM sodium ascorbate and 10 mM phenazine metasulphate (PMS) were added, while for the low-temperature measurements cryoprotectants were added as in Ihalainen et al. (2000). Low-temperature steady-state absorption and linear dichroism (LD) spectroscopy was performed as described in Ihalainen et al. (2000). Time-resolved fluorescence spectroscopy was performed with a synchroscan streak and CCD camera as described by Gobets et al. (2001), using excitation pulses at 475 nm or 710 nm. The samples were placed in a 2 mm spinning cell with rotation speed of 30 Hz. The detected two-dimensional images were

analyzed using a model with parallel decaying components, which yield decay-associated spectra (DAS). The instrument response function was modeled as a Gaussian with a FWHM of 3.9 ps in case of a 200 ps time base, or 10 ps in case of a 800 ps time base.

Results and discussion

Fig. 1A shows 5 K absorption spectra of PSI-200 particles from wild-type (WT) *Arabidopsis* and several mutants. The spectra were normalized to the area of the Chl Q_y-absorption band peaking at about 680 nm. All spectra are rather similar, which is not surprising, because the PSI-K and PSI-L subunits of *Synechococcus* bind only a few pigments and because the PSI-G and PSI-N subunits (which are not present in cyanobacteria) are not expected to bind many pigments either.

The absorption difference spectra (Fig. 1B) show, however, some clear differences between WT and mutant spectra. The PSIminG-WT difference spectrum shows that a carotenoid with the absorption maximum of 506 nm is missing in the absence of PSI-G. The missing carotenoid is probably β-carotene, because β-carotene is the only carotenoid in the PSI core complex. No clear changes are seen in the Chl Q_y-bands, which suggests that PSI-G does not bind Chl molecules.

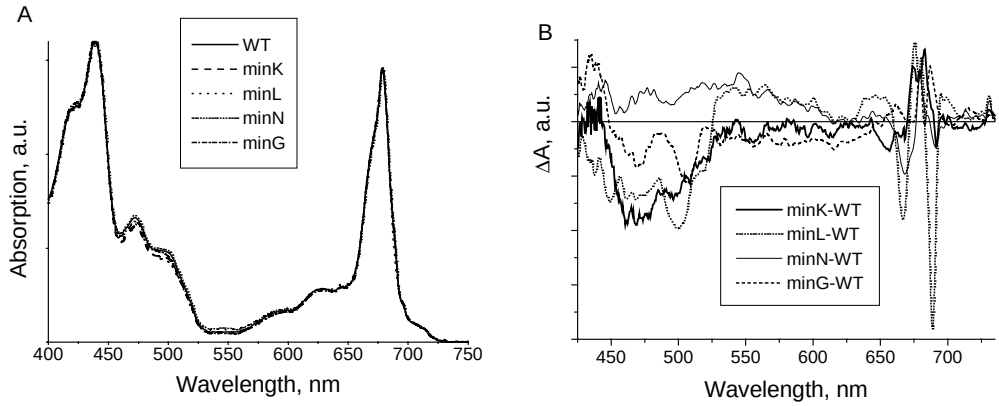

Fig. 1. Absorption spectra of PSI complex and its mutants lacking K-, L-, N-, and G-subunit at 5 K (A). Difference spectra between mutants and WT absorption (B).

The PSIminK-WT absorption difference spectrum shows a negative signal between 450 nm and 550 nm, as well as negative and positive signals around 650 and 670-680 nm, respectively. These differences are in line with earlier results (Jensen et al., 2000), which indicate that part of the Lhca2 and Lhca3 subunits of the LHCI antenna is missing in the absence of PSI-K. The PSIminL-WT absorption difference spectrum shows that a carotenoid band at 500 nm and chlorophyll bands at 666 nm and 689 nm are missing. In a similar analysis, Soukolis et al. (1999) observed lack of Chls absorbing close to 700 nm in PSI from *Synechocystis* PCC 6803 without PSI-L. The most recent structural data (Jordan et al., 2001) reveal that PSI-L of *Synechococcus elongatus* binds indeed 3 Chls and at least one β-carotene molecule. The PSIminN-WT difference spectrum shows only some small deviations in the Chl *a* Q_y- absorption region. This suggests that the absence of PSI-N has minor effects on the pigment network of PSI.

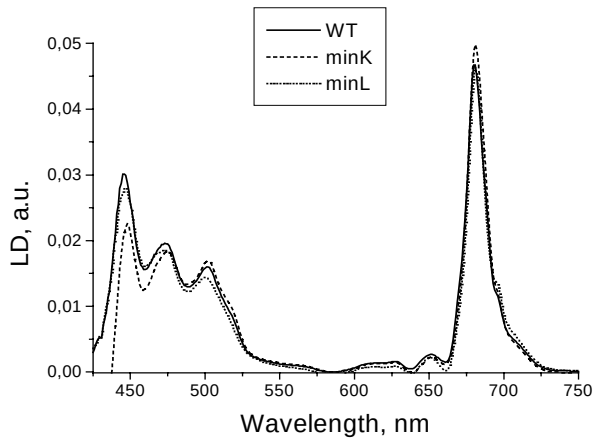

Fig. 2. 5 K LD spectra of PSI-200 complexes from *Arabidopsis* and its mutants lacking PSI-K or PSI-L.

Fig. 2 shows 5 K LD spectra from PSI-200 particles from the WT and the minK and minL plants. Also these spectra were normalized to the area of the Chl Q_y-band and also these spectra are very similar. The deviation below 500 nm in the minK samples is probably caused by a scattering artefact.

Fig. 3 shows DAS-spectra of PSI-200 from wild-type *Arabidopsis* after 475 nm (A) and 710 nm (B) excitation. The 475 nm pulse mainly excites carotenoids and Chl *b* molecules, whereas the 710 nm pulse mainly excites the 'red' pigments of PSI. In both cases about 65 % of excitation energy is absorbed by LHCI and about 35 % by the PSI-core. The data detected after 475 nm excitation could be fitted satisfactorily with six components. The fastest component (solid line) has a lifetime of 0.6 ps and probably arises from relaxation from higher Chl states to the Q_y-state and from energy transfer from carotenoids to Chl *a*. The 4 ps and 20 ps components (dashed and dotted lines, respectively) show the average times of excitation equilibration between 'bulk' and 'red' pigments. The positive spectral components of 50 ps (dash-dotted line) and 120 ps (thick solid line) corresponds to the time of the trapping of the excitation by charge separation in the reaction center of PSI. The sixth component has a lifetime of 4.9 ns (thin solid line) and originates from chlorophylls which are unable to transfer excitation energy to reaction center. For the 710 nm data analysis five components were required to describe the data. The fastest component after 475 nm excitation was missing, in line with its attribution to Chl relaxation and/or Car → Chl energy transfer. The fastest component after 710 nm excitation is about 3 ps and corresponds to equilibration between the excited 'red' chlorophylls and 'bulk' chlorophylls. All slower components are similar to those observed with 475 nm excitation. Similar lifetimes were found after fitting data obtained from PSI-200 particles from all mutants.

The shapes of the long-living DAS components are reproduced in Fig. 4. The larger amplitude in the mutants without PSI-G (Fig. 4B) and PSI-K (Fig. 4A) suggests less efficient energy transfer from the peripheral antenna to the PSI core complex, in line with the idea that PSI-K (Jensen et al., 2000) and PSI-G are needed for a stable interaction between LHCI and the PSI core complex. The smaller amplitudes of the long-living components in PSI-200 without PSI-L can, however, not easily be explained by a more efficient energy transfer from the peripheral antenna to the PSI core, because PSI-L and LHCI are located at different sides of PSI-200 (Boekema et al., 2001). It is possible that in the absence of PSI-L the trapping of the excitation energy by P700 occurs more efficiently in view of the absence of three rather remotely located chlorophylls (in PSI from *Synechococcus elongatus*).

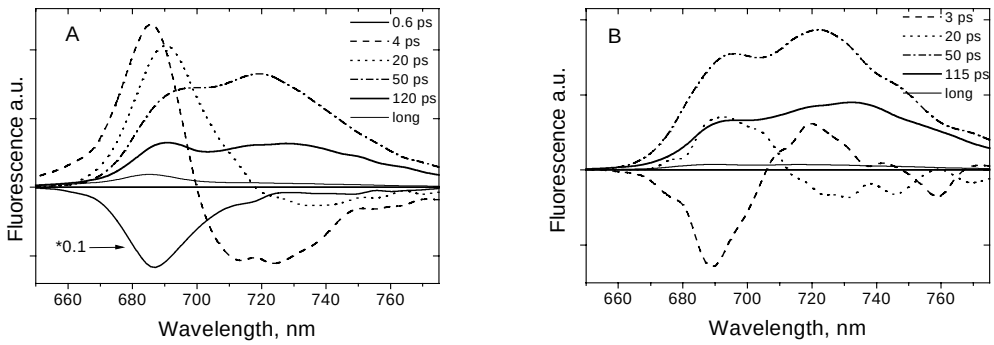

Fig. 3. Decay-associated spectra of fluorescence decay of PSI-200 complexes from WT *Arabidopsis thaliana* after 475 nm (A) or 710 nm (B) excitation.

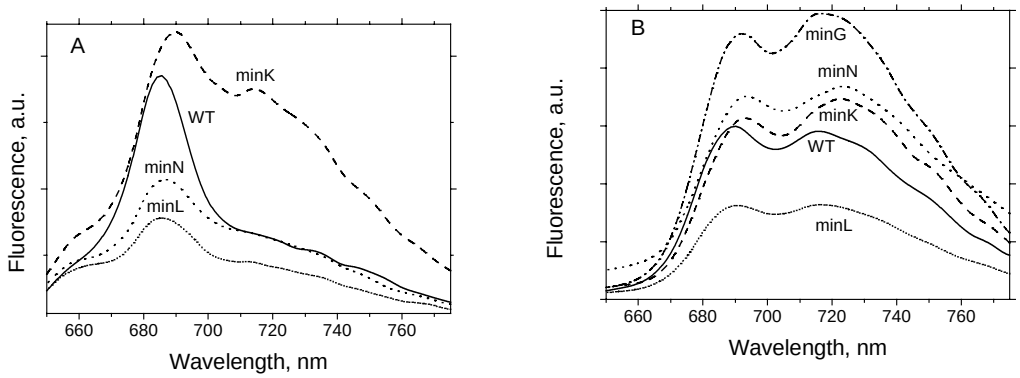

Fig. 4. Decay-associated spectra of long-living fluorescence components of PSI-200 complexes from *Arabidopsis* and various mutants upon 475 nm (A) and 710 nm (B) excitation.

Acknowledgement:

The visit of J.A.I to Amsterdam was supported by the European Science Foundation via the program Femtochemistry and Femtobiology (ULTRA).

References

Boekema EJ, Jensen PE, Schlodder E, van Breemen JFL, van Roon H, Scheller HV, Dekker JP (2001) *Biochemistry* **40**, 1029-1036.

Gobets B, van Stokkum IHM, Rögner M, Kruip J, Schlodder E, Karapetyan NV, Dekker JP, van Grondelle R (2001) *Biophysical Journal* **81**, 407 – 424.

Ihalainen JA, Gobets B, Sznee K, Brazzoli M, Croce R, Bassi R, van Grondelle R, Korppi-Tommola JEI, Dekker JP (2000) *Biochemistry* **39**, 8625-8631.

Jensen PE, Gilpin M, Knoetzel J, Scheller HV (2000) *Journal of Biological Chemistry* **275**, 24701-24708.

Jordan P, Fromme P, Witt HT, Klukas O, Saenger W, Krauss N (2001) *Nature* **411**, 909-917
Scheller, H.V., Jensen, P.E., Haldrup, A., Lunde, C. and Knoetzel, J. 2001 *Biochim. Biophys. Acta.* In press.
Soukoulis V, Savikhin S, Xu W, Chitnis PR, Struve WS (1999) *Biophysical Journal* **76**, 2711-2715.