DYNA – Exchange Grant Acceptance – 759

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Time duration : 1 August 2005 until 14 September 2005
(the time duration of the visit has been extended by 3 days
in order the experiments to be finished)

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Purpose of the visit

Purpose of the visit is the establishment of co-operation between the Vrije Universiteit Amsterdam, the Netherlands, the University of Thessaly, Greece and the Technical Educational Institute of Thessaloniki, Greece.

Future collaboration with host institution (if applicable)

Future collaboration will take place between the Biophysics Department of the Vrije Universiteit Amsterdam, the Netherlands the Biochemistry Biotechnology Department of the University of Thessaly, Greece and the Technical Educational Institute of Thessaloniki, Greece.

Projected publications/articles resulting or to result from your grant

We intend to submit a paper or a letter based on this work.

Other comments (if any)

I am grateful to ESF for giving me the chance to work as a visiting post-doc in the Vrije Universiteit Amsterdam in an effort to initiate collaboration between Universities and Institutes of Greece and the Netherlands. Thank you!

Description of the work carried out during the visit and - Description of the main results obtained

Role of the PSI-J subunit of photosystem I in energy transfer between the peripheral and core antenna complexes

Abstract

We measured 5K absorption and fluorescence spectra of photosystem I (PSI) wild-type and PSI-J depleted mutant of tobacco form *Arabidopsis thaliana*. Steady state fluorescence measurements show a broadening and a slight blue shift in the mutant fluorescence spectrum. Time-resolved measurements with picosecond time resolution at room temperature, reveal similar lifetimes of ~65 ps, a timescale that may indicate that the J-protein does not provide an effective energy transfer route between the peripheral and core antenna.

Introduction

In plants the core of photosystem I (PSI) consists of at least 14 different protein subunits named PSI-A to PSI-L, PSI-N and PSI-O. The low molecular mass membrane integral subunits do not directly bind co-factors involved in electron transport but instead fulfill other functions such as stabilization of the core antenna system, binding of the peripheral antennas and interaction with soluble electron transporters.

In the lab of Dr Poul Eric Jenssen in Copenhagen , *Arabidopsis thaliana* plants were prepared in which the chloroplast encoded PSI-J subunit has been eliminated by chloroplast transformation using a knockout allele for targeted disruption of the tobacco *psaJ* gene. PSI-J is a 5 KDa protein which spans the thylakoid membrane once, binds 2 chlorophyll molecules and is located close to PSI-F (*Ben Schem et al., 2003*). Plants devoid of PSI-J were shown to be fully viable and fertile. The absence of PSI-J does not affect the binding of LHCI to PSI, since the functional antenna size of PSI is unaffected by the elimination of PSI-J. Instead, in vitro NADP+ photo-reduction measurements

clearly indicate a role of PSI-J in electron transfer efficiency (*P.E.Jenssen et al., manuscript in preparation*)

In this proposal, we investigate the role of PSI-J in the energy transfer from the peripheral to the core antenna. Absorption and fluorescence spectra of PSI particles obtained from the PSI-J minus mutant and from the wild type at liquid helium temperatures. Hence we may be able to establish the absorption characteristics of the missing chlorophylls at liquid helium temperatures in the particles of the mutant. Similar work done before on mutants without PSI-L or PSI-G established absorption characteristics of pigments bound to these subunits. (*Ihalainen et al. 2002*).

Time-resolved fluorescence of the mutant PSI particles were recorded and were compared with the results obtained before with wild-type PSI particles. (*Ihalainen et al., 2002, 2005a, 2005b*). The particles were excited with short (~100fs) laser pulses at 400 nm and we have analyzed the fluorescence transients in the red region of the spectrum with a Steak camera set up (*Gobets et al. 2001*). The Streak camera set up allows detection of the fluorescence transients with a time resolution of about 4 ps with a high signal to noise ratio. The transients have been analyzed with global analysis (*van Stokkum et Al., 2004*), and in particular the transient that in wild types has a lifetime of ~65 ps has been investigated, as this transient defines the energy transfer from LHCI to PSI (*Ihalainen et. al 2005ab*). A slowing down of this transient in the mutant would indicate to which extend the chlorophylls bound to PSI-J are important for the energy transfer between the peripheral and core antenna.

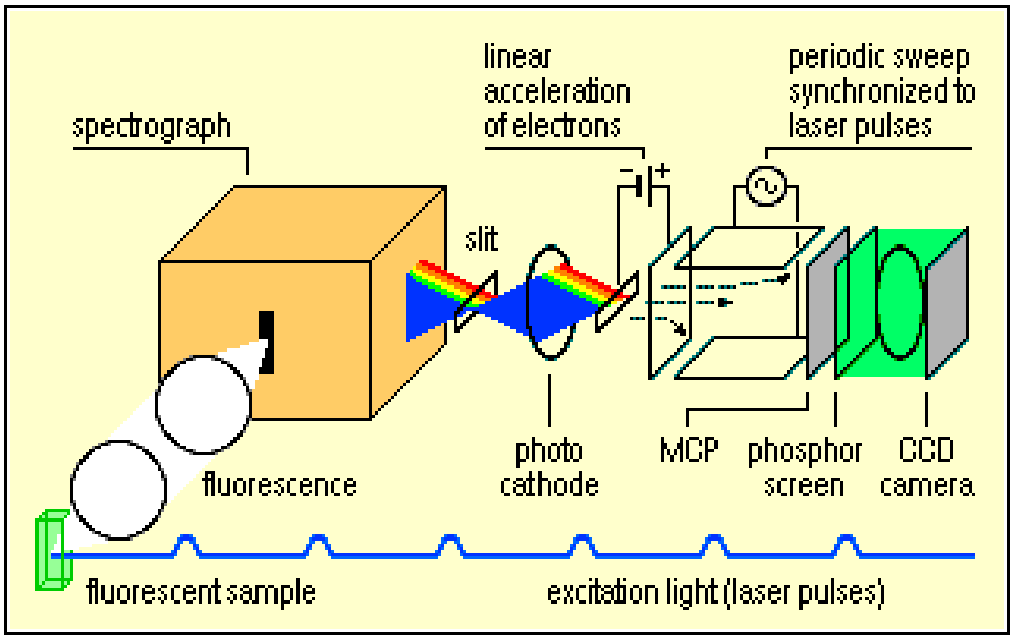
Materials and Methods

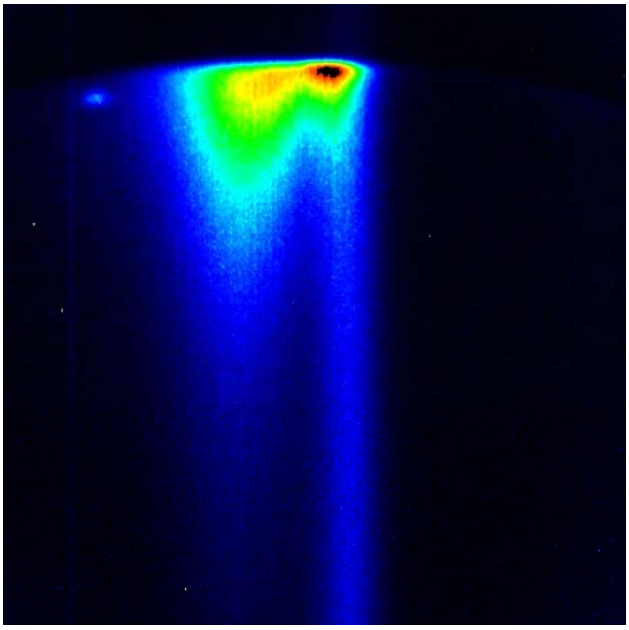
For the spectroscopic measurements, the isolated complexes were diluted in 20mM Bis-Tris (PH 6.5), 20 mM NaCl and 0.06% b-DM to an optical density of 0.6 at 680 nm. Measurements have been performed on two different batches of samples. For the low temperature steady-state measurements samples contained 66 % (v/v) glycerol was added as cryoprotectant and were played in a helium-bath cryostat (Ultreks, Ukraine) which was cooled down to 5K. For the time-resolved fluorescence measurements, 10 mM sodium ascorbate and 10μM phenazine metasulphate were added.

Steady state emission spectroscopy was performed with a ½ m imaging spectrograph and a CCD camera (Chromex ChromcamI) with a spectral resolution of 0.5 nm. A tungsten halogen lamp (Oriel) with a band-pass filter transmitting at 420 nm was used for excitation and the emission spectra were corrected for the wavelength dependent sensitivity of the detection system.

The time resolved measurements were performed with a streak camera described in detail by Gobets et al.(2001). Short excitation pulses of 400 nm (~100 fs) with vertical polarization were generated using a Titanium:sapphire laser (Coherent, VITESSE) with

regenerative amplifier (Coherent, REGA) and a double pass optical parametric amplifier (Coherent OPA) and a Berek compensator. The repetition rate was 150 KHz and the pulse energy of ~3 nJ in the sample. The fluorescence was detected at right angle with respect to the excitation beam through a polarizer at magic angle using a Chromex 2501S spectrograph and a Hamamatsu C5680 synchrotron streak camera. Streak images were recorded with a cooled Hamamatsu C4880 CCD camera. The exposure time per image was 10 minutes. The instrument response function was modeled as a Gaussian with FWHM of 4,5 ps. All emission spectra were corrected for the spectral sensitivity of the apparatus. The streakcamera set up and an image follow.





Results

Absorption spectra at 5K

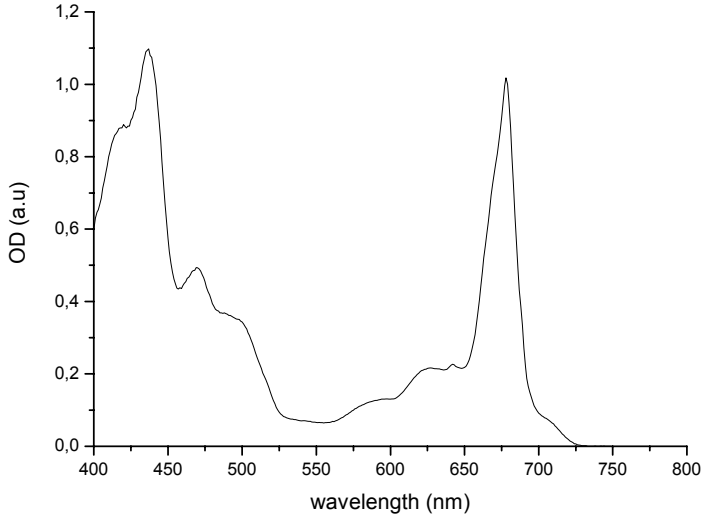


FIGURE 1
The 5 K absorption spectra of the wild type PSI. Spectrum has been normalized at 680 nm of the Q_Y absorption band

In Fig.1 the 5K absorption spectra of PSI wild is depicted. The 5 K absorption spectrum of the mutant is very similar. Spectra are normalized at the area of the Chl Q_y absorption band. There is an obvious similarity (not shown) between these spectra as also observed in previous work on mutants (*Ihalainen et al. (2002*). In order to extract more information we constructed the 5K difference absorbance spectra as shown in Fig.2. Preliminary analysis of the difference absorbance spectra PSI-J mutant minus difference spectrum shows clear minima and may suggest the exact wavelengths where the PSI-J bind Chl*a* molecules absorb (not shown). However, further analysis (correction for the scattering background) is necessary before stating these exact wavelengths.

Steady state emission at 5K

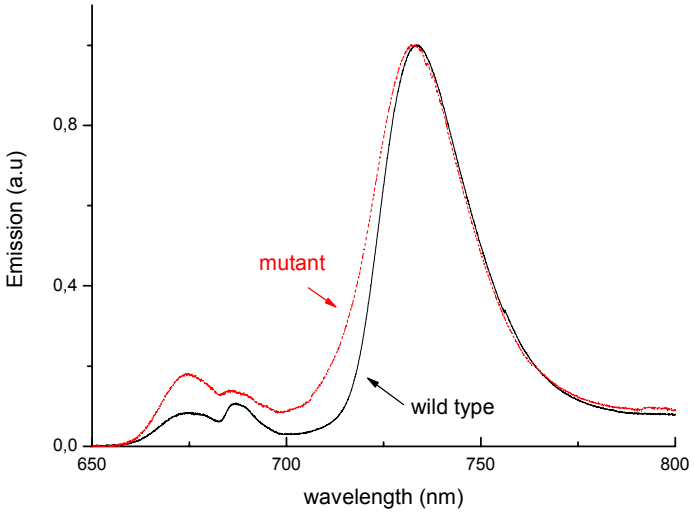


FIGURE 2
The 5 K emission spectra of the PSI wild-type and the PSI-J mutant. The spectra are normalized to the red wing of the spectra.

Fig. 2 shows the 5K fluorescence emission spectra of isolated PSI wild type (solid line) and PSI-J depleted (dashed line) particles from tobacco after Chl*a*-Soret band excitation at 420 nm. Green plant exhibits a red emission maximum at about 735 nm caused by the red-most pigments. The bands near 675 and 687 nm may be attributed to unconnected chlorophylls. Comparison between two different batches of samples showed that the spectral characteristics of these bands depend on sample preparation. The wider by 3 nm and somewhat blue shifted (by ~ 1nm) spectrum of the mutant indicates that there is a slightly more effective transfer of the excitation from the peripheral antenna to the core antenna in the wild type. This suggests also that the J- protein could contribute to the mechanism of the energy transfer of excitation to the core antenna but may not make not a major pathway as also suggested in the paper of Sener et. al (Sener *et. al 2005*)

Time-resolved fluorescence of PSI-J wild type and PSI-J mutant from tobacco.

Excitation dynamics in isolated PSI complexes were studied by measuring time-resolved fluorescence spectra after ultrashort 400 nm excitation pulses. At 400 nm Chl*a* pigments are mainly excited and accordingly to the stoichiometry of the pigments of green plant PSI-LHCI, about 70% of the excitations are transferred to the PSI core and about 30% to LHCI. For both the mutant and the wild-type, the images from both time bases were integrated together and analyzed using a global analysis method which estimated the decay associated spectra (DAS). The decay associated spectra (DAS) correspond to the amplitudes of the exponential decay components and the estimated DAS of the wild type and the mutant are shown in Fig.3. Data are fitted with five (sub)ps and two ns components as listed in Table 1 in similarity to the ones found in PSI complexes from *Chlamydomonas reinhardtii and A. thaliana* (*Ihalainen 2005ab, 2002*).

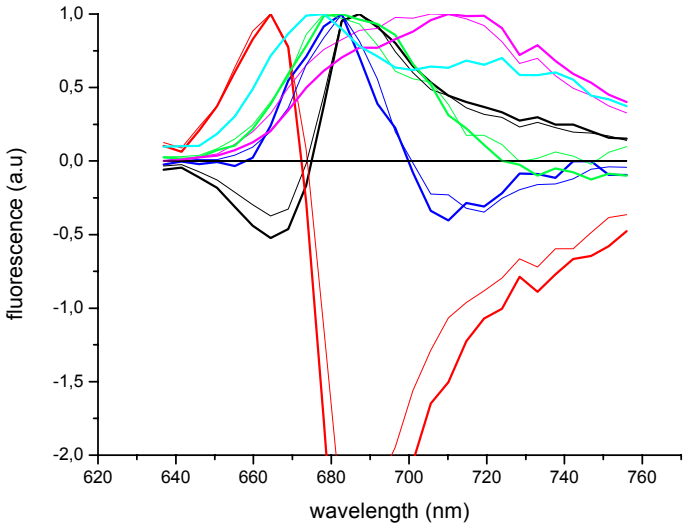


FIGURE 3
DAS of fluorescence decay of PSI wild-type and the PSI-J mutant

Table 1

Wilt type	0.64 ps	0.72 ps	5.1 ps	16.6 ps	61 ps	5 ns
J-mutant	0.64 ps	0.72 ps	5.1 ps	20.7 ps	62 ps	5 ns

The fastest component has a subpicosecond lifetime and exhibits a strong negative band in the Chl*a* Q_y absorption region. This is ascribed to the transition from the Soret-states to the Chl*a* Q_y –states.(*Gobets et al. 2001, Ihalainen 2005ab*) .

The second subpicosecond component represents the energy transfer rate from Chl *b* to Ch l*a*. Chl *b* molecules are possible to gain some excitation either directly via the laser pulse or via the Chl *a* Soret- to Chl *b* Soret transition. The energy transfer rate from Chl *b* to Chl *a* pigments is ultrafast and has been reported to range between 500 fs and 2 ps (*Gobets 2001*). As proved out this component was necessary in order to have a good fitting

The third component of ~5 ps represents energy equilibration between bulk chlorophylls and the red pigments of the PSI-LHCI. The size and spectrum is similar to previous studies characterized by a positive

The fourth component has a lifetime of about 20 ps differs slightly for the wild type and the mutant, it is positive at all wavelengths and it has been assigned as a trapping time of most of the PSI core pigments.

The fifth component of about 62 ps, differs slightly for the wild type and the mutant, it is positive at all wavelengths and indicates trapping of excitation mainly from LHCI pigments.

The last component of ~5 ns has a low amplitude and originates from uncoupled LHCI and uncoupled chlorophylls, that are unable to transfer their excitation energy to the reaction center

Acknowledgements

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