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Mutations of ligands to connecting chlorophylls perturbs excitation dynamics in the core antenna of PSI from *Chlamydomonas reinhardtii*

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Introduction

The structure of the PS I core antenna at 2.5 Å resolution is now available (Jordan *et al.* 2001). Ninety-six chlorophylls (Chls) have been identified in the crystals obtained from the cyanobacterium *Synechococcus elongatus*. Six Chls constitute two branches of initial electron transfer cofactors (ETC). The rest play a role of antenna. Almost all of the antenna Chls are located more than ~18 Å away from ETC. Only two Chls named aC-A40 and aC-B39 or connecting Chls (cCs) are closer to ETC: 12.8 Å and 10.9 Å, respectively. This structural organization suggests that cCs functionally bridge excitation energy transfer between the PS I bulk antenna and ETC. To test this hypothesis a double mutant of a green alga *Chlamydomonas reinhardtii* was construct that changed two histidine residues coordinating the two connecting Chls to leucines with the aim producing mutants in which the cCs are perturbed or removed. Consequently, energy transfer between antenna and ETC is expected to be impaired. The mutant and WT controls were studied by dynamic hole burning spectroscopy at RT and 10 K.

Materials and methods

Preparation of PS I particles. PS I particles from *Chlamydomonas reinhardtii* strains CC 2696 and KRC S1-3A (154-1A) Cbn1 fud7 (connecting Chls WT strain), as well as connecting Chls mutant obtained from the latter strain, were prepared from thylakoid membranes using a mild detergent isolation procedure described by Krabben et al. (2000). In the mutants, two histidines, His-730 of PsaA and His-714 of PsaB, were replaced by two leucines.

Femtosecond transient absorption measurements. 20 mM sodium ascorbate and 10 μM phenazine methosulfate (PMS) were added to samples to ensure efficient rereduction of oxidized primary donor. Additionally, at RT, the sample was placed in a transparent wheel rotating with a rotation frequency of ~2 Hz, so that each laser flash excited a fresh sample. For measurements at 10 K the sample was diluted with glycerol (1:2) and placed in a flat cuvete. The OD of the sample was typically between 1 and 1.2 at ~675 nm both at RT and 10 K. The experimental pump/probe setup was described by Freiberg et al. (1998). Spectrally

narrow (FWHM of ~5 nm) laser pulses with a ~150 fs duration were used to excite the sample with a repetition rate of 1 kHz. Low excitation energy was applied to avoid singlet-singlet annihilation. Transient absorbance spectra in the region between 630 and 750 nm were collected on two time scales: from –1 ps to 5 ps with a step size of 54 fs and from 5 ps to 100 ps with a step size of 2 ps. Decay associated spectra (DAS) were calculated from global fitting accounting for deconvolution of the recorded signals with the instrument response function (Gaussian function with a width of ~0.3-0.4 ps). In addition, the time *vs.* wavelength absorbance change surfaces were corrected for the spectral dispersion of the probe beam.

Results

Room temperature measurements. PS I particles were excited at 670 nm, 680 nm, 695 nm and 700 nm. In the mutant, independent of excitation wavelength, decay associated spectra (DAS) are dominated by a non-decaying (on 100 ps time scale) component of an amplitude about 3 times bigger than the DAS of a ~20 ps component ascribed to trapping. This is in contrast to CC 2696 WT control where the trapping component dominates. The spectra of the non decaying (ND) component in the mutant have maximum between 673-683 nm and are ascribed mainly to functionally uncoupled Chls, whereas in CC 2696 WT control, ND spectra peak at ~691 and are ascribed mainly to ($P700^+$-P700). The kinetic evolution and DAS of the connecting Chls WT strain are very similar to those of CC 2696 WT. Thus, the huge amount of uncoupled Chls in the mutant indicates decreased efficiency of energy transfer from bulk antenna to the ETC.

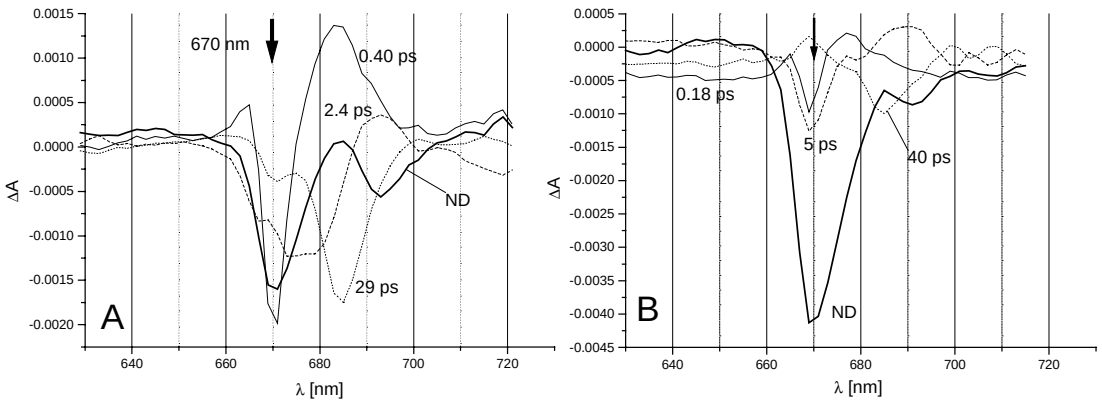

Fig. 1. Decay associated spectra found for WT (A) and mutant (B) excited at 670 nm at 10 K.

Low temperature measurements. At 10 K, the connecting Chls mutant and CC 2696 WT control samples were excited at 670 nm, 695 nm and 700 nm. Excitation at 670 nm results in four similar kinetic phases found in both preparations (Fig. 1A, 1B): two describing downhill energy transfer, one for the trapping process and one non-decaying component. Downhill energy transfer occurs biphasically, on subpicosecond (0.40 ps in WT and 0.18 ps in the mutant) and several picoseconds (2.4 ps in WT and 5.0 ps in the mutant) time scales. The slower phase describes energy transfer to more red shifted molecules compared to the subpicosecond phase, as seen from the peak positions of positive parts of the respective DAS. DAS of trapping components (29 ps in WT and 40 ps in the mutant) peak at 685 nm due to energy equilibration over a Chl pool absorbing mainly at this spectral region. In both, mutant and WT control, the ND spectrum has two maximum: at ~670 nm ascribed to uncoupled Chls and at ~692 nm ascribed to ($P700^+$-P700). However, the band at 670 nm is significantly more

pronounced in the mutant, thus confirming RT observation of a large number of uncoupled Chls. The relatively smaller but significant amplitude of the 670 nm band in WT control is probably caused by long living excited Chls that are not efficiently quenched by $P700^+$. It was shown that at low temperatures a significant amount of $P700^+$ accumulates (Schlodder et al, 1998).

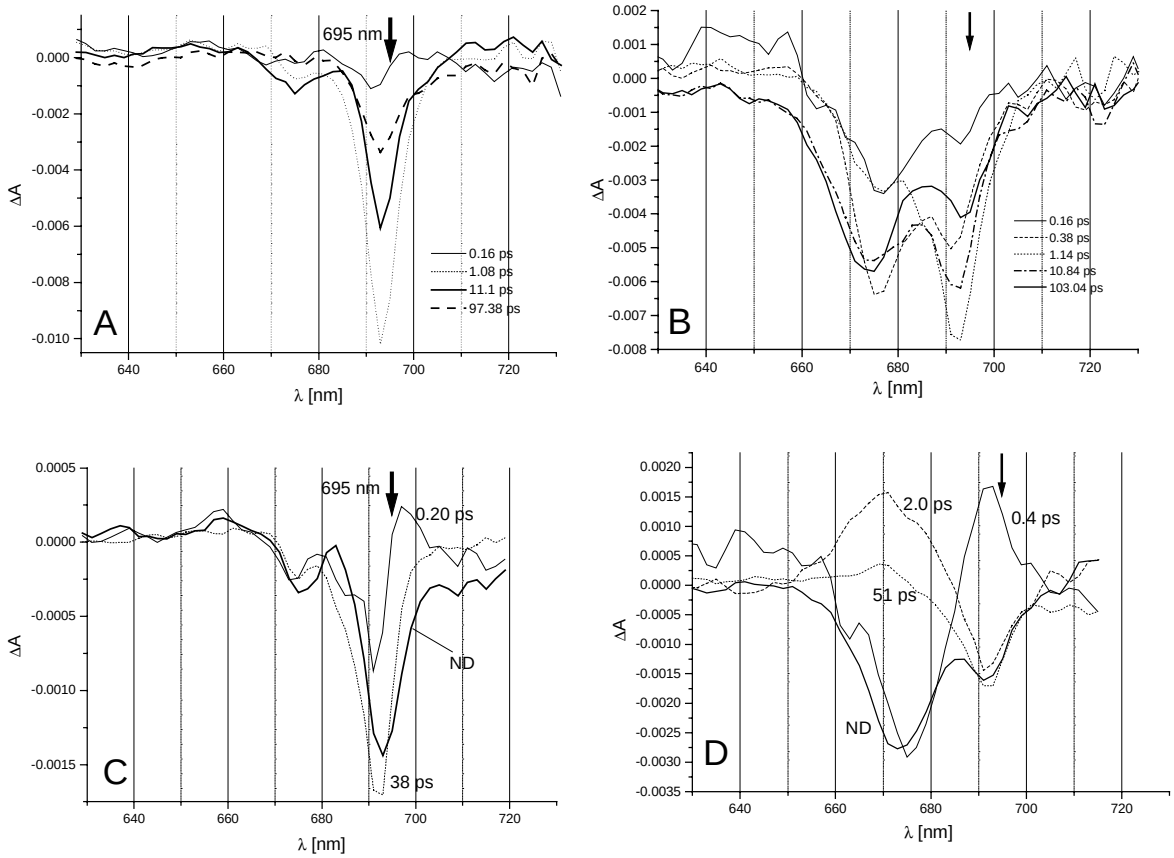

Fig. 2. Transient spectra (A, B) and decay associated spectra (C, D) for WT (A, C) and mutant (B, D) excited at 695 nm at 10 K.

Excitation at 695 nm results in quite a different evolution of spectra in WT control and mutant (Fig. 2A and 2B). In the WT, time resolved transient absorption bands are narrow and centered at ~693 nm (Fig. 2A). Only a slightly pronounced additional band is present at ~675 nm. In the mutant, initial transient spectra are very broad and structured (trace 0.16 ps in Fig. 2B). At any time, two bands centered at ~675 nm and ~693 nm can be clearly seen. The relative amplitudes of these bands is changing with time. This evolution is better seen in Fig. 2D where DAS are presented. In contrast, in WT, evolution of spectra is much more limited (Fig. 2C). Excitation at 700 nm (not shown) in WT leads to wide initial transient absorption (as seen in the mutant PS I excited at 695 nm or 700 nm), but 2 ps phase seen in Fig. 2D is not found in the WT.

Discussion

Mutation of the residues coordinating the connecting Chls has been hypothesized to diminish effectivness of energy transfer between the bulk antenna and ETC. The way to examine the energy transfer in both directions was selective excitation of either bulk Chls or ETC and observation of the subsequent excitation dynamics. Pump pulses at 670 nm and 680 nm excite preferentially bulk antenna Chls, whereas these at 695 nm and 700 nm may excite the primary donor. Excitation of bulk Chls in the mutant leads to a huge amount of long living excited Chls which appear to be functionally uncoupled from the ETC (RT data and Fig. 1). This is interpreted in terms of impaired energy transfer from the bulk Chls to the ETC. Excitation at ≥700 nm (both in the mutant and WT; data not shown), and at 695 nm in case of the mutant (Fig. 2B), causes wide and structured initial transient spectra which have also been observed at RT in CC 2696 WT and interpreted in terms of direct excitation of ETC (Gibasiewicz et al.). As seen in Fig. 2D, a subpicosecond downhill energy transfer occurs (0.4 ps) followed by a 2 ps phase. In principle, the 2 ps phase could be ascribed to uphill energy transfer. However this is very unlikely at 10 K. A more probable explanation is that the appearance of photobleaching at ~670 nm is due to electron transfer. The only electron transfer reaction taking place on such a fast time scale is primary charge separation. Consequently, the species responsible for the photobleaching at 670 nm could be either $P700^{+}$ or A_0^{-}. Thus, direct excitation of the ETC in the mutant would result in impaired energy transfer to antenna.

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