Specific mutations of the PsaB methionine axial ligand to chlorophyll (A_0) of PSI in *Chlamydomonas reinhardtii*

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Introduction

Photosystem I (PSI) is a multi subunit protein-pigment complex that uses light energy to transfer electrons from plastocyanin to ferredoxin. Light energy absorbed by the antenna Chls is transferred to P700 where charge separation occurs between $P700^+$ and A_0^-. Electrons are then transferred sequentially from A_0 to A_1, F_X, F_A and F_B, and finally to the soluble acceptor ferredoxin. The 2.5 Å crystal structure of PSI indicates the presence of two potential electron transfer pathways, consisting of six chlorophylls, two phylloquinones and three Fe_4S_4 clusters (Jordan et al., 2001). The first pair of chlorophylls are P700 and the second pair auxiliary Chls. One or both of the Chl *a* molecules of the third pair of chlorophylls (ec-A_3/ec-B_3) probably represents the electron acceptor A_0. The A_0 Chl *a* molecules are coordinated by sulphur atoms of Met A688 and Met B668, respectively. This coordination is highly unusual, as interactions between the acid Mg^+ and the soft base S are weak. Coordination by Met may partially be responsible for the low redox potential of A_0. In *Chlamydomonas reinhardtii,* the ligands to A_0 are M684 and M664 of PsaA and PsaB. Because of its location in the structure, A_0 may play a role both in excitation energy transfer and electron transfer. Hence, an attempt has been made to study the effect of mutation of this Met residue in *C. reinhardtii* on the dynamics of energy and electron transfer in PSI.

Materials and methods

Site-directed mutagenesis was used to change the met 664 of PsaB either with histidine or serine using protocols described previously (Lee et al., 1998). Thylakoid membranes were isolated from wild type and mutant cells. The steady-state level of PSI was determined by LDS-PAGE. Following electrophoresis, the PSI complexes were either visualized as a green band at the top of the gel or stained with Coomassie blue (Cui et al., 1995). PSI activity was measured polarographically as light induced oxygen uptake in the presence of methyl viologen (2 mM), 2,6-dichlorophenolindol-phenol (1mM) and ascorbate (5mM). Femtosecond transient absorption measurements were performed at RT on isolated PSI samples. 20 mM sodium ascorbate and 10 µM phenazine methosulfate (PMS) were added to samples to ensure efficient rereduction of oxidized primary donor after each flash. The experimental pump/probe setup was described by Freiberg et al. (1998). Spectrally narrow (FWHM of ~5

nm) laser pulses centered at 670 nm, 695 nm and 700 nm with a ~150 fs duration were used to excite the sample with repetition rate of 1 kHz. Transient absorbance spectra in the region between 630 and 750 nm were collected up to 100 ps after excitation. Fluorescence measurements were performed with time resolution of about 5 ps as described by Causgrove et al. (1990).
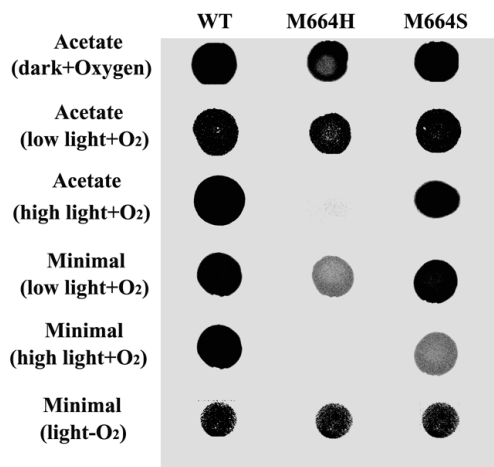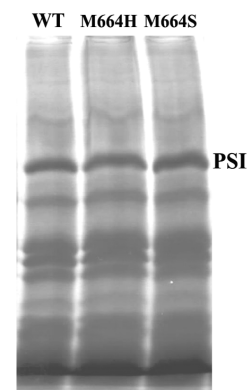

Fig.1. Spot tests of mutants of *Chlamydomonas reinhardtii* growing on acetate and minimal medium. Acetate indicates TAP medium and supports heterotrophic growth, whereas minimal medium will only support phototrophic growth. Colonies were allowed to develop in darkness and in 40 or 90 μmol photons m^{-2} s^{-1} light and in the presence or absence of oxygen as indicated.
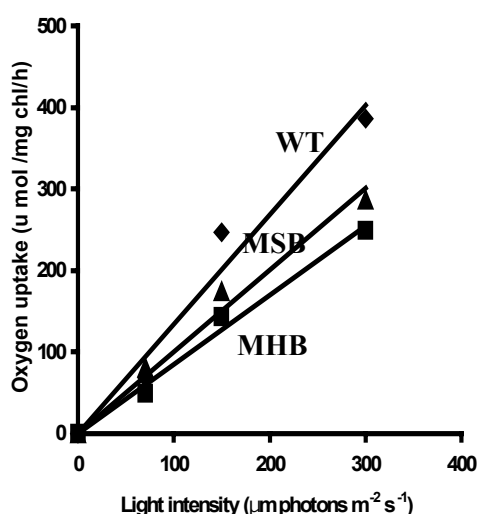
Fig. 3. Electron transfer activity and light harvesting efficiency of isolated PSI from wild type and mutants of **C. reinhardtii.**

Results

Growth characteristics. Fig.1 shows the results of spot tests of the growth of wild type and mutants under phototrophic and heterotrophic conditions. All strains were capable of heterotrophic growth in both darkness and low light (40μ mol photons m^{-2} s^{-1}) when acetate is supplied. But mutant MH (B664) could not grow heterotrophically under high light. Phototrophic growth was severely impaired in both mutants in the presence of oxygen. The histidine [MH(B664)] mutant failed to grow under high light and grew poorly under low light, whereas MS(B664) could grow slowly under high light. However, removal of the oxygen allowed the phototrophic growth of both mutants (Fig.1). This suggests a functional PSI complex is present in each mutant, but the site directed changes result in increased oxygen sensitivity.
PSI accumulation and activity. *As shown in Fig. 2, both the MH (B664) and MS (B664) mutants accumulate similar levels of PSI to wild type cells. However, the light harvesting efficiency was significantly impaired in histidine mutant (Fig. 3). Femtosecond transient absorbance measurements*. Dynamic hole-burning spectroscopy was applied to study excitation dynamics in both of the mutants at RT. Generally, similar kinetic phases were found from global analysis in the mutants as in the PS I from WT control [subpicosecond, ~2—~4 ps, 12-60 ps and non decaying (ND)]. Subpicosecond and ~2—~4 ps phases are ascribed to downhill (excitation at 670 nm) or


Fig. 2. Accumulation of PSI in thylakoid membrane of wild type and transformants

uphill (excitation at 695 nm and 700 nm) excitation energy transfer (see Fig. 4 for decay

associated spectra for PS I from histidine and serine mutants). A several tens of picoseconds phase is ascribed to trapping, and the ND spectrum to a mixture of the (P^+-P) transient absorption signal and photobleaching by the long living excited Chls. In PS I from the WT and serine mutant, the trapping time is in the range of 20-30 ps and is independent of excitation wavelength. However, in the histidine mutant, the trapping time depends on excitation wavelength and was found to be ~60 ps and ~12 ps at 670 nm and 700 nm excitation, respectively (compare Fig. 4A and 4B). In the ND spectrum, independent of excitation wavelength and sample, there are two peaks, one at ~678 nm assigned to uncoupled Chls, and the other at ~691 nm due to (P^+-P) photobleaching. In WT and in both mutants, the ratio of amplitudes at ~678 nm and ~691 nm is higher when exciting at 670 nm compared to 700 nm (Fig. 5). This is probably due to excitation of larger amounts of uncoupled Chls at 670 nm. The main difference between WT and the mutants is a higher contribution of the ~678 nm band in the ND spectra for the mutants (Fig. 5). Single photon counting (SPC) measurements also indicated a higher amount of uncoupled Chls in the mutant PS I than in the WT. In the serine and histidine mutants, about ~30% and ~40% of the total initial amplitude is due to components of lifetime >1.5 ns, whereas only ~20% of long living components was found in wild type PSI.
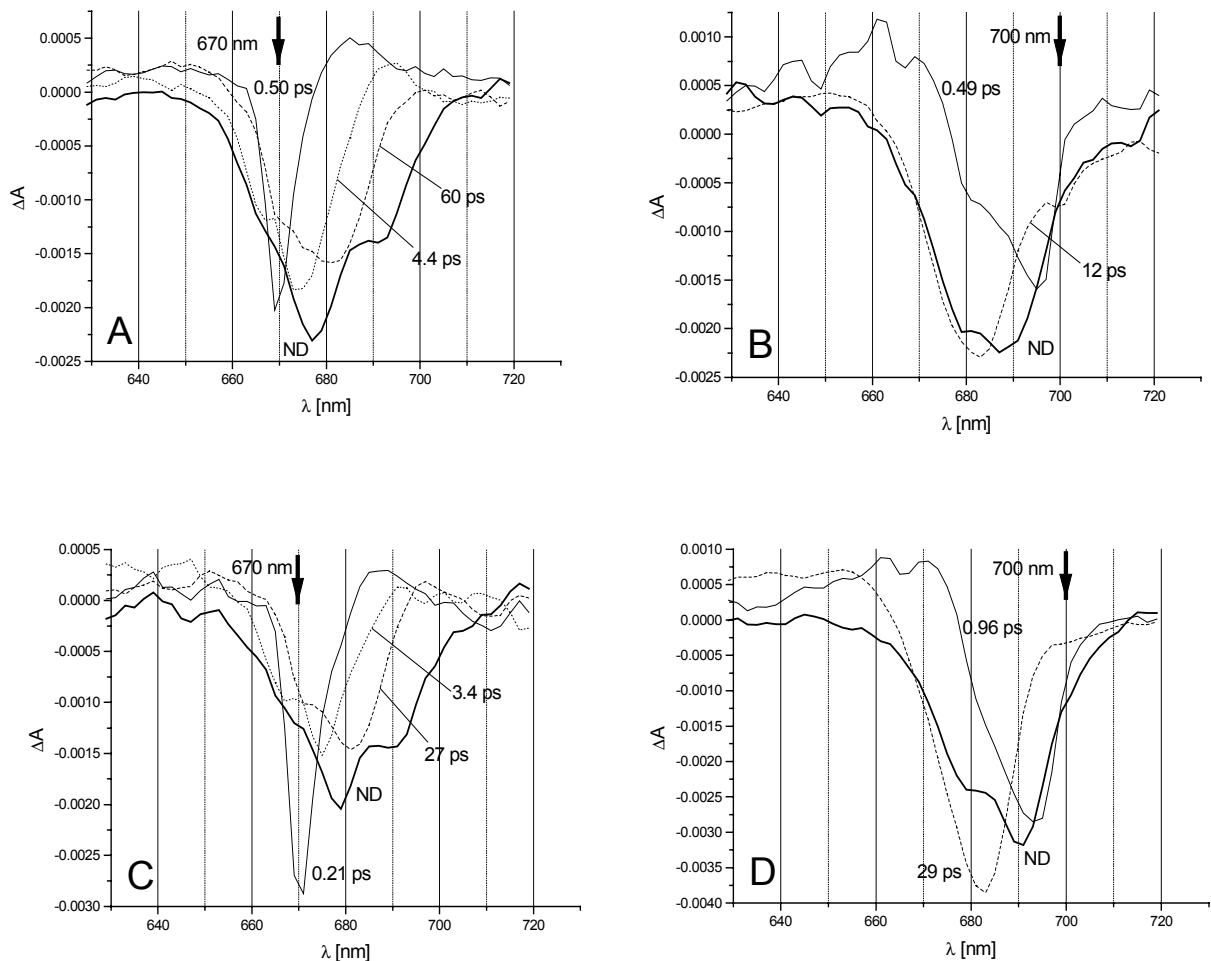
Fig. 4. Decay associated spectra of PS I histidine mutant (A, B) and serine mutant (C, D) at excitation wavelengths 670 nm and 700 nm.
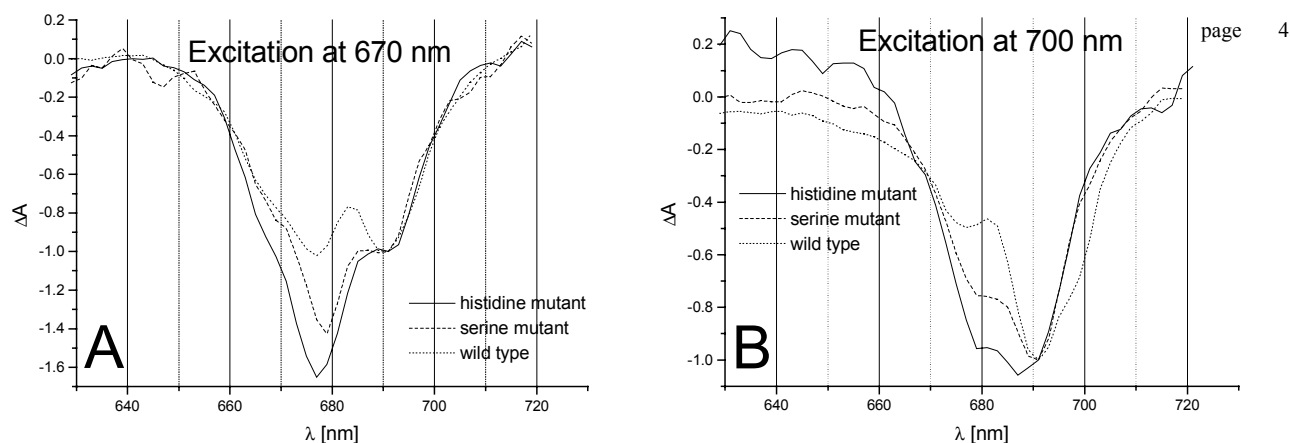
Fig. 5. Comparison of non decaying spectra normalized to the same amplitude at 691 nm for PS I mutants (replotted from Fig. 1) and PS I WT at excitation wavelengths 670 nm (A) and 700 nm (B).

Discussion

Replacement of methionine 664 results in significant alteration in growth phenotype. However, both mutants grow anaerobically suggesting that they have increased sensitivity to photo oxidative damage (Fisher et al., 1997 and Purton et al., 2001). The precise cause of photoinhibtion of photosystem I is unclear. The fact that the both MH(B664) and MS(B664) mutants grow under anoxygenic conditions indicates the growth inhibition is likely due to transfer of electrons to oxygen. Both mutants accumulate similar levels of PSI to wild type but exhibit a lower rate of electron transfer activity. Moreover, the light harvesting efficiency was also significantly impaired in both mutants. The cause of growth inhibition and decrease in light harvesting efficiency may be related to altered physical properties A_0. Comparison of transient absorption spectra of PSI from WT and mutants reveals some distinct differences. The main difference between WT and mutant is higher contribution of a ~678 nm band in the ND spectra for the mutants excited at 670 nm   (Fig. 5). Both femtosecond and SPC measurements indicate that there are more uncoupled Chls in the mutants, especially in histidine mutant, than in the WT. In the histidine mutant, selective excitation of bulk Chls at 670 nm results in an increasing of the trapping time (Fig. 4), perhaps indicating a worse coupling between antenna and electron transfer chain (ETC). An alternative or additional explanation for slower trapping may be a lower rate of primary charge separation between primary electron donor (P700) and mutated A_0 Chl.

Acknowledgement

References

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