CHAPTER TEN

$A_0 \rightarrow A_1$ Electron Transfer in *Chlamydomonas reinhardtii* PS I with Replaced A_0 Axial Ligand

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Abstract Replacement of methionine, the natural axial ligand to the primary electron acceptor (A_0) in Photosystem I, with a series of different amino acids results in dramatic increase of the A_0^- lifetime from ~20 ps in wild type to a few nanoseconds in the mutants in the case of *Chlamydomonas reinhardtii* (Ramesh et al. 2004, 2007). This effect is similar independently if the mutation affects A-side or B-side A_0. This observation confirms an existence of two equivalent primary electron acceptors in both symmetric branches of Photosystem I in *Chlamydomonas reinhardtii*, which makes this photosystem unusual among other photosystems (from purple bacteria, PS II), which are essentially unidirectional. However, it is still not clear if the bidirectionality of electron transfer in Photosystem I is complete, i.e. if the electron from A_0^- reaches A_1 in both branches or takes another route in the "non-active" branch. In order to solve this issue, in this contribution we attempted to compare kinetics of A_0^- reoxidation to the kinetics of A_1^- formation in the case of B-side A_0 mutant with methionine replaced by serine.

Keywords Photosystem I, electron transfer, primary electron acceptor A_0, mutants, femtosecond transient absorption

Introduction

In the literature there is no consensus on a few very basic issues related to the primary electron transfer reactions in Photosystem I. First one is the nature of the primary electron donor. Traditionally, a chlorophyll *a* dimer called P700 is thought to play this role. However, in a recent model it was proposed that the first electron transfer reaction in PSI occurs from an accessory Chl to the primary electron acceptor, Chl A_0 (Holzwarth et al. 2006).

Another important issue is the secondary electron transfer from Chl A_0 to phylloquinone A_1. In normal PSI this reaction occurs within ~20 ps (Hastings et al. 1994; Brettel and Vos 1999) which is a value similar to that of exciton lifetime in PSI core. It was hypothesized that methionine axial ligand to Chl A_0 was responsible for the fast electron transfer between

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J.F. Allen, E. Gantt, J.H. Golbeck, and B. Osmond (eds.), *Photosynthesis. Energy from the Sun: 14th International Congress on Photosynthesis*, 65–68.

A_0^- and A_1 (Ramesh et al. 2007). Indeed, replacement of methionine ligand with His, Ser, Leu, and Asn resulted in increased lifetime of A_0^-. In the case of cyanobacterial PSI, electron transfer from A_0^- to A_1 with methionione in branch A (but not in branch B) replaced by Leu or Asn was measured to be 100 ps (Dashdorj et al. 2005). The lifetime of A_0^- in PSI from *Chlamydomonas reinhardtii* was reported to be extended to 1–2 ns in the samples with the methionine replaced by His, Ser, and Leu in either of two branches of electron transfer cofactors (Ramesh et al. 2007). However, in the case of *C. reinhardtii* it was not shown that after 1–2 ns the electron from A_0^- was indeed transferred to A_1. Therefore, in this contribution we describe our attempt to measure the kinetics of both decay of A_0^- and formation of A_1^-.

Materials and methods

The transient absorption measurements were performed for MS(B664) mutant of PSI in which the methionine axial ligand to primary electron acceptor A_0 (M, the 664-th residue in subunit B of PSI) was replaced by serine (S). The sample was excited by flashes provided by NOPA (noncollinear optical parametric amplifier) and absorbance changes were probed by white light generated in a calcium fluoride plate. The 800-nm laser pulses with ~100-fs duration produced by Spectra Physics 1-kHz laser system were used for pumping NOPA and for white light generation. The transient absorption signal was detected by the CCD camera. The experiment was carried out in room temperature with time window limited by 1-ns delay line. The excitation energy was estimated to 0.5 μJ/pulse. The primary donor was kept neutral during repetitive excitation by addition of 20 mM sodium ascorbate and 20 μM phenazine methosulfate (PMS).

Results and discussion

The decay of A_0^- can in principle be measured both in Q_y and Soret regions as a decay of photobleaching caused by reoxidation of A_0^- (Hastings et al. 1994; Mi et al. 1999; Ramesh et al. 2004, 2007). Two panels in Fig. 1 show transient absorption spectra measured in Q_y region, 300 ps and 1 ns after excitation of MS(B664) PSI mutant at 400 nm. For comparison, transient absorption spectra measured 300 ps after excitation of PSI WT at 695 nm are shown. The difference between these two traces with a minimum at ~682 nm in each of the panels is assigned to accumulation of (A_0^- - A_0) in the mutated branch B (compare to Ramesh et al. 2004, 2007). Interestingly, the maximum of ($P700^+$-P700) photobleaching is red-shifted in the case of the mutant to 697–698 nm (691 nm in WT). This phenomenon was not observed before for any A_0

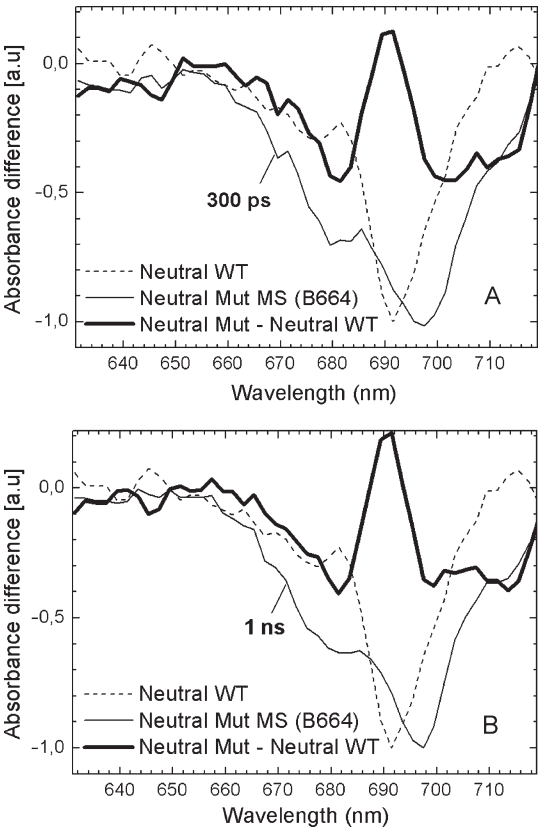

Fig. 1 Absorption difference spectra of MS(B664) (thin solid lines), recorded 300 ps (**A**) and 1 ns (**B**) after ~100-fs excitation at 400 nm at room temperature. Absorption difference spectrum of WT (dashed lines) was recorded after ~150-fs excitation at 695 nm at room temperature. The (Neutral Mut – Neutral WT) difference (thick solid lines) should correspond to accumulation of (A_0^- - A_0) in the mutated branch B