S22-006

The analysis of site-directed mutants in the glutamate 189 and histidine 190 in the D1 protein of photosystem II

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Keywords: PSII, charge separation, reaction center, *Chlamydomonas*

Introduction

The primary charge separation in photosystem (PS) II takes place at the special pair of Chl *a* (P680) in the reaction center pigment-protein complex that consists of the D1 and D2 proteins [Nanba & Satoh 1987][Tomo et al. 1997]. The D1 and D2 proteins show some homologies in their amino acid sequences to each other and also to the L and M subunits of the purple bacterial reaction center, whose three dimensional structure has been determined by X-ray crystallography. Recently, 2D and 3D crystallographic structural analyses of the PS II complex have been reported [Rhee et al. 1998][Zouni. et al. 2001]. These studies have demonstrated that the locations of the transmembrane helices of the D1 and D2 proteins fit those of the bacterial L and M subunits. A ligand for P680 is considered to be histidine198 of the D1 protein. A ligand for an accessory chlorophyll has been assumed to exist on the *cd*-helix of the D1 protein, although it was not determined unequivocally. In the present study, we characterized several mutants in the putative *cd*-helix of the D1 protein of *Chlamydomonas reinhardtii* at glutamate-189 and histidine-190, and studied the effects of amino acid substitutions on the PSII functions. The results indicate that both charge separation efficiency and oxygen evolution capability are markedly modified in mutants E189D and H190R.

Materials and methods

Introduction of the specific codon changes on the *psbA* gene were previously described [Svensson et al. 1998]. The transformants of a *psbA* deletion strain was characterized by oxygen evolution assay and thermoluminescence measurements. For preparing the reaction center samples, the mutagenized *psbA* genes were introduced into a photosystem I deficient strain carrying a His-tagged D2 protein. PS II reaction center was isolated using a Ni^{2+} chelate column and a DEAE anion exchange column according to the methods of Sugiura et al. and Nanba & Satoh. Oxygen evolution of *Chlamydomonas* cells was measured with a Clark-type oxygen electrode in TAP medium. Thermoluminescence glow curves were recorded with a home-built apparatus [Ono 2000]. Cells suspended in TAP medium were incubated in the dark for 20 min at 5 ˚C, added 10μM DCMU, and then excited at –10˚C by a saturating Xe flash (≤ 3μs duration). Time resolved fluorescence spectra were collected in photon counting mode with a model C4334 Streakscope (Hamamatsu Photonics). Signal was acquired at a frequency of 1MHz, synchronized with a laser diode pulse (650nm) for excitation. Photochemical accumulation of the reduced pheophytin was monitored by absorption change at 450 nm during illumination of saturating continuous light (> 650

nm) at 15 ˚C. The reaction mixture contained methyl viologen (2μM) and sodium-dithionite (2mg/ml).

Results

Table 1 shows the effects of mutations on the capability of photoautotrophic growth and oxygen evolution in *Chlamydomonas* cells. Mutant E189Q was able to grow photoautotrophically with slightly decreased oxygen evolution. Mutant E189L showed considerably reduced rate for photoautotrophic growth and oxygen evolution. Mutants E189D and H190R were absent of oxygen evolution and did not grow photoautotrophically. Figure 1 shows the thermoluminescence (TL) glow curves of wild-type and mutant cells used in this study. The wild-type cells showed TL Q-band at approximately 15 ºC, arising from $S_2Q_A^-$ charge recombination in the presence of DCMU. Mutant E189L showed a TL band with decreased intensity and slightly elevated peak temperature. Mutants E189D and H190R showed no TL band corresponding to the Q-band, indicating that S_2-state is not formed in these mutants, in consistent with no oxygen evolution capability in these mutants. For further detailed characterizations, the PS II reaction center (His-tagged) was isolated using Ni^{2+} and DEAE anion exchange columns. The absorption maximum in red-region spectra of the H190R and E189L reaction centers were shifted toward blue by 2-3nm compared to the wild-type center (data not shown).

Table 1
Photoautotrophic growth and oxygen evolving activities of the D1 mutant cells

Strain	Photoautotrophic Growth	O2 Evolution (μmol/mg chl/h)(%)
WT	++	181 (100)
E189Q	++	124 (69)
E189L	+/–	57 (31)
E189D	–	– (0)
H190R	–	– (0)

Figure 3 shows the effect of H190R mutation on the accumulation of the anionic radical of pheophytin during continuous illumination at 15 ˚C. The amount of pheophytin$^-$ accumulated in the mutant reaction center was 60 % of that in the wild-type reaction center. The decrease in the amount of pheophytin$^-$ accumulation may be ascribed to some alterations in P680 induced in the mutant. The change of the properties of PS II reaction center in the mutant is compatible with the findings that time-resolved fluorescence spectra showed that the fluorescence (at 695 nm) lifetime at 77K was increased by twofold in E189D, E189L and H190R cells (data not shown).

Discussion

The present results demonstrate that the oxygen evolving activity was lost and no S_2-state related TL band was induced in mutants E189D and H190R of the D1 protein, indicating that the oxygen evolving complex (OEC) is not functional in these mutants. It has been reported that mutation of histidine 190 resulted in change in the redox potential of Yz [Hays et al. 1998][Mamedov et al. 1998]. Since glutamate 189 is an amino acid residue adjacent to histidine 190, mutation of glutamate 189 may also affect the redox properties of Yz to impair the OEC function. Conceivably, changes of Yz may be ascribed to the impairment of OEC in mutants E189D and H190R. Apparently, Q or L substitutions at E189 will not affect the Yz properties. Interestingly, the present results showed that the functions of the reaction center are also modified in mutants E189D, E189L and H190R, as revealed by the increase of fluorescence lifetime and the decrease of the photo-accumulation of pheophytin$^-$ in mutant H190R. These may be attributable to lower efficiency of the charge separation activity by alteration of the redox potential of P680, induced by structure modifications of P680 and/or accessory chlorophyll. Since mutant E189L evolves oxygen, these changes occurred in the reaction center may not responsible for the impairment of OEC function in the mutants primarily.

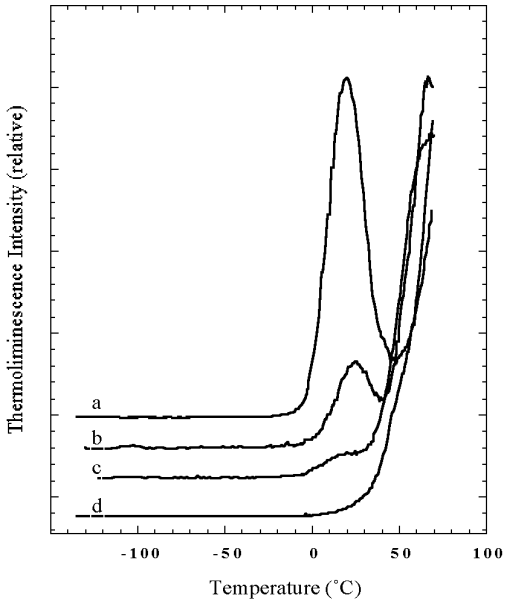

Fig.1 Thermoluminescence glow curves after a single turnover flash illuminatiopn
Q-band of control (a);E189L (b);E189D (c) and H190R (d)

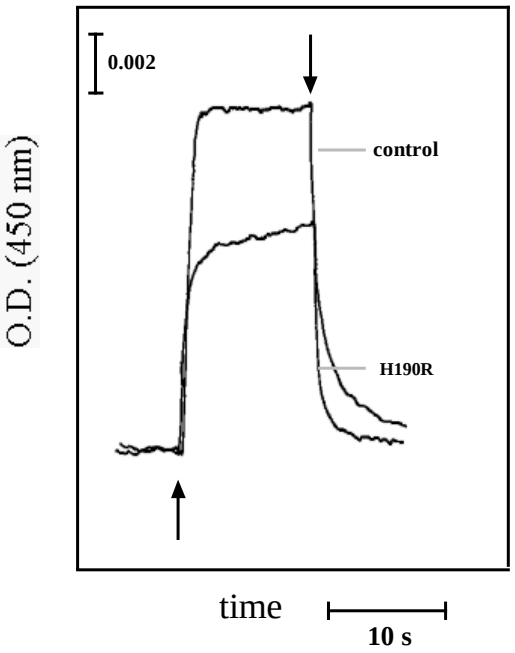

Fig. 2 Kinetic responce of the absorbance changes at 450 nm. Arrows downward and upward indicate the time that the light was switched on and the time it was switched off, respectively.

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