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Contribution of SQDG in photosystem II of *Chlamydomonas reinhardti*

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Introduction

Photosystems (PS) are supermolecular complexes constructed with many protein subunits, chlorophylls, quinones and metals, therefore, multiple interactions like chlorophylls-protein interaction should be required for their functions. Photosystems are embedded in thylakoid membranes of photosynthetic organisms, and lipid-protein interactions are also crucial for their functions. Thylakoid membranes are mainly consist of monogalactosyl diacylglycerol and digalactosyl diacylglycerol, phosphaticylglycerol and sulfoquinovosyl diacylglycerol (SQDG). The composition of lipid classes in thylakoid membranes is almost conserved among O_2 evolving type photosynthesis organisms. The amount of SQDG is almost under 10% of thylakoid membrane lipids, but sulfoquinovose residue is distinctive from other glyocolipids. SQDG defective mutant (*hf*-2) of *Chlamydomonas reinhardtii* was isolated by UV irradiation (*Sato, N. et al.,1995a*). The growth rate in *hf*-2 was slightly slower than that in the wild type and O_2 evolving rate measured with *p*-benzoquinone in *hf*-2 was 60% to that in the wild type (*Sato, N. et al.,1995b*). Additionally, thylakoid membranes in *hf*-2 were extremely curved.

Materials and Methods

Materials and culture: *Clamydomonas reinhardtii* CC125 (mt+) and *hf*-2 (mt-) in F5 population were basically used in this report. The culture was as written in (*Sato et al., 1995a*) except bubbling with 2% CO_2. Cells for an observation with a transmission electron microscopy were cultured with an ordinary air containing 0.04% CO_2.

Conditions of photoinhibition and recovery: Cells corresponding to 1μg chlorophyll·ml^{-1} were exposed with each light intensity in oblong glass vessels at 28 ˚C with bubbling as photoinhibitory treatments. In the recovery experiments, cells incubated under growing light (90μE m^{-2} s^{-1}) at 28 ˚C, with bubbling after cells treated with 2500μE m^{-2} s^{-1} until PSII activity decreased to 50%.

Measurement of PSII activity: PSII measurements in cells was as written in (*Sato et al., 1995a*), and the preparation of thylakoid membranes for PSII measurements was according to (*Roffey et al., 1994*). PSII activity in thylakoid membranes was also determined with essentially the same as that in cells. 2,6-dichlorophenolindophenol (DCIP) reduction rate was

measured spectroscopically with Mn-depleted thylakoid membranes in the presence of diphenylcarbazide (DPC). The values of these reactions are the means of two independent experiments.

Observation with transmission electron microscopy: The observation with transmission electron microscopy was performed according to (*Nozaki, H. et al,* 1994) with minor modifications.

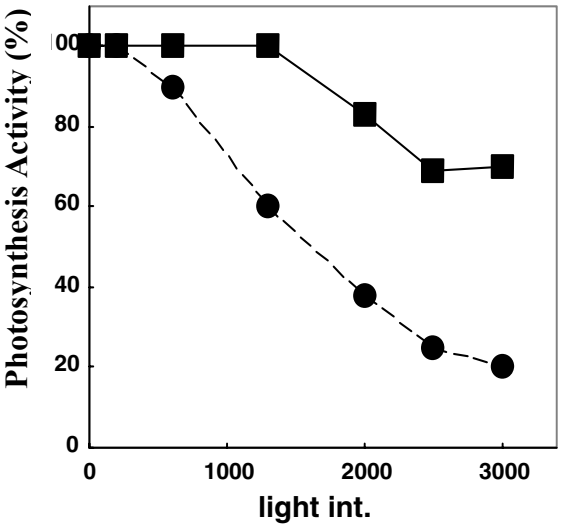

Fig. 2 Photoinhibition after 30 min exposure to different light intensity. Photosynthetic activities were measured with 5mM $NaHCO_3$ in wild type (squares) and *hf*- 2 (circles). Values are the means of two independent experiments.

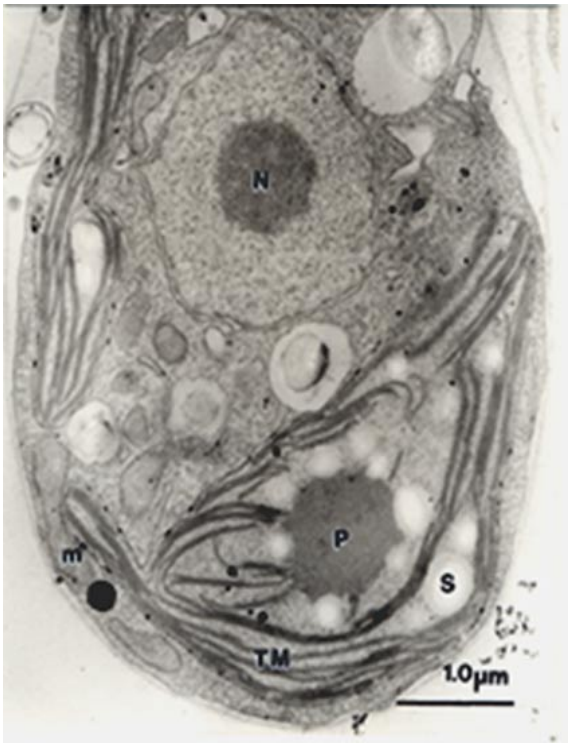

Fig.1 Transmission electron micrographs of *hf*-2 in *C.reinhardtii* N:nucleus, P:pyrenoid, TM:thylakoid membranes, m:mitochondrion

Results and Discussion

Genetic background of *hf*-2 was ensured by crossings with the wild type. As the results, *hf*-2 showed lowered PSII activity compared with the wild type (Table1), though, there was no difference in the innercelluler structure between *hf*-2 and the wild type (Fig.1). The growth rate of *hf*-2 delayed slightly compared with the wild type under 2% CO_2 condition where release CO_2 limitation. Since *hf*-2 seemed to be sensitive to the high light under growth condition, photosynthetic activity was measured in cells that treated with each light intensity for 30min (Fig.2). The inhibition of photosynthetic activity was enhanced in *hf*-2 compared with the wild type as higher light intensity and *hf*-2 suffered from photoinhibition at only 500μE·m^{-2}·s^{-1}. Time course of PSII activity at 500μE·m^{-2}·s^{-1} showed that photoinhibition was occurred not only in hf-2 but also in the wild type (Fig.3). The loss of PSII activity in hf-2 was greater than that in the wild type in the presence or absence of lincomycin. Preliminary experiments showed

Reaction (donor-acceptor)	CC125	*hf*-2
H_2O-MV[a]	286	212
H_2O-BQ[a]	269	189
DPC-DCIP[b]	64	64

a:μmoles 0_2 mg Chl^{-1} hr^{-1}
b:μmoles reduced DCIP mgChl^{-1} hr^{-1}

Table1 Summary of partial reactions in the wild type and *hf*-2 in *C.reinhardtii.*

the difference clearer at 2000μE·m^{-2}·s^{-1}. Unstable PSII complex in *hf*-2 due to missing SQDG may cause faster degradation of D1 protein than the wild type. Compared with the wild type, recovery from photoinihibition in *hf*-2 was also delayed slightly at the early phase of recovery

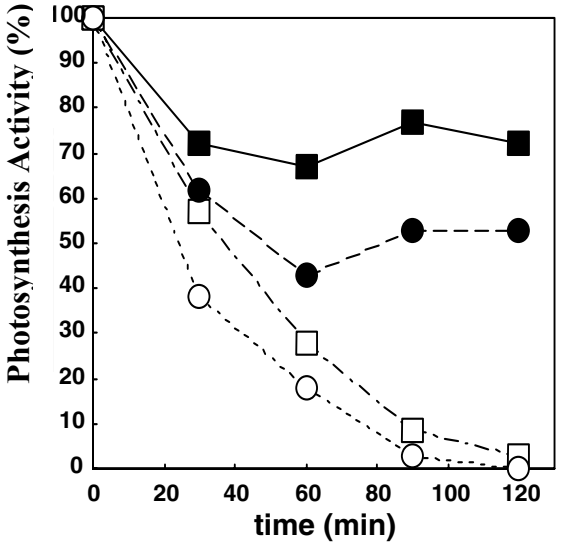

Fig. 3 Time courses of photoinhibition at 500 μE m^{-2}s^{-1} in the absence or presence of 250μg/ml lincomycin. PSII activities were mesured with 0.5mM *p*-benzoquinone in wild type (squares) and *hf*-2(circles) treated with lincomycin (open symbols) or not (closed symbols).

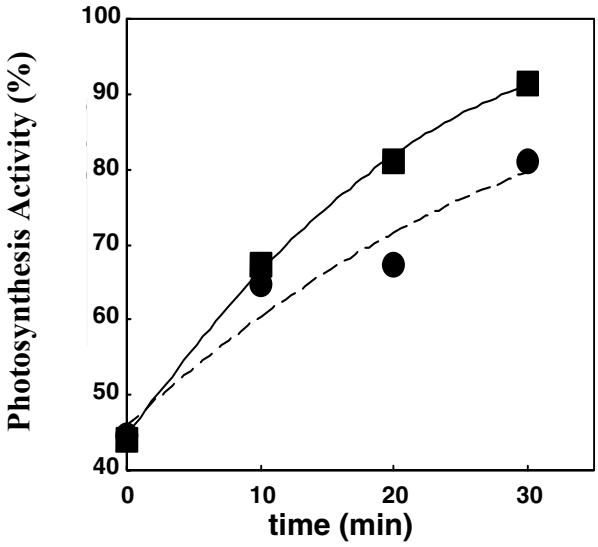

Fig. 4 Time courses of recovery at 90μE m^{-2} s^{-1} from photoinhibition in the wild type (squares) and *hf*-2 (circles). The reaction conditions were the same as the legend to Figure 3.

(Fig.4), and the recovery was inhibited by lincomycin (data not shown). D1 protein was degraded faster in *hf*-2 and its regeneration was slower than the wild type, suggesting SQDG stimulated the turnover rate of D1 polypeptide. Presumably SQDG interacts with PSII complexes. The incubation of 0.75mg chlorophyll ml^{-1} thylakoid membranes isolated from *hf*-2 with 100μM SQDG in the buffer on ice for 10 min raised the PSII activity from 210±7 μmoles O_2 mgChl^{-1} hr^{-1} to 281±8 μmoles O_2 mgChl^{-1} hr^{-1} which was almost the same rate in the wild type. The lowered PSII activity in *hf*-2 may be caused from the conformational change in PSII complex, not from the change of peptide composition in PSII complex, because there was no difference in the peptide composition between the wild type and *hf*-2 (data not shown). Furthermore, *hf*-2 is more sensitive to DCMU than the wild type, and the surroundings of Q_B site in PSII complex change (data not shown). The reduction rate of DCIP means the efficiency of electron transport from Z to Q_B in the reaction center when DPC is used as an electron donor. The electron transport from DPC to DCIP in *hf*-2 was as efficient as that in the wild type (Table1), while the environment of the Q_B site was affected in *hf*-2. The lowered PSII activity in *hf*-2 may be derived from the decline of the electron donation to Z by the conformational change in PSII complex.

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