Decrease in the efficiency of the electron donation to tyrosine Z of photosystem II in an SQDG-deficient mutant of *Chlamydomonas*

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Abstract Photosystem (PS) II activity of a sulfoquinovosyl diacylglycerol (SQDG)-deficient mutant (*hf*-2) of *Chlamydomonas* was partially decreased compared with that of wild-type. The susceptibility to 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) was also modified in the mutant. Photometric measurements in the isolated thylakoid membranes of *hf*-2 revealed that the lowered activity in the mutant was derived from a decrease in the efficiency of the electron donation from water to tyrosine Z, not from the efficiency of the electron transport from Q_A to Q_B. This result was confirmed by the decay kinetics of chlorophyll fluorescence determined in vivo. We conclude that SQDG contributes to maintaining the conformation of PSII complexes, particularly that of D1 polypeptides, which are necessary for maximum activities in *Chlamydomonas*.

Key words: Sulfoquinovosyl diacylglycerol; Photosystem II; Thylakoid membrane; Glycolipid; *Chlamydomonas*

1. Introduction

Photosynthetic organisms obtain the chemical energy for their activities by the conversion of light energy. Since the energy conversion is driven by the photosynthetic electron transport on thylakoid membranes of chloroplasts, the organization and dynamics of photosynthetic electron transport are never accomplished without a close relation between protein complexes of the photosynthetic electron transport chain and lipids of thylakoid membranes [1]. Sulfoquinovosyl diacylglycerol (SQDG) is a unique glycolipid with a sulfoquinovose residue present in thylakoid membranes of chloroplasts, Rhizobiaceae and most cyanobacteria [1,2]. SQDG usually forms less than 10% of the total glycolipids. We have isolated an SQDG-deficient mutant of *Chlamydomonas reinhardtii* (*hf*-2) to learn the role of SQDG in the photosynthetic electron transport. The growth rate of *hf*-2 was slower than that in the wild-type, and the photosystem (PS) II activity was about 60% of that of the wild-type when it was measured with *p*-benzoquinone as an electron acceptor. The lowered activity in *hf*-2 was restored by the addition of SQDG to the cells, so that SQDG was indispensable for the maximum PSII activity in *Chlamydomonas* [3].

The lowered activity in *hf*-2 was a consequence of the impairment in the reaction center of PSII, judging from the fact that the antenna size of PSII and the efficiency of the electron transport from antenna chlorophylls to the reaction center of PSII were not altered [2]. Neither a lack of any polypeptides in the PSII complex nor a decrease in the amount of PSII complex was observed in *hf*-2 [3], although the amount of lipids around PSII complexes was increased [4].

Taking the high sensitivity of the PSII activity to 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) in *hf*-2 cells into consideration, SQDG may interact with the PSII complex to maintain the complex in the proper conformation in thylakoid membranes. In fact, the incubation of thylakoid membranes of *hf*-2 with SQDG on ice resulted in an increase of the PSII activity without de novo protein synthesis [5]. We will propose the hypothesis that SQDG is indispensable for maximum PSII activity in *Chlamydomonas*.

2. Materials and methods

C. reinhardtii CC125 (mt+) and *hf*-2 (mt−) were grown under the same conditions as described in [5]. Culture medium was supplemented with 8 μM SQDG when indicated. The measurements of PSII activities in thylakoid membranes were carried out as described in [5] using 0.5 mM quinones or 1 mM ferricyanide as an electron acceptor. The activities of PSII that had been treated with NH_2OH or NaCl were measured with *p*-benzoquinone according to [6]. The reduction rate of 2,6-dichlorophenol indophenol (DCIP) was measured by the two-wavelength double beam spectrophotometer (Model 356, Hitachi, Japan). Mn-depleted membranes were obtained by the method described in [7]. Reaction mixture contained 50 μM DCIP, 1 mM diphenylcarbazide (DPC), thylakoid membranes corresponding to 1.5 μg chlorophyll/ml, 25 mM HEPES (pH7.6), 1 mM $MgCl_2$. In the case of Mn-depleted membranes, 0.4 M sorbitol was added to the mixture. Fluorescence measurements were performed using a PAM fluorometer (PAM101/102/103, Heinz Walts, Effeltrich, Germany) according to [8]. To measure the fluorescence decay, a single actinic flash was given by the attached XE-STC xenon flash lamp unit (Heinz Walts). Cells corresponding to 1 μg chlorophyll/ml were incubated for 5 min in the dark before measurements.

3. Results

To investigate the surroundings of Q_B site, PSII activities of

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Abbreviations: SQDG, sulfoquinovosyl diacylglycerol; PSII, photosystem II; DCIP, 2,6-dichlorophenol indophenol; DPC, diphenylcarbazide; PG, phosphatidylglycerol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea

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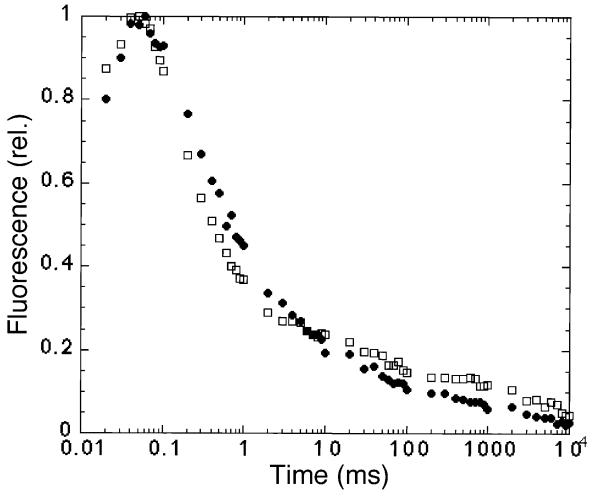

Fig. 1. The decay of chlorophyll fluorescence in the wild-type (open squares) and *hf*-2 (closed squares) of *C. reinhardtii*. Each curve was normalized to the maximum fluorescence.

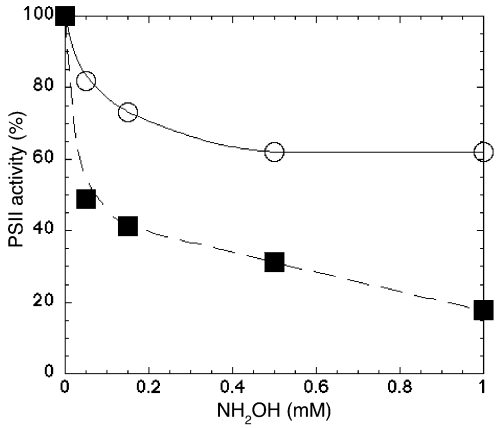

Fig. 2. Dependence of PSII activities on the concentration of NH_2OH in the treatment of thylakoid membranes of the wild-type (open circles) and *hf*-2 (closed circles) in *C. reinhardtii*. Values are given as means. The value of PSII activity as 100% is 318 μmol O_2/mg Chl/h in the wild-type, 233 μmol O_2/mg Chl/h in *hf*-2.

isolated thylakoid membranes were measured with various quinones (Table 1). Various derivatives of benzoquinone show different affinities to the Q_B site, which are dependent on the substitution of the quinone ring with halogen or methyl residues [9]. PSII activity in *hf*-2 was 50–60% of that in the wild-type when it was measured with ferricyanide, *p*-benzoquinone or dichloroquinone, while the activity was not so much different from that of the wild-type when measured with dimethylquinone or phenylquinone. These results suggested that the environment of the Q_B site in *hf*-2 was altered due to the lesion of SQDG. However, the lowered PSII activity was not derived from the change of Q_B environment. The rate of electron transfer from Q_A to Q_B did not decrease significantly in *hf*-2 when determined by the decay kinetics of chlorophyll fluorescence after excitation by single-turnover flash (Fig. 1, Table 2). The photosynthetic parameters of *hf*-2 determined by measurement of chlorophyll fluorescence were also similar to that of the wild-type (data not shown). Especially, F_v/F_m, a parameter for maximum quantum yield of PSII, was 0.74 in *hf*-2, almost equal to 0.79 in the wild-type in spite of the lowered O_2-evolving rate in the mutant. This result suggests that *hf*-2 may have a defect in the O_2-evolving complex, not in the PSII reaction center itself.

To elucidate the cause of the lowered O_2-evolving rate, the reduction rate of DCIP by PSII was measured. The reduction rate of DCIP in *hf*-2 was 66% of that in the wild-type (Table 3), which was coincident with the result of the measurements of the O_2-evolving rate with *p*-benzoquinone and ferricyanide (Table 1). The addition of DPC, an artificial electron donor to tyrosine Z of PSII, to the reaction mixture increased the reduction rate of DCIP in *hf*-2, although there was no effect on the reduction rate in the wild-type. Moreover, when SQDG was added to the culture of *hf*-2, the lowered reduction rate of DCIP was restored and the effect of DPC on the rate of DCIP reduction observed in *hf*-2 disappeared.

Apparently, the site of the defect in *hf*-2 is the oxygen-evolving machinery, and the defect is due to the lack of SQDG. In Mn-depleted thylakoid membranes with no activity in oxygen evolution, the rate of electron transport from DPC to DCIP was similar between the wild-type and *hf*-2. The results also support the fact that the site of the defect in the mutant is not in the PSII reaction center itself. The decay kinetics of chlorophyll fluorescence (Fig. 1, Table 2) also supports the proposed mechanism of the defect.

NH_2OH eliminates Mn molecules from the O_2-evolving complex on the donor side of PSII. Compared with the wild-type, *hf*-2 showed high sensitivity to NH_2OH (Fig. 2). Treatment with 100 mM NaCl, which removes the 18 kDa and 24 kDa extrinsic polypeptides on the donor side of PSII but does not alter the content of Mn and 33 kDa extrinsic polypeptide, decreased the oxygen-evolving activity by 45% in *hf*-2 while the decrease in the activity of the wild-type by the same treatment was only 15%. The concentration dependence of the oxygen-evolving rates in NH_2OH or NaCl treatments of the thylakoid membranes in the wild-type were consistent with previous reports [6,10]. These results suggest that Mn molecules and two extrinsic polypeptides on the donor side of PSII are unstable in *hf*-2 owing to the lesion of SQDG.

Table 1
PSII activities determined with several electron acceptors in thylakoid membranes of the wild-type and *hf*-2 in *C. reinhardtii*

	CC125 (μmol O_2/mg Chl/h)	*hf*-2 (μmol O_2/mg Chl/h)
Ferricyanide	222 ± 24	145 ± 16 (65%)
p-Benzoquinone	269 ± 13	189 ± 17 (70%)
2,5-Dimethylbenzoquinone	182 ± 9	206 ± 7 (113%)
2,6-Dimethylbenzoquinone	187 ± 0	199 ± 14 (106%)
2,6-Dichlorobenzoquinone	118 ± 5	56 ± 8 (47%)
Phenylbenzoquinone	67 ± 9	57 ± 8 (85%)

Values are given as means ± S.D.

Table 2
Half-lives and amplitudes of each component of the fluorescence kinetics due to the oxidation of Q_A^- reduced by a flash excitation in the absence of DCMU

Strain	Fast phase $t_{1/2}$ (μs)	Middle phase $t_{1/2}$ (ms)	Slow phase
Wild-type	140 (55.6%)	1.69 (21.0%)	constant (23.4%)
hf-2	167 (45.1%)	1.96 (37.1%)	constant (17.9%)

Decay half-lives and amplitudes were calculated according to [16].

4. Discussion

The lowered PSII activity in *hf*-2 was mainly ascribed to a decrease in the efficiency of the electron transport on the donor side. Despite the change in the surroundings of the Q_B site, the electron transport from Q_A to Q_B on the acceptor side of PSII was not altered (Tables 1 and 2, Fig. 2) so that the defect should be on the donor side of PSII. DPC is usually unable to donate electrons to tyrosine Z in the functional PSII complex due to the obstruction by the Mn atoms bound to D1 polypeptide. The fact that DPC can donate electrons to Z in thylakoid membranes of *hf*-2 must be due to the partial elimination of Mn molecules or an unusual change in the conformation of the PSII complex. We assumed that there was no difference in the number of Mn atoms bound to D1 polypeptide between the wild-type and *hf*-2, since the decrease in the PSII activity of *hf*-2 was not critical for survival.

Presumably, the change in the conformation of the PSII complex, particularly D1 polypeptide, influences the properties of the Mn atoms in *hf*-2. As a consequence, the efficiency of the electron donation from Mn to Z would be decreased. In *hf*-2 fed with SQDG, the susceptibility to DCMU was restored [5] and the effect of DPC which compensates for the decreased activity in the electron donation from Mn to Z disappeared (Table 3). The binding site of Q_B and Z is known to locate on the same polypeptide, D1 polypeptide (PsbA protein) of PSII. Coupling of the reactions on the donor side and on the acceptor side was reported for the process of photoactivation or for the effect of a site-directed mutation of the D1 polypeptide of *Chlamydomonas* [7,11]. The unusual conformational change of D1 polypeptide in *hf*-2 may cause the decrease in the electron donation to Z as well as the increase in the affinity of DCMU to the Q_B site. Altered conformation of the PSII complex in *hf*-2 could also increase the accessibility of NH_2OH and NaCl to the reaction center ([5], Fig. 2). Under high light condition, *hf*-2 was slightly more sensitive to photoinhibition, and the recovery was a little slower than in the wild-type [12]. The D1 polypeptide of the reaction center in PSII seems to be one of the most severely modified subunits in *hf*-2.

A small amount of SQDG associated with PSII complex is involved not in the assembly of the PSII complex, but in the maintenance of the proper conformation [5]. A candidate for the specific binding site of SQDG is the D1D2 heterodimer of the PSII reaction center, and a part of SQDG seems to be tightly bound so as not to be washed away with organic solvents [13]. It is unclear whether SQDG was bound to the PSII reaction center by fatty acids or by the sugar molecule. However, it was shown that the sulfonate residue and the sugar part of SQDG were required for the maintenance of maximum PSII activity [5]. These parts of SQDG may contribute to the most suitable conformation of the PSII complex, particularly of D1 polypeptide. The other acidic lipid, phosphatidylglycerol (PG), may not bind to the site as a substitute for SQDG in *hf*-2, owing to the unique 3-*trans*-hexadecenoic acid in PG. Other glycolipids, monogalactocyl glycerol and digalactocyl glycerol, cannot take the place of SQDG because of a lack of a negative charge, even if they could bind to the binding site.

In *hf*-2, the altered conformation of the D1 polypeptide may result in a change of the surroundings of the Q_B site and in a decrease in the efficiency of the electron donation from Mn to Z due to the loss of SQDG bound to the D1D2 heterodimer. The number of SQDG in a PSII complex is about 26 molecules [3] in *Chlamydomonas*, therefore multiple binding sites will exist. In *hf*-2, the O_2-evolving rate measured with dimethylquinones or phenylquinones as an electron acceptor was equal to that in the wild-type in contrast to the results with *p*-benzoquinone (Table 1). Previously, O_2-evolving rates of *hf*-2 and the wild-type were measured with various quinones in cells [4]. A conflict with the results in previous reports perhaps was caused by the permeability of dimethylquinones to the cell membranes. Dimethylquinones show a higher affinity for quinone dissolved in thylakoid membranes than that bound to the Q_B site in the PSII complex [9]. Judging from the increased affinities of dimethylquinones, the hydrophobicity and/or negatively charged surface of the membrane in the region near PSII complexes seems to be altered in *hf*-2, due to the decrease in the amount of SQDG and also possibly due to the increase in the amount of PG. Maintaining the total level of acidic lipids in thylakoid membranes is very important when the amount of SQDG or PG is altered [14]. The levels of acidic lipids in thylakoid membranes affected the efficiency of the electron transport (Table 1) [15]. Acidic lipids

Table 3
Efficiency of the electron transport on the donor side in the wild-type and *hf*-2 in *C. reinhardtii*

Electron donor	H_2O (μmol reduced DCIP/mg Chl/h)	H_2O+DPC[a] (μmol reduced DCIP/mg Chl/h)	DPC[b] (μmol reduced DCIP/mg Chl/h)
CC125	141 ± 6	132 ± 15	64 ± 5
hf-2	77 ± 3	111 ± 5	64 ± 7
hf-2+SQDG[c]	109 ± 15	112 ± 15	–

The results are means ± S.D. of three independent experiments.
[a]DPC was added to thylakoid membranes in the reaction buffer.
[b]DPC was added to thylakoid membranes whose O2-evolving complexes were inactivated by elimination of Mn.
[c]*hf*-2 was cultured in the presence of 8 μM SQDG.

could function to optimize the flow of electron transport in thylakoid membranes through the change in the hydrophobicity or the negatively charged surface of membranes.

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References

[1] Siegenthaler, P.A. and Murata, N. (1998) Lipids in Photosynthesis: Structure, Function and Genetics, Kluwer Academic, Dordrecht.
[2] Benson, A.A., Daniel, H. and Wiser, R. (1959) Proc. Natl. Acad. Sci. USA 45, 1582–1587.
[3] Sato, N., Sonoike, K., Tsuzuki, M. and Kawaguchi, A. (1995) Eur. J. Biochem. 234, 16–23.
[4] Sato, N., Aoki, M., Maru, Y., Sonoike, K., Minoda, A. and Tsuzuki, M. (2003) Planta 217, 245–251.
[5] Minoda, A., Sato, N., Nozaki, H., Okada, K., Takahashi, H., Sonoike, K. and Tsuzuki, M. (2002) Eur. J. Biochem. 269, 2353–2358.
[6] Kuwabara, T. and Murata, N. (1983) Plant Cell Physiol. 24, 741–747.
[7] Roffey, R.A., Kramer, D.M., Govindjee and Sayre, R.T. (1994) Bichim. Biophys. Acta 1185, 250–270.
[8] Sonoike, K., Hihara, Y. and Ikeuchi, M. (2001) Plant Cell Physiol. 42, 379–384.
[9] Sato, K., Oh-hashi, M., Kashino, Y. and Koike, H. (1995) Plant Cell Physiol. 36, 597–605.
[10] Cheniae, G. and Martin, I. (1971) Plant Physiol. 47, 568–575.
[11] Rova, M., Mamedov, F., Magnuson, S., Fredriksson, P.O. and Styring, S. (1998) Biochemistry 37, 11039–11045.
[12] Minoda, A., Sonoike, K., Nozaki, H., Okada, K., Sato, N. and Tsuzuki, M. (2001) Proceedings of the 12th International Congress on Photosynthesis, pp. S5–S39.
[13] Voß, R., Radunz, A. and Schmid, G.H. (1992) Z. Naturforsch. 47c, 406–415.
[14] Yu, B., Xu, C. and Benning, C. (2002) Proc. Natl. Acad. Sci. USA 16, 5732–5737.
[15] Droppa, M., Horvath, G., Hideg, E. and Farkas, T. (1995) Photosynth. Res. 46, 287–293.
[16] Hatano-Iwasaki, A., Minagawa, J., Inoue, Y. and Takahashi, Y. (2001) Biochim. Biophys. Acta 1504, 299–310.