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Photosynthetic characteristics of a mutant of *Chlamydomonas reinhardtii* impaired in fatty acid desaturation in chloroplasts

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Abstract

The photosynthetic apparatus of a mutant of *Chlamydomonas reinhardtii*, *hf-9*, impaired in fatty acid desaturation at the $\omega 6$ position of fatty acids of chloroplasts was investigated. Measurement of photosynthetic activities revealed that both PS I and PS II activities were reduced in *hf-9*. However, little alteration occurred in the contents and subunit assemblies of the PS I complex, PS II core complex and light-harvesting complex of PS II. Lipids bound to these chlorophyll–protein (CP) complexes in *hf-9* were shown to contain decreased levels of 16:4(4,7,10,13) and 18:3(9,12,15), with accumulation of 16:1(7) and 18:1(9), as compared with in the parent. Highly unsaturated fatty acids of chloroplast lipids may be required for the normal functions of PS I and PS II, by associating with these complexes.

Keywords: Fatty acid desaturation; Photosystem I; Photosystem II; (*C. reinhardtii*)

1. Introduction

Chloroplast membranes of plants are composed of thylakoid, inner and outer envelope membranes. Thylakoid membranes are responsible for photosynthetic electron transport and photophosphorylation systems, while inner and outer envelope membranes are the sites of lipid biosynthesis [1]. The major lipids comprising thylakoid membranes are three glycolipids specific to chloroplasts, i.e., monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG) and sulfoquinovosyl diacylglycerol (SQDG), and phosphatidylglycerol (PG), which is also distributed in other membrane systems such as mitochondrial membranes [2]. MGDG and DGDG, abundant chloroplast lipids, possess highly unsaturated fatty acids, such as α-linolenic acid (18:3(9,12,15)) [1], as predominant fatty acid components. Thus, it is an intriguing question how highly unsaturated fatty acids contribute to the functions of thylakoid membranes.

The alterations in thylakoid membrane functions were examined after catalytic hydrogenation of highly unsaturated fatty acids of lipids in intact chloroplasts or proteoliposomes. The activity of photosynthetic electron transport in chloroplasts and the rate of ATP–P_i exchange of the CF_0–CF_1 complex reconstituted from chloroplast lipids were reduced, with saturation of 18:3(9,12,15) of constituent lipids [3,4]. These results suggest that thylakoid membranes require highly unsaturated fatty acids for the normal photosynthetic functions.

On the other hand, the photosynthetic function and chloroplast ultrastructure have been characterized in three distinct mutants of *Arabidopsis thaliana* with decreased contents of highly unsaturated fatty acids such as 18:3(9,12,15) in chloroplasts, for investigation of the physiological significance of highly unsaturated fatty acids

Abbreviations: Chl, chlorophyll; CBB, Coomassie brilliant blue; CP complex, chlorophyll–protein complex; DCIP, 2,6-dichlorophenolindophenol; DGDG, digalactosyl diacylglycerol; DGTS, diacylglyceryl trimethylhomoserine; LDS, lithium dodecyl sulfate; LHC II, light-harvesting complex of PS II; MGDG, monogalactosyl diacylglycerol; PAGE, polyacrylamide gel electrophoresis; PC, phosphatidylcholine; PG, phosphatidylglycerol; SQDG, sulfoquinovosyl diacylglycerol; TLC, thin-layer chromatography.

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[1] Fatty acids are denoted by the numbers of carbon atoms and double bonds.

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[5–7]. These mutants deficient in the desaturation of fatty acids at the ω3, ω6, or ω9 position in chloroplasts, retaining 50% to 70% of the 18:3(9,12,15) content of chloroplast lipids, showed little alteration in various photosynthetic activities, indicating that, at least, the extent of the 18:3(9,12,15) content loss in these mutants had almost no effect on photosynthetic activities.

We previously isolated a mutant of *Chlamydomonas reinhardtii* affected in the desaturation at the ω6 position of fatty acids of chloroplasts. The mutant, designated as *hf-9* showing a pronounced decrease in the 18:3(9,12,15) content of chloroplast lipids (containing less than 30% of the wild type level), is more suitable than comparable mutants of *A. thaliana* isolated so far for investigation of the roles of highly unsaturated fatty acids in chloroplasts [8]. In this study, photosynthetic apparatus such as the photosynthetic electron transport system is characterized in *hf-9*.

2. Materials and methods

2.1. Algal culture

Cells of *Chlamydomonas reinhardtii* 6145c (*nit1-305*) *mt*$^-$ and its mutant, *hf-9*, were grown in oblong glass vessels under constant fluorescent illumination (7 W m^{-2}) at 28°C, with aeration with air containing 1.5% CO_2. As the culture medium, 3/10 HSM medium [9] was used.

2.2. Measurement of photosynthetic activity

Cells at the logarithmic growth phase were harvested by centrifugation at 3000 × *g* for 5 min and then resuspended in 50 mM Tricine/KOH (pH 7.5), at the concentration of 60 μg Chl ml^{-1}. Three photosynthetic properties, i.e., photosynthetic O_2 evolution coupled with CO_2 fixation, and PS I and PS II activities, were determined in these cells, as Sato et al. described [10]. Photosynthetic O_2 evolution coupled with CO_2 fixation was measured in whole cells with CO_2 as the electron acceptor. PS I activity was measured in sonicated cells with reduced form of 2,6-dichlorophenol-indophenol (DCIP) and methylviologen as the electron donor and acceptor, respectively. PS II activity was measured in whole cells with *p*-benzoquinone as the electron acceptor. O_2 evolution or uptake was measured with a Clark-type electrode (Rank Brothers, London, UK). The electrode chamber was filled with 5 ml of reaction mixture, kept at 25°C, and illuminated with a tungsten projector lamp (128 W m^{-2}, unless otherwise indicated).

2.3. Preparation of thylakoid membranes and CP complexes

Thylakoid membranes were prepared by floatation on sucrose gradients [11] with some modifications as follows: all the buffers contained a protease inhibitor, phenylmethylsulfonyl fluoride (1 mM), and the cells were disrupted with a sonicator in place of a French pressure cell, as described above. The purified thylakoid membranes were stored at −80°C until use.

The purified thylakoid membranes were incubated for 1 h at 0°C in a buffer containing 0.5% (w/v) dodecyl-β,D-maltoside, 50 mM Tris-HCl (pH 7.5), and 10 mM NaCl to a final Chl concentration of 0.5 mg ml^{-1}, and then centrifuged at 5500 × *g* for 5 min to precipitate insoluble material. The supernatant was subjected to disk or slab dodecyl-β,D-maltoside-PAGE, as described by Sato et al. [10].

2.4. Analyses of proteins

Thylakoid membranes were solubilized in 5% (w/v) LDS, 60 mM dithiothreitol and 30% (w/v) sucrose to a final Chl concentration of 0.5 mg ml^{-1}, or the gel containing CP complexes was excised and immersed in 5% SDS (w/v) and 2.5% (v/v) 2-mercaptoethanol. These treatments were carried out for at least 1h at room temperature. Thylakoid membranes equivalent to 5 μg of Chl or excised gels were then subjected to SDS-PAGE, as described by Ikeuchi and Inoue [12]. Gels were stained with Coomassie brilliant blue (CBB) or Silver Stain 'DAIICHI' (Daiichi Pure Chemicals, Tokyo, Japan).

2.5. Analyses of lipids of CP complexes

The gel containing CP complexes was excised, homogenized with a motor-driven Teflon pestle in cold buffer containing 20 mM Tris-HCl (pH 7.8), and then centrifuged at 20000 × *g* for 30 min at 4°C. The supernatant was then centrifuged at 160000 × *g* for 12 h at 4°C. The pellet was resuspended in cold buffer containing 50 mM Tris-HCl, 10 mM NaCl and 0.5 M sucrose, and then stored at −80°C until use. The total lipids were extracted from each CP complex, according to the method of Bligh and Dyer [13]. Fatty acid methyl esters were prepared from total lipids by treatment with 5% anhydrous methanolic-HCl, and then analyzed by capillary gas-liquid chromatography as described previously [14].

3. Results

3.1. Photosynthetic activity

It was previously shown that a mutant of *C. reinhardtii*, *hf-9*, accumulated 16:1(7) and/or 18:1(9), with concomitant decreases in highly unsaturated fatty acids such as 16:4(4,7,10,13) and/or 18:3(9,12,15), in major lipids comprising chloroplast membranes, as compared with the parent [8]. To investigate the effects of low contents of highly unsaturated fatty acids on photosynthesis, we firstly com-

Table 1
Photosynthetic characteristics in *C. reinhardtii 6145c* (*nit1-305*) and *hf-2*

Measurement		6145c	*hf-9*
Doubling time (h)		7.2	10.4
Chl *a*/*b* ratio		2.30± 0.16	2.23± 0.15
Photosynthetic O_2 evolution [a]		152 ±10	29 ± 1
PS I activity [a]	(+NH_4Cl)	489 ±26	305 ± 5
	(−NH_4Cl)	306 ±42	194 ±14
PS II activity [a]	(+NH_4Cl)	229 ± 9	46 ± 5
	(−NH_4Cl)	151 ± 3	39 ± 4

The values are the means ± S.D. for three independent experiments, except that the doubling time was measured in one experiment. Photosynthetic O_2 evolution including CO_2 fixation was measured in a reaction mixture containing cells equivalent to 12 μg Chl ml^{-1}, 10 mM Tricine/KOH (pH 7.5), and 1 mM $NaHCO_3$. PS I activity was assayed in a mixture composed of sonicated cell suspension equivalent to 2.4 μg Chl ml^{-1}, 20 mM Mes-Tris buffer (pH 8.1), 21 μM DCIP, 2 mM sodium ascorbate, 80 μM methylviologen, 6.0 μM DCMU and 0.4 mM KCN, in the presence and absence of 2 mM NH_4Cl. PS II activity was examined in a reaction mixture consisting of cell suspension equivalent to 12 μg Chl ml^{-1}, 20 mM Mes-Tris buffer (pH 8.1), and 300 μM *p*-benzoquinone in the presence and absence of 2 mM NH_4Cl.
[a] The values are expressed in μmol O_2 (mg Chl)$^{-1}$ h^{-1}.

pared the doubling time of growth, Chl *a*/*b* ratio and photosynthetic activity at the logarithmic growth phase between the parent and *hf-9* (Table 1). The growth rate (the reciprocal of the doubling time) was lower in *hf-9* than in the parent, which was reflected by the 81% reduction of the photosynthetic O_2 evolution rate coupled with CO_2 fixation in *hf-9*. On the other hand, the Chl *a*/*b* ratio was indistinguishable between the two. We then measured PS I and PS II activities in the parent and *hf-9* to determine which was responsible for the decrease in the photosynthetic O_2 evolution rate of *hf-9*. *hf-9* showed 37% and 80% decreases in PS I and PS II activities measured in the presence of an uncoupler, NH_4Cl, respectively, as compared with the parent. These results indicated that the photosynthetic O_2 evolution in *hf-9* was limited mainly by PS II activity, but not by PS I activity. It was also observed that, with no addition of the uncoupler, the PS I and PS II activities of *hf-9* decreased by 37% and 74%, respectively, as compared with those of the parent.

The light-response curve of PS II activity of the parent and *hf-9* is presented in Fig. 1. Light-saturated level of PS II activity was much lower in *hf-9* than in the parent, which indicated a decrease in the activity of the PS II reaction center complex of *hf-9* (Fig. 1a), corresponding to the result in Table 1. This decrease cannot be ascribed to the simple decrease of PS II unit, since the initial slope of the light-response curve, representing relative quantum yield of PS II, showed little difference between *hf-9* and the parent (Fig. 1b).

3.2. Protein analyses of CP complexes

We then investigated whether or not *hf-9* was affected in the contents of CP complexes responsible for PS I and PS II activities. Fig. 2 compares the protein patterns of thylakoid membranes on SDS-PAGE between the parent and *hf-9*. With the exception that *hf-9* was almost devoid of a protein of 14 kDa, the parent and *hf-9* showed little alteration in protein patterns. Some of the proteins were recognized as subunits comprising the PS I complex (CP I apoprotein, 60 kDa), PS II core complex (CP III apoprotein, 47 kDa), and light-harvesting complex of PS II (LHC II; 30 kDa, 27 kDa, 25 kDa), from their molecular masses. The results indicated that the contents of these CP complexes were little affected in *hf-9*.

It is possible that some other subunits were lost from the CP complexes of *hf-9*. However, it was difficult to analyze the subunit compositions of CP complexes in detail by SDS-PAGE of thylakoid membranes. We then isolated each CP complex by dodecyl-β,D-maltoside-PAGE of thylakoid membranes from the parent and *hf-9* to compare the subunit patterns (Fig. 3). Specific subunits were observed in CP complexes of the parent, as described previously [10]: the PS I complex contained CP I apoprotein (60 kDa), and at least three subunits ranging from 19 to 24 kDa, the PS II core complex was composed of at least seven subunits such as apoproteins of CP III and CP IV (47 kDa and 42 kDa, respectively), and the D1 and D2 proteins (31 kDa and 34 kDa, respectively), and two

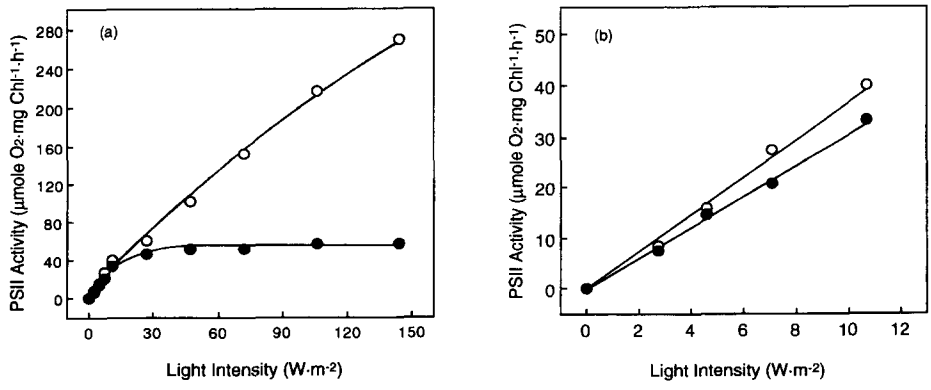

Fig. 1. Light-intensity dependency of PS II activity in *C. reinhardtii* 6145c (*nit1-305*) and *hf-9*. (a) PS II activity was measured with *p*-benzoquinone as described in Table 1. (b) PS II activity at low light intensities in a is shown. ○, 6145c; ●, *hf-9*.

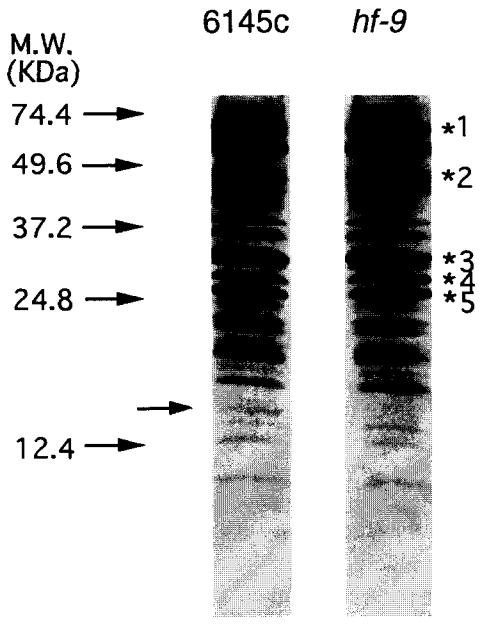

Fig. 2. SDS-PAGE of thylakoid membranes of *C. reinhardtii* 6145c (*nit1-305*) and *hf-9*. Thylakoid membranes from 6145c and *hf-9* equivalent to 5 μg Chl were subjected to SDS-PAGE, and then the gel was stained with CBB. The subunits of CP complexes are numbered. 1, two subunits of the PS I complex; 2, a subunit of the PS II core complex; 3 to 5, subunits of LHC II. A small arrow indicates a protein, the content of which was decreased in *hf-9*.

distinct LHC II consisted of two subunit (30 kDa and 27 kDa) and one subunit (25 kDa), respectively. No subunits were missing from any CP complex of *hf-9*, suggesting that *hf-9* was little affected in the subunit assemblies of these CP complexes. The 14-kDa protein missing from *hf-9* may not be a component of either the PS I or PS II complex, and was not further pursued.

3.3. Analyses of constituent fatty acids of lipids bound to CP complexes

CP complexes, isolated by dodecyl-β,D-maltoside-PAGE of *C. reinhardtii* thylakoid membranes, are considered to retain functionally important lipids [10]. Clarification of the fatty acid compositions of lipids bound to CP complexes will provide a clue for understanding the roles of highly unsaturated fatty acids in thylakoid membranes. Table 2 shows the compositions of major fatty acids of total lipids from thylakoid membranes and each CP complex prepared by dodecyl-β,D-maltoside-PAGE, in the parent and *hf-9*. Highly unsaturated fatty acids such as 16:4(4,7,10,13) and 18:3(9,12,15) of thylakoid membranes were abundant in the parent, whereas they decreased with the accumulation of 16:1(7) and 18:1(9) in *hf-9*. This is consistent with the previous finding that chloroplast lipids

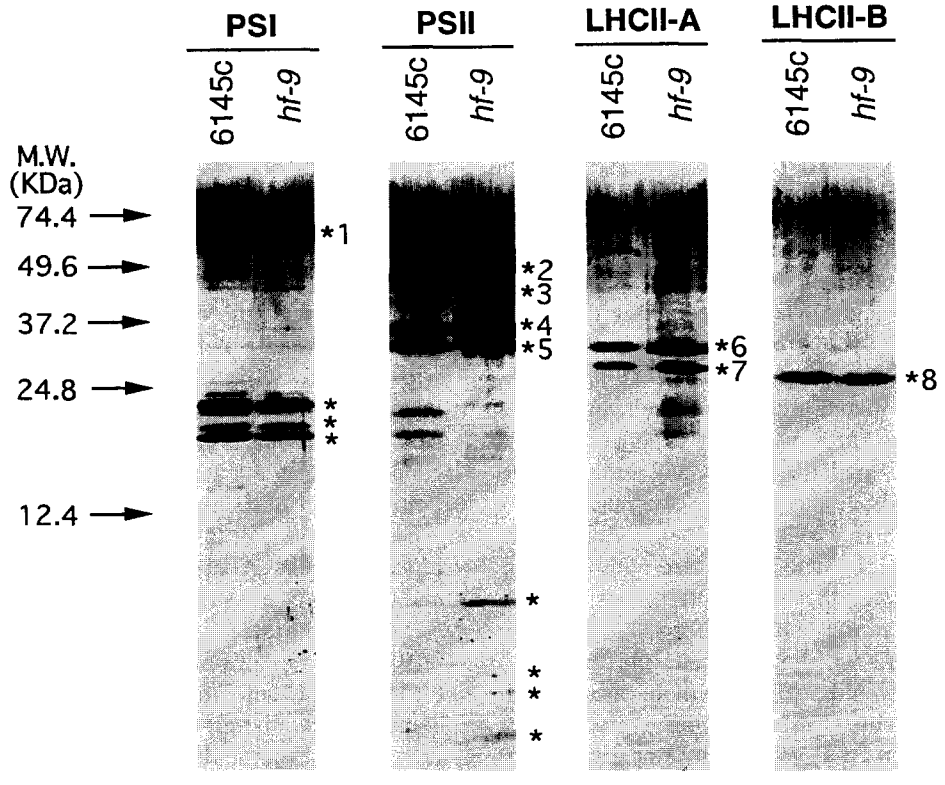

Fig. 3. SDS-PAGE of CP complexes of *C. reinhardtii* 6145c (*nit1-305*) and *hf-9*. Thylakoid membranes of *C. reinhardtii* 6145c and *hf-9* equivalent to 12.5 μg Chl were separated into CP complexes on a disc gel (5 cm long × 0.5 cm diameter) by dodecyl-β,D-maltoside-PAGE. Gel containing CP complexes was excised, and then subjected to SDS-PAGE. The gel was stained with silver. Stars indicate the protein bands corresponding to the subunits of CP complexes. 1, apoprotein of CP I; 2, apoprotein of CP III; 3, apoprotein of CP IV; 4, D2 protein; 5, D1 protein; 6 to 8, LHC II subunits.

Table 2
Compositions of major fatty acids of total lipids affected in thylakoid membranes and CP complexes from *C. reinhardtii 6145c* (*nit1-305*) and *hf-9* (data given in mol%)

Fatty acid	T.M.		PS I		PS II		LHC II-A		LHC II-B	
	6145c	*hf-9*	6145c	*hf-9*	6145c	*hf-9*	6145c	*hf-9*	6145c	*hf-9*
16:1(7)	3.0	17.9	6.4	21.3	3.8	17.0	4.4	15.1	4.3	11.0
16:4(4,7,10,13)	17.9	1.0	16.9	2.1	19.1	1.2	15.9	1.2	12.6	2.1
18:1(9)	7.2	35.5	9.1	36.7	7.3	34.3	7.6	30.9	9.0	25.9
18:3(9,12,15)	27.0	1.6	21.7	2.5	26.3	2.2	23.0	1.4	16.0	1.1

exhibited lower unsaturation levels of fatty acids in *hf-9* than in the parent [8]. The parent also exhibited large amounts of 16:4(4,7,10,13) and 18:3(9,12,15) in the PS I complex, PS II core complex and LHC II, as well as in thylakoid membranes. This inferred that lipids containing highly unsaturated fatty acids play some functional role in PS I and PS II. In contrast, these CP complexes of *hf-9* accumulated 16:1(7) and 18:1(9), with decreases in 16:4(4,7,10,13) and 18:3(9,12,15).

4. Discussion

The abundance of highly unsaturated fatty acids in chloroplast membrane lipids raised a question about their roles in photosynthesis. We investigated the photosynthetic characteristics of *hf-9*, a mutant of *C. reinhardtii* impaired in the desaturation at the $\omega 6$ position of fatty acids of chloroplasts (Table 1). *hf-9* showed a lower growth rate than the parent, which corresponded to a decrease in the rate of photosynthetic O_2 evolution coupled with CO_2 fixation of *hf-9*. In the parent, the rates of electron transfer through PS I and PS II became 1.5- to 1.6-fold higher with the addition of NH_4Cl. On the other hand, in *hf-9*, PS I activity increased 1.6-fold whereas PS II activity became only 1.2-fold on its addition. The minor increase in *hf-9* PS II activity may be due to that PS II activity, responsible for the H^+ gradient through thylakoid membranes, was as much as 80% lower in *hf-9* than in the parent. The similar extent of increase in PS I activity upon addition of NH_4Cl were observed for *hf-9* and the parent, indicating that thylakoid membranes of *hf-9* were not so injured as to greatly reduce the H^+ gradient by the low unsaturation level of chloroplast lipids. In the presence of an uncoupler, NH_4Cl, the PS I and PS II activities of *hf-9* were reduced by 37% and 80%, respectively, as compared with those of the parent, which indicated that PS II activity is the main factor for the decrease in the photosynthetic O_2 evolution rate of *hf-9*. Other parts of photosynthetic processes affected in *hf-9*, if any, would not have a more pronounced lesion than PS II activity. The deleterious effect of the desaturation mutation on PS II activity is comparable with the observation that PS II activity was more seriously damaged than PS I activity when unsaturated fatty acids of *Pisum sativum* chloroplasts were saturated with a catalyst [3].

Although the light-saturated level of PS II activity was greatly suppressed in *hf-9* (Fig. 1a, Table 1), quantum yield of PS II was not largely affected (Fig. 1b). This is quite contrasting with the result of *hf-2*, an SQDG-defective mutant of *C. reinhardtii*. *hf-2* showed decreases in PS II activity at all light intensities examined, as compared with the parent, suggesting its inactivation as all-or-none type [10]. The defect in PS II of *hf-9* seems to be caused not by all-or-none type inactivation, but by slow-down of some step in PS II electron transfer. It was previously reported that the saturation of fatty acids in *Pisum sativum* chloroplasts by catalytic hydrogenation brought about inefficient electron flow from Q_A to the PQ pool [15]. The PS II reaction center of *hf-9* may also be defective in the same electron flow. Further experiments should be carried out for determination of the precise damaged site of PS II in *hf-9*.

The similar quantum yield of PS II for *hf-9* and the parent suggests that the number of PS II units or energy transfer from LHC II to PS II core was little altered in *hf-9*. This assumption is supported by little effect of *hf-9* mutation on the contents of PS II core and LHC II subunits in thylakoid membranes (Fig. 2), and also on Chl *a*/*b* ratio (Table 1). *hf-9* showed a decrease of 80% in oxygen evolution coupled with CO_2 fixation under light-saturated condition, owing to the damage in PS II, as compared with the parent (Table 1). However, the extent of decrease in the growth rate (the reciprocal of the doubling time) was not so remarkable as that in the oxygen evolution coupled with CO_2 fixation. This can be ascribed to the similar quantum yield of PS II, since the doubling time was determined in relatively low growth irradiances. Thus, it is concluded that the low unsaturation level of chloroplast lipids of *hf-9* leads to the lesions in the PS I complex and, especially, the PS II complex. This results in impaired photosynthetic O_2 evolution coupled with CO_2 fixation and eventually a decrease in the growth rate.

Despite the defects in the PS I and PS II activities of *hf-9* (Table 1), our preparation of CP complexes responsible for these activities showed little alteration in their contents and subunit compositions (Figs. 2 and 3). These facts suggest that highly unsaturated fatty acids of chloroplast lipids are little concerned in the subunit construction of CP complexes. However, lipids co-purified with CP complexes prepared by dodecyl-β,D-maltoside-PAGE exhibited the accumulation of 16:1(7) and 18:1(9) with de-

creased levels of highly unsaturated fatty acids such as 16:4(4,7,10,13) and 18:3(9,12,15) in *hf-9*, as compared with in the parent (Table 2). We previously showed that the lipids retained by these purified CP complexes reflected boundary lipids rather than the membrane matrix ones [10]. Thus, it was inferred that the association of CP complexes with lipids involving highly unsaturated fatty acids is important for normal functions of CP complexes. Highly unsaturated fatty acids may be responsible to conform PS I and PS II complexes for the full activities.

hf-9 was defective in a 14-kDa protein of thylakoid membranes (Fig. 2). This protein may be labile in lipid environment with reduced unsaturation levels. It should be examined in the future whether this protein is involved in the photosynthetic activities affected in *hf-9* or not.

A mutant of *C. reinhardtii* named *hf-2* defective in SQDG synthesis showed a decrease of 40% in PS II activity, with little effect on PS I activity [10], although this mutant also retained normal contents of the CP complexes responsible for PS I and PS II activities. This was accounted for by association of SQDG of the wild type of *C. reinhardtii* with the PS II complex, but not with the PS I complex. In *hf-9*, PS I activity was reduced by only 37%, whereas PS II activity was by as much as 81% (Table 1). The much larger number of lipid molecules bound to the PS II complex than to the PS I complex suggested that lipids contribute more greatly to the expression PS II activity than to that of PS I activity [10]. This may be reflected by the more deleterious effects of the lipid mutations of both *hf-2* and *hf-9* on PS II activity than on PS I activity. It was noted that both PS I and PS II activities were more seriously damaged in *hf-9* than in *hf-2*. MGDG and DGDG, with large amounts of highly unsaturated fatty acids, constitute as much as 50% to 80% of the total lipids bound to the CP complexes responsible for PS I and PS II activities, while SQDG occupies 20% of such lipids at most [10]. The change in the unsaturation levels of major lipid constituents such as MGDG and DGDG may damage the conformation of CP complexes more severely than a defect in a minor lipid constituent such as SQDG.

So far, in several mutants of prokaryotic and eukaryotic photosynthetic organisms, decreased levels of highly unsaturated fatty acids have shown little effect on photosynthesis. The mutants of *A. thaliana* were deficient in $\omega 3$, $\omega 6$ or $\omega 9$ desaturation of fatty acids in chloroplasts [5–7]. However, the mutants retained substantial contents of highly unsaturated fatty acids in chloroplasts: the chloroplast $\omega 6$ desaturation mutant, which was unable to desaturate 18:1(9) to 18:2(9,12) in chloroplast lipids, e.g., showed more than 70% of the wild-type level of 18:3(9,12,15) in major chloroplast lipids such as MGDG and DGDG, since $\omega 6$ desaturation in ER is active and its product, 18:2(9,12), is transported to chloroplasts to be further desaturated [16]. In contrast, *hf-9*, with a defect in chloroplast $\omega 6$ desaturation, exhibited a much more pronounced effect on the 18:3(9,12,15) content of chloroplast lipids (Table 2, see thylakoid membranes), probably owing to the absence of a transportation system for 18:2(9,12) from ER to chloroplasts [8]. The additional decrease in the 18:3(9,12,15) content in the chloroplast $\omega 6$ desaturation mutant of *A. thaliana* on the introduction of another mutation, such as a deficiency in ER $\omega 6$ desaturation [17], would result in large alterations of photosynthetic electron transport activities.

A mutant of the cyanobacterium, *Synechocystis* PCC6803, named Fad12, defective in $\Delta 12$ desaturation in MGDG, SQDG and PG, was also indistinguishable from the wild-type in the photosynthetic electron transport rates under the optimal growth condition, although it completely lacked major unsaturated fatty acids such as 18:2(9,12) and 18:3(6,9,12) [18–20]. MGDG, the most abundant lipid in thylakoid membranes, is occupied mainly by 16:0 in cyanobacteria (22% to 58% of constituent fatty acids; [21,22]). In contrast, MGDG predominantly comprises highly unsaturated fatty acids such as 18:3(9,12,15) in green plants [23]. The unsaturation level of lipids required for photosynthesis may be distinguished between green plants such as *C. reinhardtii* and cyanobacteria.

Abnormal thylakoid membrane ultrastructure was revealed in several mutants of *C. reinhardtii* defective in photosynthetic electron transport activities, owing to the loss of proteins concerned in these activities: hyper-stacked thylakoid membranes were typical of PS I mutants, while unstacked thylakoid membranes were characteristic of PS II mutants [24,25]. Electron microscopy previously showed that *hf-9*, damaged mainly in PS II (Table 1), contained hyper-stacked thylakoid membranes similar to mutants previously reported to lack PS I subunits [8]. However, this abnormal ultrastructure of *hf-9* thylakoid membranes is not caused by the miss of PS I subunits, since no notable changes occurred in the subunit construction of *hf-9* CP complexes (Figs. 2 and 3). LHC II is considered to play a role in thylakoid membrane stacking [26], while it was shown that the LHC II content was not affected in *hf-9*, on analyses of the Chl a/b ratio and protein composition of thylakoid membranes (Table 1 and Fig. 2). A mutant of *C. reinhardtii* lacking 16:1(3*t*) of PG contained unstacked thylakoid membranes, and the stacking of thylakoid membranes was restored on the supplying of PG containing 16:1(3*t*) during cell growth [27]. This suggested that 16:1(3*t*) of chloroplast PG participate in the organization of thylakoid membranes into stacked and unstacked regions. Probably, highly unsaturated fatty acids of chloroplast lipids also participate in the organization, directly, or indirectly through the control of components responsible for the membrane stacking, such as LHC II.

Since the membrane fluidity is affected by the unsaturation level of fatty acids of membrane lipids, the involvement of fatty acid desaturation in adaptation to low-temperature is expected. The mutant of *Synechocystis* PCC6803 defective in $\Delta 12$ desaturation was reported to be

cold-sensitive [18]. The mutants of *A. thaliana* impaired in the ω6 or ω9 desaturation of chloroplasts showed chlorosis in developing leaves, but not in mature ones [28]. Since *hf-9* exhibited much larger effects on the unsaturation levels of chloroplast lipids than the mutants of *A. thaliana*, *hf-9* will be useful for elucidation of the roles of highly unsaturated fatty acids of green plants in adaptation to low temperature.

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References

[1] Douce, R. and Joyard, J. (1990) Annu. Rev. Cell Biol. 6, 173–216.
[2] Moore, T.S. (1982) Annu. Rev. Plant Physiol. 33, 235–259.
[3] Horváth, G., Droppa, M., Szitó, T., Mustárdy, L., Horváth, L.I. and Vigh, L. (1986) Biochim. Biophys. Acta 849, 325–336.
[4] Pick, U., Weiss, M., Gounaris, K. and Barber, J. (1987) Biochim. Biophys. Acta 891, 28–39.
[5] McCourt, P., Kunst, L., Browse, J. and Somerville, C.R. (1987) Plant Physiol. 84, 353–360.
[6] Hugly, S., Kunst, L., Browse, J. and Sommerville, C. (1989) Plant Physiol. 90, 1134–1142.
[7] Kunst, L., Browse, J. and Somerville, C. (1989) Plant Physiol. 91, 401–408.
[8] Sato, N., Tsuzuki, M., Matsuda, Y., Ehara, T., Osafune, T. and Kawaguchi, A. (1995) Eur. J. Biochem. 230, 987–993.
[9] Sueoka, N. (1960) Proc. Natl. Acad. Sci. USA 46, 83–91.
[10] Sato, N., Sonoike, K., Tsuzuki, M. and Kawaguchi, A. (1995) Eur. J. Biochem. 234, 16–23.
[11] Chua, N.H. and Bennoun, P. (1975) Proc. Natl. Acad. Sci. USA 72, 2175–2179.
[12] Ikeuchi, M. and Inoue I. (1988) Plant Cell Physiol. 29, 1233–1239.
[13] Bligh, E.G. and Dyer, W.J. (1959) Can. J. Biochem. Physiol. 37, 911–917.
[14] Tsuzuki, M., Ohnuma, E., Sato, N., Takaku, T. and Kawaguchi, A. (1990) Plant Physiol. 93, 851–856.
[15] Horváth, G., Melis, A., Hideg, É., Droppa, M. and Vigh, L. (1987) Biochim. Biophys. Acta 891, 68–74.
[16] Browse, J., Kunst, L., Anderson, S., Hugly, S. and Somerville, C.R. (1989) Plant Physiol. 90, 522–529.
[17] Miquel, M. and Browse, J. (1992) J. Biol. Chem. 267, 1502–1509.
[18] Wada, H. and Murata, N. (1989) Plant Cell Physiol. 30, 971–978.
[19] Gombos, Z., Wada, H. and Murata, M. (1991) Plant Cell Physiol. 32, 205–211.
[20] Mamedov, M.D., Hayashi, H. and Murata, N. (1993) Biochim. Biophys. Acta 1142, 1–5.
[21] Zepke, H.D., Heinz, E., Radunz, A., Linsheid, M. and Pesch, R. (1978) Arch. Microbiol. 119, 157–162.
[22] Murata, N., Wada, H. and Gombos, Z. (1992) Plant Cell Physiol. 33, 933–941.
[23] Quinn, P.J. (1987) Biochem. Soc. Trans. 15, 86–91.
[24] Goodenough, U.W. and Levine, R.P. (1969) Plant Physiol. 44, 990–1000.
[25] Levine, R.P. and Duram, H.A. (1973) Biochim. Biophys. Acta 325, 565–572.
[26] Staehelin, L.A. and Arntzen, C.J. (1983) J. Cell Biol. 97, 1327–1337.
[27] Trémolières, A., Roche, O., Dubertret, G., Guyon, D. and Garnier, J. (1991) Biochim. Biophys. Acta 1059, 286–292.
[28] Hugly, S. and Sommerville, C. (1992) Plant Physiol. 99, 197–202.