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Effect of chlorophyll-protein complex I deficiency on the physiological character of a *Chlamydomonas reinhardtii* mutant

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Abstract

In the mutant CC-1047 of *Chlamydomonas reinhardtii*, LDS-PAGE showed that the chlorophyll-protein complex I (CPI) is almost absent. The mutant could not grow in a culture medium without organic carbon source while the wild type (WT) *C. reinhardtii* grew quickly. When an organic carbon source was added into the culture medium, the mutant grew almost as well as WT. The rate of photosystem 1 (PS1) electron transport (DCPIP→MV) and the rate of whole chain electron transport (H_2O→MV) of chloroplasts of the CC-1047 mutant were both lower than those of WT. The photophosphorylation activity, photosynthetic O_2 evolution rate, and rate of $NADP^+$ photoreduction of CC-1047 were also much lower than the activities of WT. There were some differences in ATPase activity between the mutant and WT. Two different activation ways were used to activate the latent ATPase using methanol and dithiothreitol (DTT) as activation substrate. More methanol and DTT were required for the mutant than WT to obtain the maximum activity. Thus the photosynthetic apparatus could not operate normally when CPI was absent because of the abnormal PS1 electron transport. Meanwhile, the other adjacent complexes of the thylakoid membrane, for example, ATP synthase complex, were slightly affected.

Additional key words: ATPase; electron transport; NADP; cyclic and non-cyclic photophosphorylation; photosystems 1 and 2; proteins.

Introduction

There are four multi-subunit membrane-protein complexes embedded in the thylakoid membrane of higher plants and green algae: photosystem 1 (PS1) complex, photosystem 2 (PS2) complex, cytochrome (cyt) b_6f complex, and ATP synthase complex. PS1 and PS2 are photon traps and transducers of their energy through electron transport into chemical energy forms, ATP and NADPH. The electrons transferred from PQH_2 to cyt b_6f are immediately passed onto cyt *f* and then to plastocyanin, which passes the electrons to PS1. PS1 reaction centre (P700) accepts the electrons from plastocyanin and then transfers them to ferredoxin.

PS1 is embedded in the thylakoid membrane and consists of two large homologous subunits encoded by the chloroplast genes *psa*A and *psa*B and several small subunits encoded by the chloroplast genes *psa*C, *psa*I, *psa*J as well as nuclear genes *psa*D, *psa*E, *psa*F, *psa*G, *psa*H, *psa*K, *etc.* The primary donor chlorophyll (Chl), P700, two intermediate acceptors, A_0 and A_1, and the iron-sulfur center X, as well as the core antenna pigment molecules are bound to the heterodimer of the two large subunits (Takahashi *et al.* 1991). Two additional electron acceptors, F_A and F_B, are co-ordinated by an 8-kDa polypeptide encoded by the chloroplast gene *psa*C. PsaF subunit plays an important role in docking plastocyanin to the PS1 complex.

Native, "green gel" electrophoresis, by which Chl-protein complexes can be solubilised and separated with a minimum loss of non-covalently bound Chl, is a very important tool to study the thylakoid membrane composition, organisation, and biogenesis (Thornber 1986). Thornber *et al.* (1967) and Ogawa *et al.* (1966) demonstrated that Chl *a* and *b* in thylakoid membranes were associated with proteins to form discrete Chl-protein complexes. These workers fractionated an anionic-detergent extract of thylakoid membranes by polyacrylamide gel electrophoresis and obtained three bands that they designated as CPI, CPII, and free pigment zone, in the order of increasing electrophoretic mobility. CPI is a Chl-*a*-protein complex that represents the core of PS1

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complex. CPII is believed to function as a harvester of photon energy for both photosystems (Thornber 1975). Three new Chl-protein complexes, designated as CPII, CPIV, and CPV, appeared in the low temperature gel. CPIII and CPIV are likely to be associated with the PS2 reaction centre. CPV may play the role of harvester of photon energy (Delepelaire and Chua 1981). A new Chl-protein complex, CPO, which is a part of the PS1 antenna in *C. reinhardtii* has been isolated by Wollman and Bennoun (1982).

Numerous photosynthetic mutants have been of great help in understanding the operation of the photosynthetic apparatus and of the relationship between structure and function of thylakoid membranes. A mutant of *Chlamydomonas*, CC-2341, which has a frame-shift mutation in the *psa*B gene, is non-photosynthetic (Webber *et al.* 1993). Takahashi *et al.* (1991) reported that the two reaction centre polypeptides encoded by *psa*A and *psa*B were de-stabilised in the transformants of *C. reinhardtii* as a result of the absence of the *psa*C product when its gene was disrupted with the *aad*A expression cassette. Transformants were unable to grow on the minimal medium lacking acetate and were deficient in PS1 activity. Farah *et al.* (1995) found that the *psa*F mutant could still assemble a functional PS1 complex and was capable of photoautotrophic growth. However, the rate of electron transfer from plastocyanin to $P700^+$ was much lower in the mutant than in the wild type, indicating that the PsaF subunit plays an important role in docking plastocyanin to the PS1 complex. A *Synechococcus* sp. strain PCC7002 Δ*psa*B::*cat* can grow photoheterotrophically with glycerol as the carbon source. In the absence of the PsaA and PsaB proteins, the amount of several other PS1 proteins did not reach their normal levels. No light state transition could be detected in the mutant. The absence of PS1 reaction centre has no apparent effect on PS2 assembly and activity (Shen and Bryant 1995).

In this paper, a mutant strain *C. reinhardtii* CC-1047 obtained by UV irradiation was studied in comparison with its wild strain (WT) CC-125. WT cells were grown in Tris-acetate-phosphate (TAP) medium to a density of 10^6 cells per cm^3 and 10 cm^3 of the culture were exposed to UV radiation (253.7 nm) of 0.17 W m^{-2} for 1 min with constant agitation (Girard *et al.* 1980). Previous works on this mutant were focused on the genetic analysis or the discussion of whether a mutant lacking PS1 could perform photoautotrophic growth (Girard *et al.* 1980, Lee *et al.* 1996, Cournac *et al.* 1997, Redding *et al.* 1999). In this communication, we examined the effect of CPI-deficiency on the physiological mechanism in the mutant alga and the function of the adjacent protein complex of the thylakoid membrane.

Materials and methods

Algae: *C. reinhardtii* wild type CC-125 and the mutant CC1047 (Girard *et al.* 1980) were a generous gift from Dr. James W.F. Lee (Oak Ridge National Laboratory, USA) and Dr. Elizabeth Harris (*Chlamydomonas* Genetics Center, Duke University, USA). Cultures were grown in a minimal liquid medium with 2 kg m^{-3} sodium acetate at 25 °C under an irradiation of 100 μmol m^{-2} s^{-1}. Growth was monitored at 750 nm with a spectrophotometer everyday.

Isolation of chloroplasts was done according to Selman-Reimer *et al.* (1981) with the following modifications: cells were collected by centrifugation (1 000×*g*, 5 min) and washed with 50 mM Tris-HCl (pH 8.0) buffer. The cells were re-suspended in 50 mM Tris-HCl (pH 8.0) buffer, containing 0.5 mM $MgCl_2$, 10 mM NaCl, 0.4 mM sucrose, and sonicated thrice by a sonicator (*Shanghai Sonicator Instrument Company*) at 0 °C, 80 W, for 30 s each time. The suspension was centrifuged at 2 000×*g* for 5 min to remove unbroken cells. Chloroplasts were pelleted by centrifugation at 8 000×*g* for 10 min. The pellets were re-suspended with Tris buffer as above. The Chl content was measured spectrophotometrically according to Arnon (1949).

LDS-PAGE analysis of the thylakoids was carried out according to Wollman and Bennoun (1982). Thylakoid membranes were treated with 1 % LDS for about 1 h at 4 °C before electrophoresis and then electrophoresis was carried out using 5 % resolving gel and 12 % stacking gel.

Oxygen evolution rate of algae was measured with a Clark-type oxygen electrode (Wei *et al.* 1988a). The reaction mixtures contained 50 mM Tris-HCl, pH 7.4, 10 mM $NaHCO_3$, and algae cells containing 20 μg Chl. The actinic irradiance was about 1 000 μmol m^{-2} s^{-1}.

Electron transport rate of algae chloroplasts was determined by measuring oxygen evolution with a Clark-type oxygen electrode under saturating irradiance (about 1 000 μmol m^{-2} s^{-1}) and 25 °C. PS2 electron transport rate was assayed with H_2O as electron donor and with BQ (benzoquinone) as electron acceptor in the reaction mixture containing 50 mM Tris-HCl (pH 8.0), 5 mM NaCl, 5 mM $MgCl_2$, 0.5 mM BQ, and chloroplasts containing 20 μg Chl. PS1 electron transport rate was assayed with 2,6-dichlorophenolindophenol (DCPIP) as electron donor and with methyl viologen (MV) as electron acceptor in a reaction mixture containing 50 mM Tris-HCl (pH 8.0), 5 mM NaCl, 5 mM Mg_2Cl, 2 mM MV, 10 μM 3-(3',4'-dichlorophenyl)-1,1-dimethylurea (DCMU), 0.2 mM DCPIP, 5 mM Vc, 2 mM NaN_3, and chloroplasts containing 20 μg Chl. The whole chain (PS2+PS1) electron transport rate was assayed with H_2O as electron donor and MV as electron acceptor in a reaction mixture

containing 50 mM Tris-HCl (pH 8.0), 5 mM NaCl, 5 mM Mg_2Cl, 2 mM MV, 2 mM NaN_3, and chloroplasts containing 20 μg Chl.

Photoreduction of $NADP^+$ of alga chloroplasts: The basic method was that of Zanetti and Curti (1980). The difference in absorption at 340 nm in light and in the dark measured with spectrophotometer was taken as the reduction of $NADP^+$. Chloroplasts containing 20 μg of Chl were added into the reaction mixture containing 50 mM Tris-HCl (pH 8.0), 0.5 mM $NADP^+$, 10 mM $MgCl_2$, 30 μM ferredoxin (Fd), and algal chloroplasts with 20 μg Chl.

Photophosphorylation activity was measured according to Wei *et al.* (1990) and Allnutt *et al.* (1991) in 1 cm^3 reaction mixtures, each containing 50 mM Tris-HCl (pH 8.0), 5 mM NaCl, 5 mM $MgCl_2$, 2 mM Na_2HPO_4, 1 mM ADP, 0.3 M sucrose, 1 mM FeCy (ferricyanide) for non-cyclic or 0.05 mM PMS (phenazine methosulfate) for cyclic photophosphorylation and chloroplasts containing 20 μg Chl. ATP content was measured by the luciferin/luciferase luminescence assay (Allnutt *et al.* 1991).

Measurement of chloroplast ATPase activity: Chloroplasts were activated by light + dithiothreitol (DTT) according to Wei *et al.* (1988b). Chloroplasts containing 20 μg Chl were irradiated for 5 min under a photon flux density of 1 500 μmol m^{-2} s^{-1} in 1.0 cm^3 of a medium containing 50 mM Tris-Cl (pH 8.8), 2 mM $MgCl_2$, 10 mM NaCl, 0.1 mM PMS, and different DTT concentrations (10, 15, 20, 25, and 30 mM, respectively). Then ATP was immediately injected to a final concentration of 5 mM ATP and the reaction was allowed to proceed for 5 min in the dark at 37 °C. The reaction was stopped with 0.2 cm^3 of 20 % trichloroacetic acid, and then the inorganic phosphate content in the reaction mixture was determined.

The assay for methanol-stimulated ATPase of chloroplast was similar to that of Wei *et al.* (1988b). Chloroplasts containing 20 μg Chl were added to the reaction mixture containing 50 mM Tris-Cl (pH 8.0), 2 mM $MgCl_2$, 10 mM NaCl, 5 mM ATP. Methanol concentration in the reaction mixture was 30, 35, 40, 45, or 50 %. The reaction was allowed to proceed in the dark at 37 °C for 2 min, and then stopped with 0.2 cm^3 of 20 % trichloroacetic acid. The inorganic phosphate produced in the reaction mixture was measured according to Taussky and Shorr (1953).

Results

LDS-PAGE analysis of thylakoid membranes from *C. reinhardtii*: Three green bands found on the unstained LDS-PAGE gel pattern of thylakoid membrane of WT *C. reinhardtii* were identified as CPI, CPII, and FP (free Chl) (Fig. 1, *A1*), which was consistent with the results of Thornber (1986). Only two green bands were found on the gel pattern of the mutant, *i.e.* CPII and FP. Only trace of a green band was seen on the counterpart of WT (Fig. 1, *A2*). After being stained with Coomassie Brilliant Blue, a faint band was observed at this position (Fig. 1, *B2*). Hence there was almost no CPI in the mutant CC-1047. Moreover, Chl and the protein were absent. No other obviously different position between the mutant and WT was found.

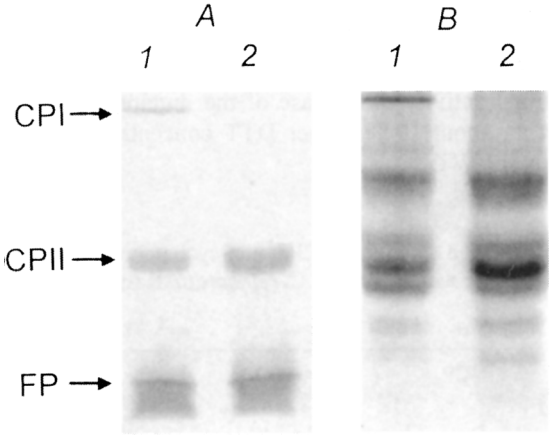

Fig. 1. LDS-PAGE of the thylakoid membrane from wild type (*1*) and mutant type (*2*) of *C. reinhardtii*. The gel was unstained (*A*) or stained with Coomassie Brilliant Blue (*B*). LDS-PAGE was carried out according to Wollman and Bennoun (1982).

Observation of *C. reinhardtii* growth: The growth curves of WT and CC-1047 were tested with different culture media (Fig. 2). The growth of WT showed a slow-fast-slow feature on the minimal medium without organic carbon source. It grew faster when sodium acetate was added to the minimal medium (Fig. 2, curves *A* and *B*). Under the same condition, CC-1047 cells grew very

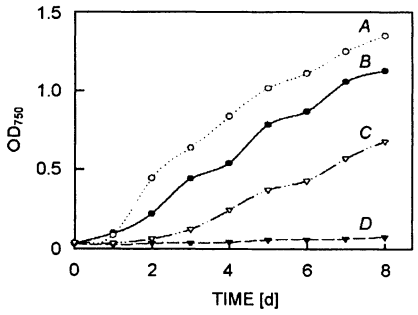

Fig. 2. Growth curves of *C. reinhardtii*. (*A*) Wild type (WT) in minimal culture medium + sodium acetate. (*B*) WT in minimal culture medium. (*C*) Mutant in minimal culture medium + sodium acetate. (*D*) Mutant in minimal culture medium.

slowly, but maintained their green colour for a long time. On the minimal medium with sodium acetate, the mutant cells grew normally, though at a much lower rate (Fig. 2, curves *C* and *D*).

Change in oxygen evolution ability: In the presence of 10 mM $NaHCO_3$, the O_2 evolution rates of the mutant was only 12.5 % of that of WT (Fig. 3*A*).

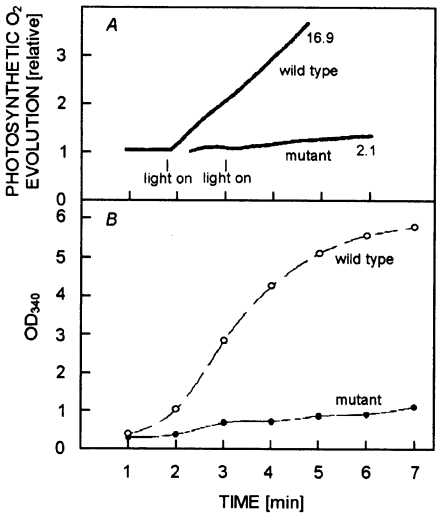

Fig. 3. Photosynthetic O_2 evolution [μmol(O_2) kg^{-1}(Chl) s^{-1}] (*A*) and photoreduction of $NADP^+$ in light (*B*) of *Chlamydomonas reinhardtii*.

Electron transport: The production of NADPH, ATP, and O_2 in thylakoid membrane involves the transfer of electrons through a chain of electron carriers between H_2O and $NADP^+$, designated as non-cyclic electron transport. Both the whole chain (PS2+PS1) and PS1 electron transport rates in mutant chloroplasts were slower than in chloroplasts of WT (Table 1), while the PS2 electron transport rate of the mutant was close to that of WT. The whole chain (PS2+PS1) and PS1 electron transport rates of the mutant type chloroplasts were less than 40 % of those of WT.

Photophosphorylation activity: Non-cyclic photophosphorylation rate of the alga chloroplasts was measured with potassium ferricyanide (FeCy) as electron acceptor and cyclic photophosphorylation with phenazine methosulfate (PMS) as a cofactor. Both cyclic and non-cyclic photophosphorylation activities of the mutant type chloroplasts were lower than the activities of WT (Table 1).

Photoreduction of $NADP^+$: Fig. 3*B* shows that in the WT chloroplasts $NADP^+$ was reduced to NADPH slowly in the first 2 min of irradiation, then the rate was higher for 4~5 min, and finally slowed down again. The photoreduction rate of chloroplast $NADP^+$ in the mutant CC-1047 was much lower than that of WT.

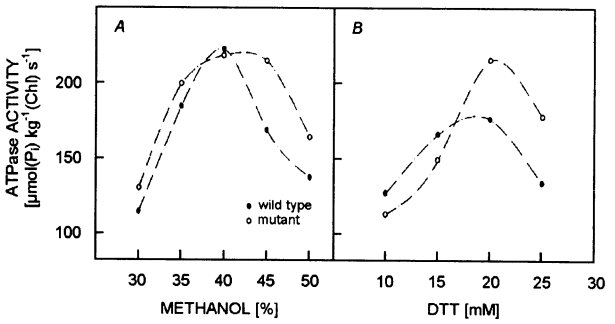

Fig. 4. The chloroplast ATPase activities of mutant and wild type *C. reinhardtii*. *A*, activated by methanol; *B*, activated by DTT + irradiation.

The ATPase activity of chloroplasts: Chloroplast ATPase is a latent ATPase that can be activated in a variety of ways to exhibit high Mg^{2+}- or Ca^{2+}-ATPase activity. Measurements by two methods were performed to observe any difference between the two kinds of chloroplast ATPase activity of the algae. Fig. 4 shows some differences in ATPase activity between WT and the mutant. When chloroplasts were activated by different concentrations of methanol, the maximal activities of ATPase in WT and mutant were very close, but more methanol was needed for exerting the maximal activity of ATPase of the mutant. When algae chloroplasts were activated by irradiation + DTT, more DTT was needed for exerting the maximal activity of chloroplasts of the mutant. However, the maximal activity of ATPase of the mutant type was found at an about 10 % higher DTT concentration than needed by the WT.

Table 1. Electron transport activities [mmol(O_2) kg^{-1}(Chl) s^{-1}] and photophosphorylation activities (NCP – non-cyclic photophosphorylation, CP – cyclic photophosphorylation) [mmol(ATP) kg^{-1}(Chl) s^{-1}] of the wild type and the mutant *C. reinhardtii*. Results of three independent measurements. WC – whole chain.

	Electron transport activity									NCP			CP		
	PS2 (H_2O→BQ)			PS1 (DCPIP→MV)			WC (H_2O→MV)								
	1*	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Wild type (WT)	6.0	3.6	4.9	31.7	21.7	22.5	15.9	9.9	11.6	16.8	21.8	27.5	24.5	43.3	33.3
Mutant	5.8	3.9	3.8	12.3	7.9	7.5	4.0	3.0	4.1	7.6	8.4	10.8	9.4	10.1	11.1
Mutant/WT [%]	96.7	108.0	77.6	38.8	36.4	33.3	25.2	30.3	35.3	45.2	38.5	39.3	38.4	23.3	33.3

Disscusion

An important feature of *C. reihardtii* is that its photosynthetic function is dispensable when a carbon source such as acetate is present in the growth medium. The cells can, therefore, be grown under three different regimes: on acetate medium in light (mixotrophic growth), on acetate medium without light (heterotrophic growth), and with CO_2 as the only carbon source in light (photoautotrophic growth) (Hippler 1998). The mutant CC-1047 used in this study could not grow normally on the minimal medium without organic carbon source, but could remain green. On the medium with sodium acetate, it grew normally (Fig. 2). Heterotrophic growth can be presumed for the growth regime of the mutant type alga.

The mutant alga could not grow photoautotrophically and its oxygen evolution activity during photosynthesis was very low, suggesting that the operation of photosynthetic apparatus in the mutant is different from the wild type. Both the photophosphorylation activity (Table 1) and the photoreduction rate of $NADP^+$ (Fig. 3*B*) of chloroplasts in the mutant were much lower than those of the WT. Therefore CC-1047 generated much less ATP and NADPH than the WT. ATP and NADPH are the "assimilation power" required to drive carbon assimilation in the dark phase. The lack of the assimilation power must cause the abnormality of carbon assimilation and therefore the mutant alga cannot grow photoautotrophically.

Our experiments showed that while the PS2 electron transport rate of the mutant CC-1047 was very close to the WT, the PS1 and whole chain electron transport rates were about only 30 % of the WT (Table 1). Hence most of the CPI-deficiency affects mainly the PS1 electron transport. Based on these facts, we conclude that the abnormal growth of CC-1047 is due to the block of the electron transport, and the site of the block is at the PS1 complex. The LDS-PAGE confirmed that the mutant was almost deficient in the Chl-protein complex I (CPI) (Fig. 1). Our previous work showed that the oxidation-reduction rate of P700 in CC-1047 was much slower than that in WT, which also supported this conclusion (Tang and Wei 2001). The phenomenon that CC-1047 also could keep green but has an extremely slow growth rate was due to the low residual activity of its photophosphorylation, photosynthetic electron transport, and oxygen evolution which can be attributed to its residual CPI.

In vitro, chloroplasts isolated from the mutant perform photophosphorylation under irradiation while their ATPase activity is much lower. They show high ATP hydrolysis activity only after activation. DTT-induced activation of latent ATPase has been attributed to the reduction of disulfide bond in the γ-subunit and may result from an interference with the ε-subunit binding interaction (Nalin and McCarty 1984). Activation of CF_1 by organic solvents (for example, methanol) also involves the dissociation of the ε-subunit (Mills 1996). Our results showed that greater amount of methanol was needed for reaching the maximal activity of the mutant chloroplast ATPase which was close to that of WT, indicating that the effect of the thylakoid membrane on the ATP synthase increased due to CPI deficiency. On the other hand, more DTT was also needed for the mutant alga chloroplasts to reach maximal ATPase activity. This suggests that the structure of ATP synthase in CC-1047 may become tighter than that of WT, so it is difficult for DTT to get an access to the disulfide bond of the γ-subunit and reduce it.

Similar to the physiological activities of photosynthesis, such as electron transport or oxygen evolution, the function of adjacent membrane ATP synthase complex could also be affected by the deficiency of the CPI. However, the function of PS2 may be little affected by CPI deficiency alone. This may be related to their distribution on the thylakoid membrane (Melis and Homann 1976). Both PS1 and ATP synthase are located in the stromal thylakoid membrane and in the marginal parts of grana thylakoid membrane so they are near to each other. If CPI is deficient, the ATP synthase may be affected too. PS2 is located in the granum thylakoid membrane, a little away from the PS1. Thus its structure and function are little affected by CPI-deficiency of the PS1 complex. These results may suggest that the deficiency of one of the membrane-protein complexes on the thylakoid may affect the microstructure and function of the other adjacent ones, especially the ATP synthase complex.

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