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Evidence for a Block between Plastoquinone and Cytochrome *f* in a Photosynthetic Mutant of *Lemna* with Abnormal Flowering Behavior

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ABSTRACT

Mutant strain 1073 of *Lemna perpusilla* is concluded to be blocked between plastoquinone and cytochrome *f* in the photosynthetic electron transport system. The location of the block is based on the following observations of activities in chloroplasts isolated from the mutant and wild-type plants. (*a*) Relative to wild type, electron flow rates from water to ferricyanide, 2,6-dichlorophenol indophenol or NADP were very low in the mutant, but rates of photosystem I-dependent electron flow and cyclic phosphorylation were high. (*b*) Chlorophyll *a* fluorescence induction curves for mutant and wild type were similar. (*c*) Silicomolybdate and lipophilic acceptors in the mutant were photoreduced at rates comparable to wild type. (*d*) Cytochrome *f* of the mutant chloroplasts was not reduced by red light, but was oxidized by red or far red light. (*e*) Reduction of the primary electron acceptor of photosystem II (Q) by ATP-driven reverse electron flow was not observed in the mutant.

The use of photosynthetic mutants has been one of the important tools leading to our present understanding of the localization and sequence of electron transfer components. Most of the photosynthetic mutants have been induced in algae (12), primarily because of the relative ease of the necessary manipulations. The Lemnaceae (duckweeds) is a family of higher plants which may be propagated vegetatively in axenic culture autotrophically as well as heterotrophically. Although difficult to use in genetic studies, the duckweeds have attributes which make them potentially valuable for studies of photosynthesis in higher plants.

Lemna perpusilla strain 1073, a clone isolated from an x-irradiated culture of wild type (strain 6746), was reported to differ in its flowering responses to light (17). Addition of the photosynthetic inhibitor DCMU to WT[2] cultures caused flowering responses that mimicked those of the mutant. It was suggested that the mutant flowering responses were the result of a photosynthetic mutation. This hypothesis is supported by the following report in which an impaired site in the photosynthetic electron transport chain of strain 1073 is demonstrated and located. A preliminary report on the lack of photosynthetic activity in the mutant has been given elsewhere (6).

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[2] Abbreviations: WT: wild type; DAD: diaminodurene; DCPIP: 2,6-dichlorophenol indophenol; PMS: phenazine methosulfate; Q: primary acceptor of photosystem II, which in its oxidized form quenches the variable fluorescence of chlorophyll.

MATERIALS AND METHODS

Cultures of *Lemna perpusilla* 6746 (WT) and mutant strain 1073 were grown on half-strength Hutner's medium (10) with 1% sucrose under continuous cool white fluorescent light (16) of about 30 ft-c at 26 C.

Chloroplasts were prepared from *Lemna* and lettuce as previously described (2), except for the addition of 0.5 mg/ml of BSA to the grinding and washing media and 10 mg/ml to the resuspension medium (7). Chl concentration was determined according to Arnon (1). The reduction of ferricyanide, DCPIP, or NADP was measured in a Cary 14 spectrophotometer with an attachment to reduce scattering effects. Oxygen uptake was measured with a Yellow Springs Instrument Clark type O_2 electrode. ATP formation was assayed as described (2). Chl fluorescence induction and ATP-driven reverse electron flow (18) were measured in the apparatus constructed by Malkin (15).

Changes in the oxidation reduction state of Cyt *f* were followed in an Aminco-Chance dual wavelength spectrophotometer (3). Silicomolybdic acid was kindly provided by Dr. G. Ben-Hayyim of Tel Aviv University.

RESULTS

The ability of mutant 1073 to photoreduce several electron acceptors in reactions requiring excitation of photosystem II, photosystem I, or both is given in Table I. Electron transport from water to ferricyanide, DCPIP, or NADP was very poor in the mutant chloroplasts relative to the WT or to lettuce. The rates of ferricyanide reduction in the WT or lettuce chloroplasts were stimulated 2- to 3-fold by the addition of the uncoupler methylamine, whereas the rate in the mutant chloroplasts was unaffected, indicating that the block was not located in the energy-converting system. In the photosystem I-sensitized electron transport from reduced DAD to O_2 (9), the mutant was about as effective as the WT.

Consistent with the above results, mutant chloroplasts were inactive in photophosphorylation coupled to electron flow from water to ferricyanide but were active in PMS-catalyzed cyclic photophosphorylation. The major block was therefore in the electron transport system close to photosystem II.

To determine whether the block was located before, at, or after photosystem II, Chl fluorescence induction was followed. Figure 1 shows that the WT and mutant had essentially identical fluorescence induction curves, which were similarly affected by the addition of DCMU. The dark recovery of the variable fluorescence yield was also similar in the wild type and mutant (not shown). Since the rise in the variable fluorescence yield upon illumination is thought to be due to the electron transport system from water to and including the "pool" of electron acceptors (14), mainly plastoquinone, it is clear from the latter observa-

tions that the electron flow from water to the plastoquinone pool is not defective in the mutant. In agreement with the electron transport data (Table I), addition of ferricyanide slowed the rise in fluorescence yield in the WT (14), but had no effect on the fluorescence curve of the mutant.

Further evidence for the site of the block in the mutant was provided by analyzing the photoreduction of the lipophilic electron acceptors DAD and phenylenediamine (11, 19) and of silicomolybdate (4, 5, 8); the first two were reported to accept electrons at the level of plastoquinone and the last at the level of Q. The addition of these electron acceptors to mutant chloroplasts increased the low rate of ferricyanide reduction to rates similar to those of WT (Table II). As expected, the reduction of the lipophilic acceptors was sensitive to DCMU, whereas that of silicomolybdate was not. The block was located beyond the site of electron acceptance by DAD or phenylenediamine but preceded that of ferricyanide.

We next tried to establish whether the photooxidation or the photoreduction of Cyt *f* was blocked in the mutant (Fig. 2). Cyt *f* of the WT showed the typical responses to red and far red illumination (3), with the former reducing Cyt *f* and the latter oxidizing it. In the mutant either red or far red light oxidized Cyt *f*, indicating that the block was located on the photoreducing side of Cyt *f* (*i.e.* between photosystem II and Cyt *f*).

Table I. *Electron Transport and Photophosphorylation of Chloroplasts Isolated from L. perpusilla Wild Type, Mutant 1073, and Lettuce*

Reaction Measured	Lettuce	*Lemna perpusilla* Wild type	*Lemna perpusilla* Mutant 1073
		μeq/mg Chl·hr	
A. Electron transport			
H_2O to ferricyanide[1]	268	159	17
+ methylamine, 5 mM	713	378	21
H_2O to DCPIP[2]		123	12
H_2O to NADP[3]	163	49	0
Ascorbate + DAD to O_2 (+DCMU)[4]		237	193
		μmoles ATP/mg Chl·hr	
B. Photophosphorylation			
H_2O to ferricyanide[5]	364	188	0
PMS + Ascorbate (+DCMU)[5]	1352	737	368

[1] Ferricyanide reduction was determined as the light-induced decrease in 420 nm absorption. Reaction mixture contained in 3.0 ml: Tricine-KOH, pH 7.8, 20 mM; NaCl, 30 mM; $K_3Fe(CN)_6$, 0.4 mM, and chloroplasts containing about 45 μg of Chl. Illumination from a tungsten lamp passed through a Corning CS 2-62 filter. The photomultiplier was protected from the actinic light by a Wratten 34 and Corning CS 4-96 filters.

[2] DCPIP reduction was measured as in 1 above, but at 620 nm, without the Wratten 34 filter, and with 16.7 μM DCPIP replacing ferricyanide.

[3] NADP reduction was measured as in 1 above, but at 350 nm using a Corning CS 7-60 filter and a saturated $CuSO_4$ solution in front of the photomultiplier and with 0.33 mM NADP and 0.36 mg of ferredoxin replacing ferricyanide.

[4] Oxygen uptake was measured in a reaction mixture as in 1 above, but with NaN_3, 0.5 mM; DAD, 0.1 mM; DCMU, 2.5 μM; and Na-Ascorbate, 5 mM, replacing ferricyanide.

[5] Reaction mixture contained in mM in 3.0 ml: Tris-HCl (pH 7.8), 20; NaCl, 30; $MgCl_2$, 5; Pi (pH 7.8), 5 (containing 2.6×10^6 cpm ^{32}P); ADP, 1; chloroplasts containing about 40 μg Chl; and where indicated $K_3Fe(CN)_6$, 0.4; PMS, 0.033; Na-ascorbate, 5; DCMU, 0.005. Samples were illuminated for 1 min at 20 C. Light intensity was 160,000 lux.

Table II. *Photoreduction of Several Electron Acceptors by Wild Type and Mutant 1073 of L. perpusilla*

Electron Acceptor	Ferricyanide Reduction Wild type	Ferricyanide Reduction Mutant
	μmoles/mg Chl·hr	
Ferricyanide[1]	95	20
+DCMU, 7 μM	16	
DAD + ferricyanide[2]	265	201
+ DCMU, 7 μM	0	0
Phenylenediamine + ferricyanide[2]	280	203
Silicomolybdate + ferricyanide[3]	200	
+ DCMU, 7 μM	206	160

[1] Same as Table I, footnote 1.

[2] Reaction mixture was the same as in 1 above except for the addition of 0.2 mM DAD or phenylenediamine and an extra 0.6 mM ferricyanide (see 19).

[3] Reaction mixture contained in 3.0 ml: HEPES-KOH (pH 6.9), 30 mM; KCl, 30 mM; $K_3Fe(CN_6)$, 0.7 mM; silicomolybdate, 0.2 mg, and chloroplasts containing 40 μg of chlorophyll.

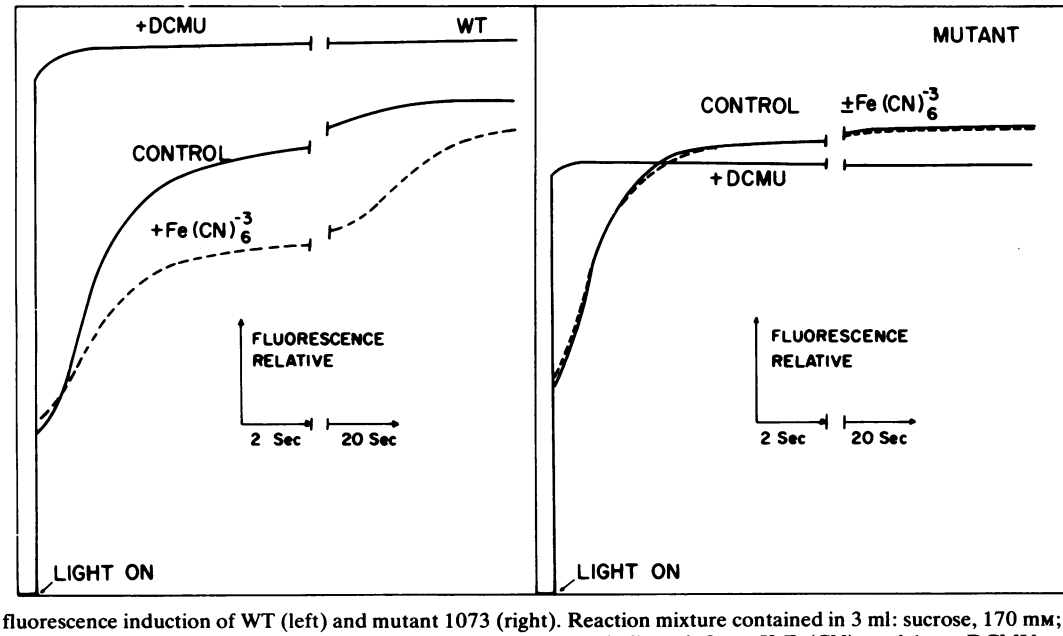

FIG. 1. Chl fluorescence induction of WT (left) and mutant 1073 (right). Reaction mixture contained in 3 ml: sucrose, 170 mM; NaCl, 80 mM; tris-HCl (pH 7.8), 40 mM; chloroplasts containing about 50 μg of Chl and, where indicated, 2 μM $K_3Fe(CN)_6$ and 1 μM DCMU.

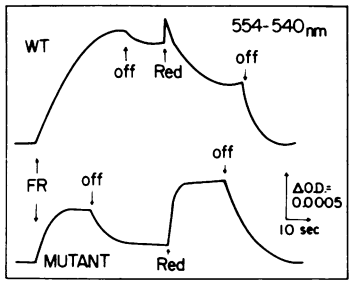

FIG. 2. Light-induced changes in the redox state of Cyt *f* of WT (top) and mutant (bottom). Reaction mixture contained in 3 ml: Tricine (pH 8.0), 15 mM; KCl, 20 mM; and chloroplasts containing 91 and 103 μg of Chl for the WT or mutant, respectively. Red light was filtered through a Schott RG 665 filter and far red light through a Baird-Atomic 730 nm interference filter.

Finally, a block at this site should inhibit the ATP-driven reverse electron flow activity (18). Wild-type chloroplasts exhibited ATP-driven reverse electron flow to an extent similar to that observed in lettuce chloroplasts (20–30% reduction of Q), while mutant chloroplasts were totally inactive. The light-triggered ATPase activity measured separately was similar in the WT and mutant, with a rate similar to that observed in lettuce chloroplasts (not shown).

DISCUSSION

Chloroplasts of the mutant strain do not photoreduce NADP, ferricyanide, or DCPIP with water as the electron donor but do have relatively high rates of photosystem I-dependent electron flow and cyclic phosphorylation (Table I). This suggests that the block is either between the two photosystems or before photosystem II. The latter possibility is excluded by the finding that the fluorescence induction curves of the mutant are normal (Fig. 1). These curves indicate that the electron flow in the mutant is intact at least up to and including the plastoquinone pool.

Further support of this conclusion is provided by the finding (Table II) that silicomolybdate can be reduced in a DCMU-insensitive reaction, and lipophilic electron acceptors like DAD and phenylenediamine can be reduced by the mutant. The former has been shown to be due to a direct reduction by Q (4, 5, 8), and the latter to reduction at the site of plastoquinone (11). The fact that Cyt *f* of the mutant is not reduced by red light but is oxidized by red or far red (Fig. 2) suggests that the defective site precedes Cyt *f*. The mutant chloroplasts do not exhibit ATP-driven reverse electron flow despite a normal light-triggered ATPase activity.

All the data suggest that the block in mutant strain 1073 is located between plastoquinone and Cyt *f*. The only photosynthetic mutant described thus far which resembles this mutant is ac-21 of *Chlamydomonas reinhardi* (13). Chloroplast fragments from ac-21, in contrast to our mutant, do photoreduce ferricyanide and DCPIP from water. A possible explanation for this difference is that the defect in ac-21 is closer to Cyt *f* in the electron transport chain. Alternatively, both mutants may have a similar block, but the treatment necessary to obtain chloroplast fragments in *Chlamydomonas* is such that ferricyanide and DCPIP can be reduced directly by reduced plastoquinone, whereas this is not the case in the *Lemna* chloroplast preparation.

This communication describes a mutant with a defect in the electron transport system in a region for which no electron transport components have been described. Further study of the chloroplast components of this mutant may help to identify the missing substance. Another problem to be resolved is the relationship between the photosynthetic defect and the flowering behavior of the mutant. Previous results (17) showed that when the mutant was grown on appropriate sucrose-supplemented media, flowering was promoted by high intensity light; WT responded similarly if treated with DCMU. It would therefore be of interest to determine whether the mutant is capable of certain photosynthetic reactions, such as cyclic phosphorylation, *in vivo* as it is *in vitro*.

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