Plant Physiol. (1980) 66, 18–24
0032-0889/80/66/0018/07/$00.50/0

# Characterization of Three Photosystem II Mutants in *Zea mays* L. Lacking a 32,000 Dalton Lamellar Polypeptide[1]

Received for publication June 29, 1979 and in revised form February 15, 1980

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## ABSTRACT

**Two fully blocked and one partially blocked photosystem II nuclear mutants have been selected in *Zea mays*. The fully blocked mutants lack photosystem II activity, variable fluorescence, the light-inducible C-550 signal, the high potential form of cytochrome *b*-559, and most or all of the low potential form of the cytochrome. The block in these mutants may primarily affect the reducing side of photosystem II, inasmuch as chloroplasts isolated from both mutants exhibit an elevated F695 fluorescence emission peak. The partially blocked mutant exhibits partial photosystem II activity and a reduction, but not the total loss of the variable fluorescence yield, the C-550 signal, and the high potential form of cytochrome *b*-559. Lamellae isolated from the fully blocked mutants are greatly deficient for a major lamellar polypeptide with an apparent molecular weight of 32,000 daltons, whereas lamellae from the partially blocked mutant show the partial loss of this same polypeptide, suggesting that the 32,000 dalton polypeptide is necessary for the proper function of photosystem II.**

Genetic mutants are useful tools for the study of photosynthesis, as has been amply documented in the classic work with algal photosynthetic mutants (22). However, with the exception of the large collection of well characterized pigment mutants in barley (17, 36), the number of photosynthesis mutants studied in any single species of flowering plant has been relatively small. To date, most higher plant photosynthesis mutants are characterized by gross pigment losses which are often accompanied by major alterations in thylakoid membrane morphology and the loss of many lamellar polypeptides (9, 15, 36, 37). Recently, a large collection of photosynthesis mutants was obtained in *Zea mays* (28) using elevated levels of Chl fluorescence to screen specifically for mutants blocked in photosynthesis (29). By employing this technique, both fully green and yellow-green nuclear mutants with blocks in electron transport, photophosphorylation, and dark reactions have been identified (26, 28).

The present study describes the characteristics of three nuclear photosynthesis mutants in maize. Two of these mutants, *hcf**-3[3] and *hcf**-19G, are fully blocked in PSII, whereas a third mutant, *hcf**-19YG, is partially blocked in PSII. All three mutations affect not only PSII activity, but also cause the loss of one or both redox forms of Cyt *b*-559 and the loss of a major lamellar polypeptide with an apparent mol wt of 32,000 daltons. Allelism tests reveal that *hcf**-3 is genetically distinct from both *hcf**-19 G and *hcf**-19YG, although the allelic relationship between the latter two mutants is unclear (20). This suggests that at least two and possibly three nuclear loci necessary for the proper assembly and function of PSII in maize plastids have been identified.

## MATERIALS AND METHODS

**Plant Material.** Maize (*Zea mays* L.) seedlings were grown to the three-leaf stage in controlled environment chambers as described previously (28). The mutants *hcf**-3, *hcf**-19G, and *hcf**-19YG were originally assigned the isolation numbers E-846, E-1257A, and E-1257B, respectively, by M. G. Neuffer, who recovered the plants after treatment of pollen with the alkylating agent ethyl diethane sulfonate (30), and who supplied the original $M_2$ seed as accession material. All mutants were originally selected on the basis of hcf upon irradiation with a long wave UV source. Inheritance and transmission studies indicate that *hcf**-3, *hcf**-19G, and *hcf**-19YG behave as would be expected for nuclear mutations (20).

**Fluorescence Induction.** Whole leaf fluorescence induction kinetics were measured by a modification of the methods of Miles and Daniel (28). Leaf segments were excised and placed in a mask exposing a 6- × 20-mm area of the upper leaf surface. The mask was placed at a 45° angle to a stable DC-powered tungsten light source. Actinic light was provided by passing light from the tungsten lamp through a blue plastic broad bandpass filter (peak transmission, 450 nm) providing an intensity of $6 \times 10^3$ erg $cm^{-2}$ $s^{-1}$ at the leaf surface. Fluorescence was measured at 45° to the leaf surface with an EMI-9558B photomultiplier (S-20 response) powered by a Pacific Photometric model 203 negative high voltage supply. The photomultiplier was protected from scattered light by a Corning 2030 and a Schott RG-N9 filter, the combination giving a sharp 10% cut off at 711 nm. The anode signal was amplified by a Keithley 414 picoammeter and displayed on a Houston 2000 X-Y recorder.

**Chloroplast Isolation.** Chloroplasts used for the measurement of photoreactions were isolated by grinding 10 g washed maize leaves in a chilled Sorvall Omni-Mixer with 60 ml of cold isolation buffer containing 30 mM Na-Tricine (pH 8.0), 0.33 M sucrose, 1 mM EDTA, 1 mM $MgCl_2$, 5 mM 2-mercaptoethanol, and 0.1% (w/v) BSA. A small amount of solid polyvinylpolypyrrolidone was added just prior to grinding. The preparation was filtered through Miracloth, centrifuged briefly at 1,500*g* to remove debris, and the chloroplasts collected from the supernatant by centrifugation at 1,500*g* for 10 min. After isolation, chloroplasts were suspended in the grinding medium and Chl determined using the absorption coefficients of MacKinney (25).

Chloroplasts used in all other assays were isolated by grinding washed maize leaves in the Omni-Mixer with 20 mM Na-Tricine (pH 7.8), 0.8 M sucrose, and 10 mM NaCl (STN medium). The

[1] This research was supported by National Science Foundation Grant PCM-76 08831.

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[3] Abbreviations: *hcf*: high chlorophyll fluorescence; DBMIB: 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCIP: 2,6-dichlorophenol indophenol; MV: methylviologen; LP: low potential; HP: high potential; ctDNA: chloroplast DNA.

homogenate was filtered through eight or more layers of Miracloth and the pellet collected by centrifugation at 4,500*g* for 8 min. The pellet was judged free of significant mitochondrial contamination by the absence of an *A* maximum at 598 nm attributable to Cyt $aa_3$ in a dithionite minus ferricyanide difference spectrum. The STN pellet was resuspended in various media as described below.

For the analysis of lamellar polypeptides, thylakoid membranes were prepared by grinding washed maize leaves in a Waring Blendor with 100 mM Na-Tricine (pH 7.8), 0.4 M sorbitol, 10 mM NaCl, and 5 mM $MgCl_2$ followed by three washes in a medium consisting of 10 mM Na-Tricine (pH 7.8) and 10 mM NaCl.

**Photoreactions.** Light-dependent electron transport was measured polarographically using a Yellow Springs Instruments #4044 Clark electrode. Actinic light ($1.9 \times 10^5$ erg $cm^{-2}$ $s^{-1}$) was provided by unfiltered light from a 750-w AC projection lamp. For measurements of $O_2$ evolution, chloroplasts were isolated as described above and aliquots containing 15 μg Chl were resuspended in a reaction mixture containing 40 mM Tricine (pH 7.8), 10 mM NaCl, 4 mM $MgCl_2$, 5 mM $NH_4Cl$, and 2 mM $K_3(FeCN)_6$ in a total volume of 5.4 ml. PSI-dependent $O_2$ uptake was measured as above, using 100 mM MV as terminal electron acceptor and 80 μM DCIP and 1 mM ascorbate as an electron donor system. PSII contributions were eliminated with 30 μM DCMU. PSII-dependent $O_2$ uptake in the presence of DBMIB and $Mn^{2+}$ was assayed as described by Miles (27).

**C-550.** The low temperature, light-induced C-550 signal was measured in a DW-2 recording spectrophotometer equipped with a low temperature accessory. Chloroplasts were isolated in STN medium and the pellet resuspended in a small volume of reaction mix containing 20 mM Hepes (pH 7.7), 0.25 mM sorbitol, and 1 mM $MgCl_2$. The suspension was made 75% (v/v) in glycerol, incubated in the dark for 5 min, and frozen with liquid $N_2$ in the dark. The baseline was recorded and corrected after 20–30 min in the dark and the light minus dark difference spectrum subsequently scanned from 538 to 575 nm. Actinic illumination ($7 \times 10^4$ erg $cm^{-2}$ $s^{-1}$) was provided for 10–20 s by white light from a microscope illuminator. Large signals were observed using a final Chl concentration of 200–500 μg/ml in a cell of 2-mm optical path length.

**Low Temperature Fluorescence Emission.** Low temperature fluorescence emission spectra from isolated chloroplasts were measured in an Aminco-Bowman spectrofluorimeter modified as described by Newman and Sherman (31). Chloroplasts were isolated in STN medium and resuspended in 50 mM $NaH_2PO_4$ (pH 6.5) and 0.15 M sorbitol. Small aliquots of the sample were added directly to 75% glycerol to give a final Chl concentration of 5–10 μg/ml. Chl was excited at 430 nm by light from a xenon lamp and the fluorescence emission recorded from 550 to 790 nm.

**Cyt Determinations.** Room temperature Cyt difference spectra were measured in an Aminco DW-2 spectrophotometer. The STN pellet was resuspended in 50 mM $NaH_2PO_4$ (pH 6.5), and 0.15 M sorbitol to a final Chl concentration of 1–2 mg/ml. Aliquots from this stock suspension were diluted to 40 μg/ml Chl in the resuspension buffer and spectra recorded in the split-beam mode.

Estimations of Cyt content were made from room temperature reduced minus oxidized difference spectra using a procedure modified after that of Henningsen and Boardman (14) with 60 μM potassium ferricyanide, freshly recrystallized 0.2 mM hydroquinone, and a few grains of solid dithionite used as redox mediators. These low concentrations of oxidant and reductants were saturating; higher concentrations resulted in apparent bleaching at the long wavelength end of the spectra. Cyt $b$-$559_{HP}$ was determined from the *A* change at 559 nm in hydroquinone minus ferricyanide difference spectra. Total Cyt *b*-559 and Cyt $b_6$ were determined from *A* differences at 559 and 563 nm, respectively, in dithionite minus ferricyanide difference spectra. Cyt *f* was determined at 554 nm as the average of the values calculated from dithionite minus ferricyanide and hydroquinone minus ferricyanide difference spectra. Cyt $b$-$559_{LP}$ was estimated as the calculated difference between total Cyt *b*-559 and Cyt $b$-$559_{HP}$. Direct calculation of Cyt $b$-$559_{LP}$ from dithionite minus hydroquinone difference spectra were not considered reliable because of the difficulty of obtaining reproducible spectra from the yellow-green mutants. In all cases the amplitude of the *A* change was calculated as the difference between the spectral envelope at the measuring wavelength and a baseline joining the troughs as about 545 and 570 nm. Cyt contents were calculated from measured *A* changes using the wavelength equations of Heber *et al.* (13).

The time course of Cyt $b_6$ reduction was followed using a modification of the procedure of Bendall *et al.* (3). The progressive reduction of Cyt $b_6$ in a dithionite minus ferricyanide difference spectrum was followed by repeatedly scanning the spectrum in the region from 535 nm to 575 nm after the addition of a few grains of solid dithionite.

**Electrophoresis.** Thylakoid membrane samples were analyzed by SDS-gradient gel electrophoresis using the discontinuous buffer system of Laemmli (18). The gel consisted of a 1.5-cm stacking gel made 5% (w/v) in acrylamide and a 9-cm analyzing gel incorporating a 10% to 15% (w/v) linear acrylamide gradient accompanied by a 0.9% to 9% (w/v) linear sucrose gradient. Gels were formed between two glass plates (either 17.5 × 20 cm or 17.5 × 30 cm) separated by 1-mm thick spacers. The sandwich was mounted and the experiment executed in a locally constructed vertical slab gel apparatus. Power was provided by either a Heathkit IP-17 constant voltage power supply or a Brinkmann 1-kv voltage and current-regulated power supply.

Samples (thylakoids isolated as described) were prepared by boiling for 2 min in the sample buffer described by Laemmli (18). Phosphorylase A (92,000 daltons), BSA fraction V (67,000 daltons), ovalbumin (45,000 daltons), chymotrypsinogen (25,500 daltons), and Cyt *c* (12,400 daltons) were added to a separate well for mol wt calibration. Gels were stained for 30–45 min in 0.1% (w/v) Coomassie brilliant blue R-250, 50% (v/v) methanol, and 7% (v/v) glacial acetic acid and were destained overnight in 20% (v/v) methanol, 7% (v/v) glacial acetic acid, and 3% (v/v) glycerol.

MV was obtained from Sigma. DBMIB was synthesized by the procedure outlined by Trebst *et al.* (38).

## RESULTS

We report here the further characterization of *hcf**-3 (28) and the initial characterization of two new mutants, *hcf**-19G and *hcf**-19YG. All three mutants appear to be similarly blocked in PSII. The designations -G and -YG refer to the seedling phenotype upon emergence; typically, *hcf**-19G and *hcf**-19YG were both slightly yellow-green at the three-leaf stage of seedling growth. For subsequent biochemical characterization *hcf**-19G and *hcf**-19YG were separated on the basis of differences in whole leaf fluorescence induction kinetics or delayed luminescence (20). In contrast, *hcf**-3 remains fully green to the three-leaf stage. All three mutants are seedling lethal, growth to the three-leaf stage presumably supported by kernel storage products.

Three lines of evidence suggest that *hcf**-3, *hcf**-19G, and *hcf**-19YG are nuclear mutants. First, all three mutants were recovered following mutagenic treatment of mature pollen grains. Since extranuclear genetic information is not passed through the pollen in maize, all mutations transmitted by the treated pollen are presumed to be nuclear. Second, all three lesions are pollen transmissible in outcrosses, arguing against maternal inheritance. Finally, *hcf**-3 and *hcf**-19YG have been mapped to the nuclear genome (19). Allelism tests indicate that *hcf**-3 is not allelic to either *hcf**-19G or *hcf**-19YG, although the genetic relationship between the latter two mutants is unclear (20). Taken together, this suggests that the mutants *hcf**-3, *hcf**-19G and *hcf**-19YG represent mutations at at least two, and possibly three, nuclear

loci.

**Photoreactions.** Chloroplasts isolated from *hcf**-3 and *hcf**-19G exhibit very low rates of $O_2$ evolution with ferricyanide as terminal electron acceptor (Table I). In contrast, chloroplasts isolated from *hcf**-19YG are only partially blocked under the same conditions. Since ferricyanide is believed to accept electrons from both photosystems under certain conditions (10), the PSII specific reaction catalyzed by low concentrations of DBMIB in the presence of $Mn^{2+}$ (27) was employed as a further test for PSII dependent electron transport. The $Mn^{2+}$-dependent PSII rates seen with chloroplasts isolated from *hcf**-3, *hcf**-19YG and wild-type sibs agree well with the rates of $O_2$ evolution seen with ferricyanide (Table I). Despite being fully blocked in PSII, chloroplasts isolated from *hcf**-3 and *hcf**-19G exhibit high rates of PSI activity. PSI-dependent electron transport is reduced in chloroplasts isolated from *hcf**-19YG relative to sib controls, but this reduction is less than that measured for PSII-dependent electron transport.

**Whole-Leaf Fluorescence Induction Kinetics.** Whole leaves excised from wild-type maize seedlings show the typical complex induction kinetics (Kautsky effect) characteristic of isolated plant and algal cells (Fig. 1). The rise to 0 represents a component of fluorescence which is insensitive to physiological processes, whereas the O-P rise, termed the variable fluorescence, is largely a reflection of the kinetics of reduction of the Q-A pool by PSII (32). Whole leaf induction transients from *hcf**-3 and *hcf**-19G show an elevated 0 level and the complete loss of the variable fluorescence yield. Transients from *hcf**-19YG also exhibited an elevated 0 level but retained a portion of the variable fluorescence yield, although the amplitude of the rise differed somewhat from planting to planting. The decreased amplitude of the O-P rise in this mutant is consistent with the observed reduction in PSII electron transport activity (Table I), while the presence of the P-S decline suggests that PSI is at least partially active. The complete loss of the variable fluorescence yield in the fully blocked mutants *hcf**-3 and *hcf**-19G suggests that primary charge separation is not possible in these mutants due either to the loss or inactivation of Q, or a similar loss of inactivation of the reaction center pigment of PSII.

Table I. *Light-dependent $O_2$ Evolution or Uptake (Mehler Reaction) by Chloroplasts Isolated from Maize Mutants and a Representative Wild-type Control*

Reaction media for the various assays are described under "Materials and Methods."

| Source | Electron Transport | | |
|---|---|---|---|
| | $H_2O \rightarrow K_3(FeCN)_6$ | $H_2O \rightarrow$ DBMIB/$Mn^{2+}$ | DCIP/Ascorbate $\rightarrow$ MV |
| | *μmol $O_2$/mg Chl·h* | | |
| Wild-type | 144 | −162 | 221 |
| *hcf**-3 | 3 (2%)[a] | 0 | 197 (89%) |
| *hcf**-19G | 7 (5%) | | 213 (96%) |
| *hcf**-19YG | 44 (31%) | −44 (30%) | 135 (61%) |

[a] Percent relative to wild-type.

**C-550.** Spectra of the light-induced low temperature C-550 signal from chloroplasts isolated from *hcf**-3, *hcf**-19G, *hcf**19YG, and control plastids are shown in Figures 2 and 3. The C-550 signal (seen as a carotenoid bandshift at 542 and 547 nm) has been shown to be a good but indirect indicator of the redox state of Q (6). At low temperature this signal is accompanied by an *A* decrease at 557 nm corresponding to the photooxidation of Cyt *b*-559$_{HP}$ (6). Large signals corresponding to the photoreduction of Q and the photooxidation of Cyt *b*-559$_{HP}$ were seen with wild-type plastids, while chloroplasts isolated from *hcf**-3 and *hcf**-19G did not show the C-550 signal or the photooxidation of Cyt *b*-559$_{HP}$ (Figs. 2 and 3). Chloroplasts isolated from *hcf**-19YG showed a reduction in the extent of the photoreduction of Q and photooxidation of Cyt *b*-559$_{HP}$ relative to sib controls.

**Low Temperature Fluorescence Emission Spectra.** Low temperature fluorescence emission spectra for plastids isolated from the fully blocked mutants *hcf**-3, *hcf**-19G, and wild-type seedlings are presented in Figure 4. At 77 K, maize plastids show the characteristic three banded fluorescence emission spectrum typical of a variety of photosynthetic organisms (33) with emission peaks at approximately 685 (F685), 695 (F695), and 735 nm (F735). In wild-type maize plastids F695 is typically seen as a small shoulder (33). Plastids from the fully blocked mutants *hcf**-3 and *hcf**-19G show a markedly increased fluorescence yield at 696 nm relative to wild-type (Fig. 4). Since F695 has been suggested to arise from pigments closely associated with the reaction center of PSII (5), these observations suggest that the loss or inactivation of Q, rather than the reaction center pigment P680, might be responsible for the observed lack of fluorescence quenching in the fully blocked mutants.

**Spectral Estimation of Cytochrome Content.** Reduced minus oxidized difference spectra reported previously (26) suggested that

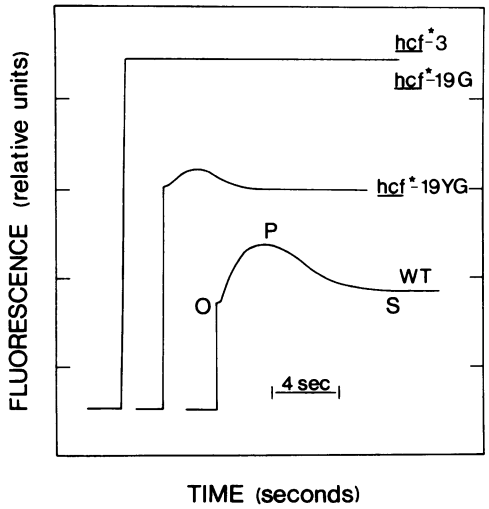
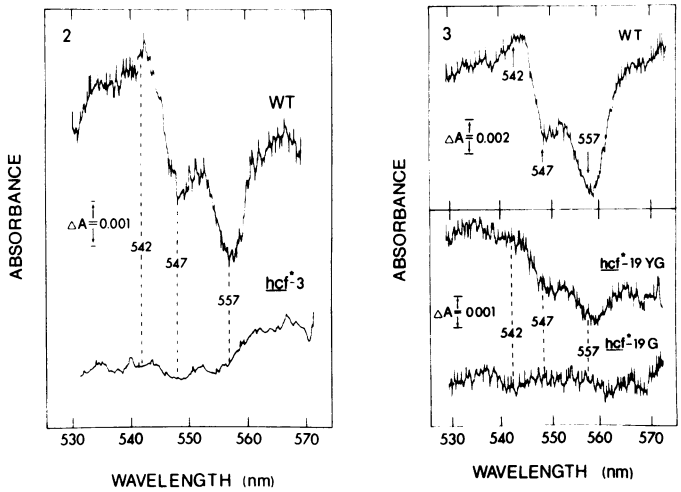



FIG. 1. Whole leaf fluorescence induction kinetics from wild-type (WT) and the high fluorescent mutants, *hcf**-3, *hcf**-19G, and *hcf**-19YG.




FIGS. 2 and 3. Light minus dark difference spectra of the C-550 change and the photooxidation of Cyt *b*-559$_{HP}$ at 77 K. Chl concentrations: WT-3, 325 μg/ml; *hcf**-3, 350 μg/ml; WT-19, 475 μg/ml; *hcf**-19YG, 200 μg/ml; *hcf**-19G, 330 μg/ml.

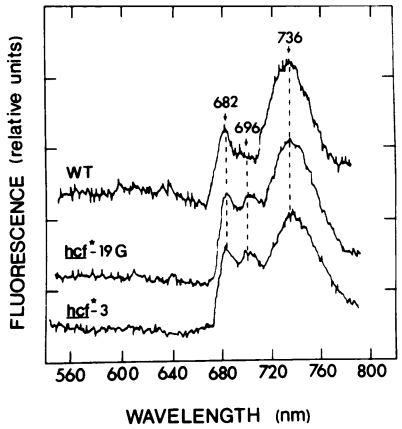
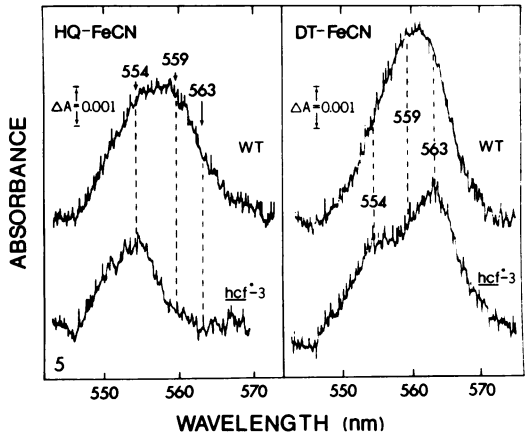
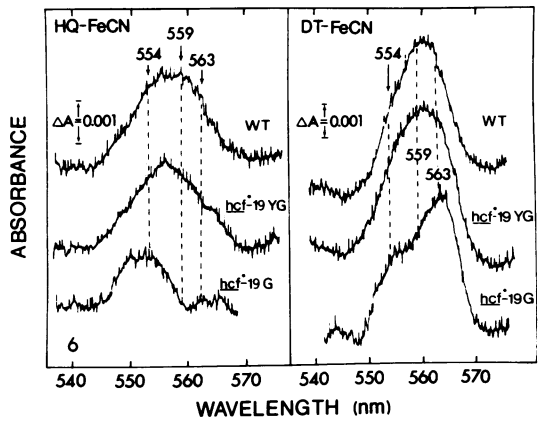



FIG. 4. Low temperature (77 K) fluorescence emission spectra from typical wild-type maize plastids and plastids isolated from the fully blocked mutants *hcf**-3 and *hcf**-19G. Chl concentration, 5–10 μg/ml.




FIGS. 5 and 6. Hydroquinone minus ferricyanide (HQ-FeCN) and dithionite minus ferricyanide (DT-FeCN) difference spectra for chloroplasts isolated from *hcf**-3, *hcf**-19YG, *hcf**-19G, and wild type sibs. Chl concentration in all cases was 40 μg/ml.

chloroplasts isolated from *hcf**-3 are deficient in the high potential form of Cyt *b*-559. Interestingly, may algal mutants blocked in PSII also exhibit the loss of one or both forms of the Cyt (4, 23, 35). Spectra presented in Figure 5 suggest that chloroplasts isolated from *hcf**-3 lack all of the high potential Cyt *b*-559 and are also deficient for most or all of the total Cyt *b*-559 pool as well. Calculations based on these and a large number of similar difference spectra indicate that the dip seen at 559 nm in dithionite minus ferricyanide difference spectra cannot be due to the loss of the high potential form alone, but that most or all of the low

Table II. *Estimated Cytochrome Content for Maize Mutants and wild-type Sibs Determined from Reduced Minus Oxidized Absorption Spectra*

All measurements were made using unstirred chloroplast suspensions with a final Chl concentration of 40 μg/ml. Samples treated with dithionite were incubated for at least 10 min prior to recording spectra. Values represent means of at least five determinations.

| Cytochrome | WT-3 | *hcf**-3 | WT-19 | *hcf**-19YG | *hcf**-19G |
|---|---|---|---|---|---|
| | | | *nmol Cyt/μmol Chl* | | |
| Cyt *f* | 1.5 | 2.1 | 1.5 | 1.8 | 2.1 |
| Cyt *b*-559$_{HP}$ | 2.2 | 0 | 2.2 | 1.6 | 0 |
| Cyt *b*-559$_{LP}$ | 1.4 | 0.6 | 0.5 | 0.9 | |
| Cyt *b*-6 | 2.6 | 3.5 | 3.6 | 4.0 | 4.3 |

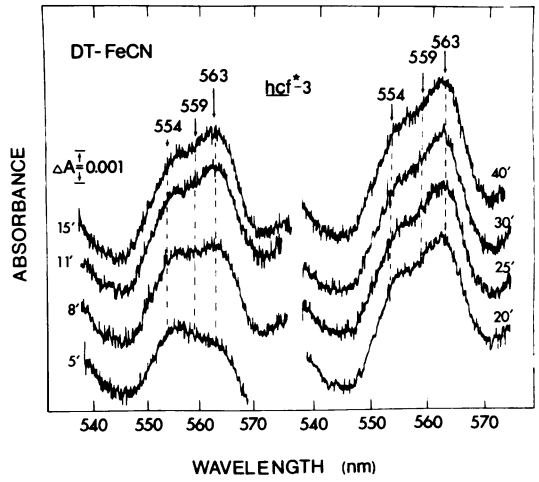



FIG. 7. Time course for the reduction of Cyt $b_6$ by dithionite in plastids from *hcf**-3. Numbers at the short or long wavelength ends of the spectra refer to time in minutes after the addition of a few grains of solid dithionite. Chl concentration, 40 μg/ml.

potential form of the Cyt is also missing (Table II).

Spectra presented in Figure 6 suggest the loss of all of the high potential and most of the low potential Cyt *b*-559 in plastids from *hcf**-19G. The small amount of Cyt *b*-559 present in the low potential form in this genetic background (0.5 nmol Cyt/μmol Chl in WT-19 *versus* 1.4 nmol Cyt/μmol Chl in WT-3) precludes an accurate measure of the extent of the loss of Cyt *b*-559$_{LP}$ in *hcf**-19G, but calculations suggest the complete loss of the low potential form. In contrast, chloroplasts isolated from *hcf**-19YG show only a partial loss of Cyt *b*-559$_{HP}$ and a normal or slightly increased content of the low potential form of the Cyt as compared to wild-type sibs (Table II).

Although it is clear that chloroplasts from *hcf**-3 and *hcf**-19G are completely deficient in Cyt *b*-559$_{HP}$, it is difficult to estimate the precise extent of the loss of the low potential form of the Cyt from difference spectra due to the existence of multiple redox potential forms of the *b*-type Cyt (8) and the large overlap between the spectral envelopes of the different chloroplast Cyt. For the same reasons it is possible that the calculated increase of Cyt *f* and $b_6$ associated with the loss of Cyt *b*-559 in *hcf**-3 and *hcf**-19G (Table II) is artifactual; these increases are not immediately apparent from the shape of the spectral envelopes.

Bendall *et al.* (3) reported a method for discriminating between Cyt *b*-559$_{LP}$ and Cyt $b_6$ based on differences in the rate of reduction of these two Cyt by dithionite. Since dithionite reduces Cyt *b*-559 more rapidly than it reduces Cyt $b_6$ (3), initial rapid scans of a dithionite minus ferricyanide difference spectrum show a peak at 559 nm; in subsequent scans the peak shifts toward 563 nm as Cyt $b_6$ becomes progressively reduced. In the case of *hcf**-3, a significant peak was never observed at 559 nm (Fig. 7),

whereas very rapid reduction of the Cyt was evident in wild-type sibs over the same time course (data not shown). Identical results were obtained with plastids isolated from *hcf**-19G (data not shown). These time course studies suggest a complete loss of Cyt *b*-559 in the fully blocked mutants *hcf**-3 and *hcf**-19G.

**Electrophoresis.** Polypeptide profiles obtained from maize lamellae typically contained 30 to 40 bands on gradient polyacrylamide gels; not all these bands are readily seen in Figure 8. Lamellae isolated from *hcf**-3 are greatly deficient for a major polypeptide with an apparent mol wt of about 32,000 daltons (Fig. 8); in some preparations, this polypeptide was completely absent. Lamellae isolated from *hcf**-3 also show decreases in the staining intensity of polypeptides with apparent mol wt of 9,000, 10,000, and 11,000 daltons as compared to normal sibs. These low mol wt polypeptides may not be specifically associated with PSII since they are completely missing from lamellae isolated from *hcf**-43, a maize mutant blocked primarily in PSI (Miles and Leto, unpublished). Small differences in staining intensity are also seen for minor polypeptides with apparent mol wt between 16,000 and 20,000 daltons; higher resolution runs on long (30 cm) slab gels revealed that lamellae from *hcf**-3 are slightly depleted in polypeptides with apparent mol wt of 19,000 and 16,000 daltons and are slightly enriched in a 17,000 dalton species (Fig. 8, inset). Alterations in this region of the gel are never as drastic as changes seen at 32,000 daltons.

Similar losses are seen in lamellae isolated from the *hcf**-19 series of mutants (Fig. 8); the 32,000 dalton polypeptide is present in the wild-type profile, is somewhat reduced in staining intensity in lamellae from *hcf**-19YG, and is almost completely absent from lamellae isolated from *hcf**-19G. The progressive loss of the 32,000 dalton polypeptide across the *hcf*-19 series of mutants parallels the progressive loss of PSII activity also seen across the series (Table I). As was seen for *hcf**-3, the staining intensity of the low mol wt triplet at 9,000, 10,000, and 11,000 daltons parallels that of the 32,000 dalton polypeptide. There are hints of alterations in the 16,000 to 20,000 dalton region of the gel, but high resolution analysis on long slab gels has not yet been carried out. None of the three PSII mutants show consistant losses of polypeptides in the 40,000 to 50,000 dalton mol wt region, the region suspected to contain the reaction center polypeptides for PSII in both *Chlamydomonas* (7) and barley (36, 37).

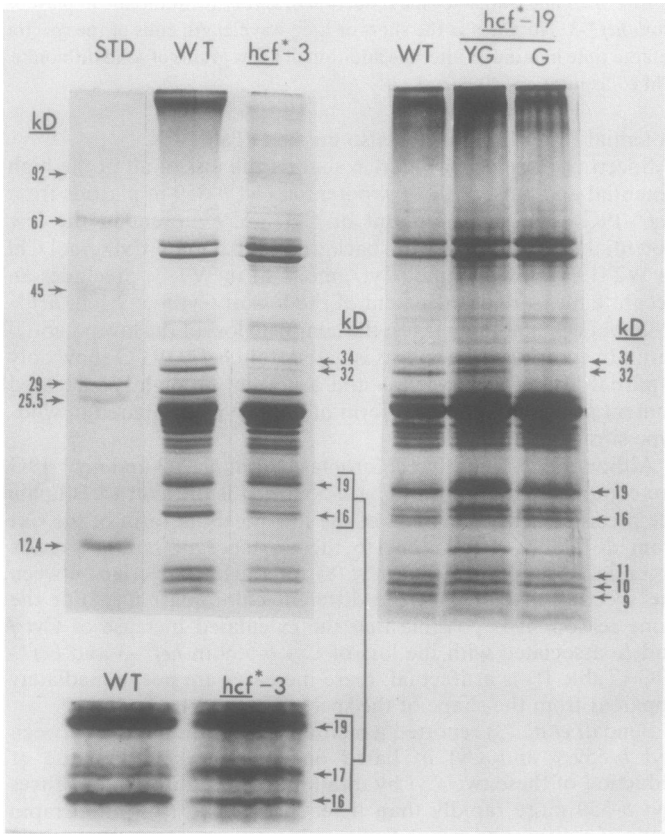



FIG. 8. Gradient polyacrylamide gel electrophoresis of lamellar polypeptides isolated from *hcf**-3, *hcf**-19YG, *hcf**-19G, and wild-type sibs. Apparent mol wt are indicated in kilodaltons (kD).

## DISCUSSION

The characteristics of the PSII mutants described here are summarized in Table III. The nuclear lesions *hcf**-3 and *hcf**-19G result in elevated levels of Chl fluorescence and the complete loss of the light-inducible C-550 signal. The high potential form of Cyt *b*-559 is missing in both mutant strains, and the low potential form of the Cyt is either greatly reduced or completely absent in these mutants. As expected, both mutants lack PSII activity (Table I). In contrast, the partially blocked mutant *hcf**-19YG retains a portion of the variable fluorescence yield, a reduced light-inducible C-550 signal, a reduction but not the total loss of Cyt $b$-$559_{HP}$, and a normal or slightly increased amount of the low potential form of the Cyt as compared to wild-type sibs. The fully blocked mutants *hcf**-3 and *hcf**-19G show a great reduction or the total loss of the 32,000 dalton lamellar polypeptide; the loss of this polypeptide is also seen, to a lesser extent, in lamellae isolated from *hcf**-19YG.

It is difficult to determine from the present data whether the lesions in the fully blocked mutants cause the loss of the entire PSII reaction center (P680 and Q), or whether the reducing side of PSII is preferentially affected. A high invarient fluorescence yield would be expected if either Q, P680, or both were lost or rendered non functional by mutation (6); the loss of the light-inducible C-550 signal would follow. Chloroplasts isolated from *hcf**-3 and *hcf**-19G exhibit an elevated F695 emission peak (Fig. 4); according to Butler (5), this emission comes from antenna pigments closely associated with PSII. Unfortunately, this still does not reveal whether P680 is present, since no low temperature fluorescence signal has been directly attributed to the reaction center Chl (5). Nevertheless, the presence of an elevated F695 fluorescence emission peak seen in the fully blocked mutants leaves open the possibility that these lesions may primarily involve the reducing side of PSII. In contrast, the $P_4$ mutant of *Euglena* (35) and several static and conditional PSII mutants in *Scenedesmus* (4) all lack F695 while exhibiting high, invariant room temperature fluorescence induction kinetics (4, 35). The genetic blocks in these algal mutants may lead to slightly different alterations in the structure of PSII than are seen in the fully blocked maize mutants.

The pleiotropic effect of mutations affecting PSII has been repeatedly observed in the photosynthetic algae, where it is well established that the mutational loss of PSII is almost always accompanied by the concomitant loss of Cyt *b*-559 (4, 7, 23, 25), and, in many cases, by the loss of lamellar polypeptides (7, 21). It is not surprising that the loss of Cyt *b*-559 accompanies the mutational alteration of PSII inasmuch as fractionation studies (1) and the direct photooxidation of the Cyt by PSII at 77 K (6) suggest a close physical relationship between the Cyt and PSII. Unfortunately, few parallel studies exist for similarly blocked mutants in flowering plants. Yellow-green sectors from the variegated tobacco mutant, NC-95 (16) and from the variegated *Oenothera* plastome mutants I alpha, II gamma, II delta, and II gamma (9) yield chloroplasts largely or completely lacking both variable fluorescence and PSII activity; however, little is known about the Cyt content, low temperature fluorescence emission characteristics, or lamellar polypeptide composition of these mutants. Pale green sectors from the plastome mutant en:viridis-1 of

Table III. *Summary Characterization of High Fluorescent PSII Maize Mutants*

All determinations relative to wild-type sib controls as described in the text.

| Characteristic | Wild-type | *hcf**-3 | *hcf**-19G | *hcf**-19YG |
|---|---|---|---|---|
| Pigmentation[a] | green | green | slightly yellow green | |
| Variable fluorescence | + | 0 | 0 | reduced |
| C-550 | + | 0 | 0 | reduced |
| F695 | + | elevated | elevated | NT[b] |
| Cyt *b*-559$_{HP}$ | + | 0 | 0 | reduced |
| Cyt *b*-559$_{LP}$ | + | absent, or greatly reduced | | slightly elevated |
| 32 kD Polypeptide | + | absent, or greatly reduced | | reduced |

[a] At the three-leaf stage.

[b] Not tested; presence inferred by PSII activity.

*Antirrhinum majus* (15) and several nuclear barley mutants (24, 36, 37) lack both PSII activity and, in each case, several lamellar polypeptides; again, fluorescence and Cyt data are lacking for nearly all of these mutants. Further work is needed to determine the extent to which all these mutants share common functional and structural characteristics.

The present study suggests a strong correlation between the loss of PSII activity and the loss of a major lamellar polypeptide with an apparent mol wt of 32,000 daltons (Fig. 8). This association is perhaps pointed out most convincingly in the *hcf**-19 family of mutants, where the progressive loss of PSII is clearly paralleled by the progressive loss of the 32,000 dalton polypeptide. A similar case was reported by Chua and Bennoun (7) for fully blocked and suppressed strains of F34, a PSII mutant in *Chlamydomonas*. In this case the partial restoration of PSII activity in the suppressed strain was correlated with the reappearance of two polypeptides with apparent mol wt of 47,000 and 50,000 daltons which were missing or greatly reduced in the nonsuppressed, fully blocked strain. This correlation was taken as strong evidence for the involvement of these polypeptides in PSII function. There is also evidence that a 46,000 dalton lamellar polypeptide, which cross-reacts with the 47,000 dalton *Chlamydomonas* polypeptide (37) is required for normal PSII activity in barley (36, 37). Several PSII-deficient barley mutants which lack the 46,000 dalton lamellar polypeptide have now been identified; in many cases the loss of the 46,000 dalton polypeptide is accompanied by the reduction or loss of a polypeptide migrating in the 32,000 dalton region of the gel (24, 36, 37). In barley these lesions are accompanied by the loss of the major subunits of the chloroplast coupling factor and several other unidentified polypeptides. Maize does not exhibit as prominent a band in the 40,000- to 50,000-dalton region as has been reported for *Chlamydomonas* and barley; however, the clear loss of the 32,000 dalton polypeptide in maize is not accompanied by the consistent loss of any polypeptides in the 40,000- to 50,000-dalton region of the gel (Fig. 8), suggesting an independent requirement of the 32,000 dalton polypeptide for PSII activity.

Much work has been devoted to understanding the control of the synthesis of the 32,000 dalton polypeptide in maize (2, 11, 12). This polypeptide appears to be derived from a 34,500 dalton precursor polypeptide which undergoes posttranslational modification (processing) resulting in a decrease in apparent mol wt to 32,000 daltons (12). The processing of the 34,500 dalton precursor species is presumably under nuclear control, since only the precursor is labeled by isolated chloroplasts, while both the precursor and the 32,000 dalton product are labeled when whole leaves are fed [$^{35}$S]methionine (12). Bedbrook *et al.* (2) have shown that during greening the accumulation of a chloroplast mRNA species hybridizing to ctDNA Bam restriction fragment 8 follows a similar time course as the *in vitro* translation of a 34,500 dalton polypeptide directed by bulk chloroplast mRNA. Taken together, this information suggests that the structural gene for the 32,000 dalton polypeptide resides on chloroplast DNA, that the transcription of the mRNA coding for the 34,500-dalton precursor is controlled by light, and that the processing of the precursor species may be under nuclear control (2). Because peptide mapping suggests that the stainable 32,000-dalton polypeptide and the 34,500-dalton polypeptide labeled *in vitro* are the same protein (12), it is possible that one or more of the nuclear maize loci described here may be necessary for the post translational modification of the precursor polypeptide.

*Acknowledgments*—The authors wish to thank B. Pill, L. Cowles, and P. Manning for expert technical assistance, L. Sherman for advice concerning gel electrophoresis, and C. J. Arntzen for the opportunity for further electrophoretic investigations.

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