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Photochemical Characteristics in a Soybean Mutant[1]

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ABSTRACT

Chloroplasts were isolated from wild type (DG) and heterozygous mutant (LG) soybean (*Glycine max*) leaves, and various biochemical functions were compared. Noncyclic electron transport, and its coupled phosphorylation, cyclic phosphorylation and H^+ ion transport in both systems, were 3 to 5 times faster in rate (on a chlorophyll basis) in the mutant plastids. On a chloroplast lamellar protein basis, the mutant plastid rates were 1.5 to 2.5 times the wild type rates.

Plastoquinone (PQ) reduction and oxidation (rates and extent) were measured by following absorbance changes at 260 nanometers with the repetitive flash technique. Mutant plastids have about a 2-fold greater apparent first order rate constant for PQ oxidation and a 3- to 5-fold larger pool of rapidly reducible PQ. Plastoquinone oxidation has been identified by other workers as the rate-limiting step in electron transport. Assuming the PQ oxidation is a first order process ($d(PQH_2)/dt = k_D[PQH_2]t$), the observed increase in k_d for the LG ($kd_{LG} \approx 2kd_{DG}$) and the greater steady state amount of rapidly turning over PQ, $[PQH_2]_{LG} > [PQH_2]_{DG}$, could account for the 3- to 5-fold greater rates of electron transport and phosphorylation found in the mutant chloroplasts.

Light saturation for noncyclic photophosphorylation and photosystem 2 plus 1 electron transport occurred at similar intensities for both LG and DG plastids. Relative quantum requirements extrapolated to zero intensity were similar in the LG and DG, although at finite light intensities the LG had a better relative quantum efficiency.

Ammonium chloride concentrations needed to inhibit cyclic photophosphorylation 50% were similar in both LG and DG plastids. Nigericin, poly-L-lysine, and chlorotri-*n*-butyltin, were needed in concentrations 5 to 10 times greater in the LG to yield 50% inhibition at comparable chlorophyll concentrations.

Many higher plant mutants exist which are depleted in chlorophyll content (14). While most of these mutants are lethal or grow very slowly, a few are able to photosynthesize and grow as rapidly as the normal.

Boardman and Highkin (1) described a barley mutant lacking chlorophyll *b* which was more susceptible to CMU[2] inhibition, appeared normal morphologically, and required a high light intensity for saturation. On a chlorophyll basis, the mutant photosynthetic rates were greater at light saturation when compared to the wild type. At low light intensities, however, the mutant had lower photosynthetic rates than the wild type.

Schmid and Gaffron (22) reported a tobacco mutant with ⅛ to 1/10 the wild type chlorophyll content which at high temperatures and high light intensity grow at the wild type rate.

A pea mutant (8) requires more light for saturation and reduces TCIP at four times the rate of the wild type. On a chlorophyll basis the mutant fixes CO_2 and reduces NADP more rapidly than the wild type. The mutant saturates at a higher light intensity when compared to the wild type (9). Smaller photosynthetic units have been suggested for these mutants.

A one-gene nuclear mutation of soybean expresses incomplete dominance in regard to chlorophyll concentration yielding three phenotypes. The heterozygous plants are a light green (LG) and the homozygotes a wild type (DG) and a lethal yellow (LY). The LG plants fix CO_2 on a per leaf area basis at a rate similar to the DG plant and are light-saturated at the same intensity which saturates the wild type (27).

Chloroplast composition and structural differences in these plants are described in the companion paper (13). On a leaf basis the LG and LY types have reduced protein, chlorophyll, and carotenoid values compared to the DG. The LG leaf has similar cytochrome and plastoquinone A + C (on a protein basis), and similar P_{700}/chlorophyll values compared to DG. There is twice as much plastoquinone A + C on a chlorophyll basis in the LG plastids compared to the DG. α-Tocopherol is nearly 5-fold greater in concentration in the LG on a chlorophyll basis. Accompanying these composition changes, the LG and LY have greatly altered thylakoid arrangements, there being very little stacked lamellae. The occurrence of significant structural and compositional differences between the wild type (DG) and the heterozygous soybean mutant (LG) provides a potentially useful probe for correlating membrane ultrastructure with chloroplast biochemistry. The functions reported herein include electron and proton transport, photophosphorylation, and the direct measurement of plastoquinone oxidation-reduction. The data suggest that the mutant chloroplasts have a significantly faster turnover time for plastoquinone, a step which Witt (26) has identified as the rate-limiting reaction for electron transport. The faster turnover time of the plastoquinone and the larger plastoquinone pool can account for the faster rates of electron transport and phosphorylation found in this mutant.

METHODS AND MATERIALS

The plant growth conditions were as in the accompanying paper (13). Chloroplasts from mature primary and trifoliate

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[2] Abbreviations: CMU: 1-(*p*-chlorophenyl)-3,3′-dimethyl urea; TCIP: 2,3′,6-trichlorophenolindophenol; DPIP: 2,6-dichlorophenolindophenol; MV: methyl viologen; pyo, pyocyanin; PSI: photosystem 1; PSII: photosystem 2.

leaves were isolated as previously described. After washing the plastids with isolation media, the plastids were resuspended in 1 to 2 ml of isolation media containing 5% Carbowax. Both types of plastids (LG and DG) were isolated simultaneously, and assays were run alternately.

The photochemical activities of the isolated plastids were assayed in a water-jacketed cuvette maintained at 15.5 C. The apparatus for recording pH changes was essentially that of Dilley and Vernon (5) with the addition of an oxygen probe (Chemtronics). The light sources, controlled by a powerstat, yielded 4.0×10^5 ergs cm^{-2} sec^{-1} at full intensity.

Hydrogen ion uptake and photophosphorylation were determined by the ΔpH technique (10, 19). Electron transport was measured by employing an oxygen electrode, calibrated after a series of measurements by correlating the amount of ferricyanide reduced (measured spectrophotometrically) with the signal obtained with the O_2 probe.

Chloroplasts containing about 100 μg of chlorophyll were added to a 10-ml reaction mixture containing 0.1 M KCl, 5 mM $MgCl_2$, and other components depending upon the assay. Photosystem 1 electron transport activity was measured with the following additions: 0.01 M Tricine, pH 8.5; 10^{-4} M DPIP; 3×10^{-3} Na ascorbate; 4×10^{-4} M MV; 5×10^{-4} M Na azide; and 10^{-5} M gramicidin D. Additional components for the photosystem 2 plus 1 electron transport assay included: 0.01 M Tricine, pH 8.5; 4×10^{-4} M MV; 5×10^{-4} M Na azide; and 10^{-5} M gramicidin D.

Cyclic photophosphorylation assays included 0.8 mM ADP, 0.8 mM KH_2PO_4 (P_i), and 3×10^{-5} M pyo. The solution was titrated to pH 8.0 to 8.5 with KOH before the addition of chloroplasts. Additions for noncyclic photophosphorylation were as follows: 0.8 mM ADP, 0.8 mM P_i, 4×10^{-4} M MV, and 5×10^{-4} M Na azide. This solution was also titrated to pH 8.0 to 8.5 prior to the addition of chloroplasts.

Carbon dioxide uptake by the terminal leaflet of the first trifoliate was determined. The leaflet attached to the plant was enclosed in a Plexiglas cuvette, and air was pumped across the leaflet at 1400 cc/min. The CO_2 concentration, monitored by an infrared gas analyzer (Mine Safety), was maintained by forcing CO_2 into the 1200-ml system by a mechanical pump. The pressure of the system was 1 mm of H_2O above ambient. The leaflets were illuminated with 300-w reflector flood lamps, and light intensity was altered by a powerstat. Light intensity was determined by a Weston-Todd foot-candle meter. The temperature of the closed system was constant at 26 C. A steady state CO_2 uptake was allowed at each light intensity prior to the experimental measurement. The measurement of the photosynthetic rate at each light intensity was determined by the CO_2 concentration decrease in the system to equilibrium. Data points were plotted on semilog paper ([CO_2] *versus* time), and initial rates were determined starting at 400 μl/liter. Following the experiments, leaflet outlines were traced on graph paper, and chlorophyll was extracted.

The oxidation-reduction kinetics of plastoquinone were determined in isolated plastids by measuring ultraviolet absorbance changes in the kinetic spectrophotometer described previously (12). A water-cooled deuterium lamp (WHS-200, Kern, Göttingen, Germany) was used as the measuring light source, in conjunction with a Bausch and Lomb 500-mm focal length monochromator. An EMI 9558-BQ photomultiplier tube was used as the detector. Schott reflection-type ultraviolet filters were used to guard the detector against the excitation light. A broad band interference filter covering the major red absorption band of chlorophyll was used for isolating the main excitation light from a Sylvania tungsten halogen lamp operated at 250 w, yielding a saturating intensity of 10^6 ergs/cm^2·sec. Supplemental background light was isolated by a 10-nm wide interference filter at 720 nm, providing a constant sink for the oxidation of the plastoquinone pool via reduction of MV. Absorbance change signals were averaged in a Fabri-tek signal averager to improve the signal-to-noise ratio; for a reasonable S/N ratio, 512 or 256 flashes were usually used for averaging. We assume that only plastoquinone undergoes rapid redox changes, and that α-tocopherol, which has a somewhat similar spectrum, is not involved. This assumption is supported by the difference spectrum in the ultraviolet region inasmuch as there is a single peak at 260 nm. If α-tocopheryl-quinone were involved in the redox changes equal ΔA would be expected at 260 and 270 nm, which was not the case, as will be shown below. We cannot, however distinguish between plastoquinone *a* and plastoquinone *c*, both of which are present.

RESULTS

Electron Transfer. The average values of photosynthetic reaction rates (cyclic and noncyclic photophosphorylation, proton uptake, photosystem 1 electron transport, and photosystem 2 plus photosystem 1 electron transport) of LG and DG isolated plastids are listed in Table I. The LG rates are three to five times greater than DG rates.

Other electron carriers such as FMN, phenothiazine methosulfate, and ferricyanide were employed in assays and gave LG rates three to five times that of DG. When cyclic photophosphorylation, PSI electron flow, or both were assayed, DCMU was not routinely included as it had no measurable effect on these rates. When only electron transport rates were measured, gramicidin was added, yielding increased rates because of its uncoupling action.

Phosphorylation. Optimal photophosphorylation rates were obtained in a pH range from 8.0 to 8.5. Electron transport rates and proton uptake activities were also assayed at this pH range. The concentration of the several components listed in the reaction mixtures (see "Methods and Materials") yielded the most active plastids.

Figure 1 shows the rate *versus* light intensity data for cyclic and noncyclic photophosphorylation as well as electron transport supported by PSI alone or PSII plus PSI. The intensities for the first measurable rates were similar for both LG and DG plastids in the four assays. Noncyclic photophosphorylation (Fig. 1A) and the full electron transport system (Fig. 1C) light-saturates near 1.5×10^5 ergs/cm^2·sec for both LG and DG plastids. The cyclic phosphorylation (Fig. 1B) and the PSI electron transport (Fig. 1D) were not saturated at the light intensities we employed. While the light intensities for the first measurable rates and for saturation were the same for both LG and DG, the LG attained a greater rate at all light intensities.

Relative Quantum Yields. Employing data from the PSII plus PSI electron transport and the noncyclic phosphorylation, light intensity/reaction velocity (*I/V*) *versus* light intensity (*I*) data were plotted. By extrapolating to zero light intensity, this technique provides an estimate of the relative quantum requirement (20). Figure 2 gives typical data revealing no difference in LG and DG plastids with regard to relative zero intensity quantum meas-

Table I. *Partial Photosynthetic Reaction Rates*

Values represent averages of measurements and their ranges at a light intensity of 4×10^5 ergs cm^{-2} sec^{-1}. The assays were as described in "Methods and Materials."

	DG	LG
	μmoles/mg chlorophyll·hr	
Photophosphorylation (cyclic)	150 ± 50	700 ± 200
Photophosphorylation (non-cyclic)	100 ± 20	350 ± 50
Proton pump, initial rate	260 ± 60	1250 ± 300
Electron transport (PS2 and PS1)	560 ± 60	2800 ± 300
Electron transport (PS1)	1400 ± 400	5000 ± 2000

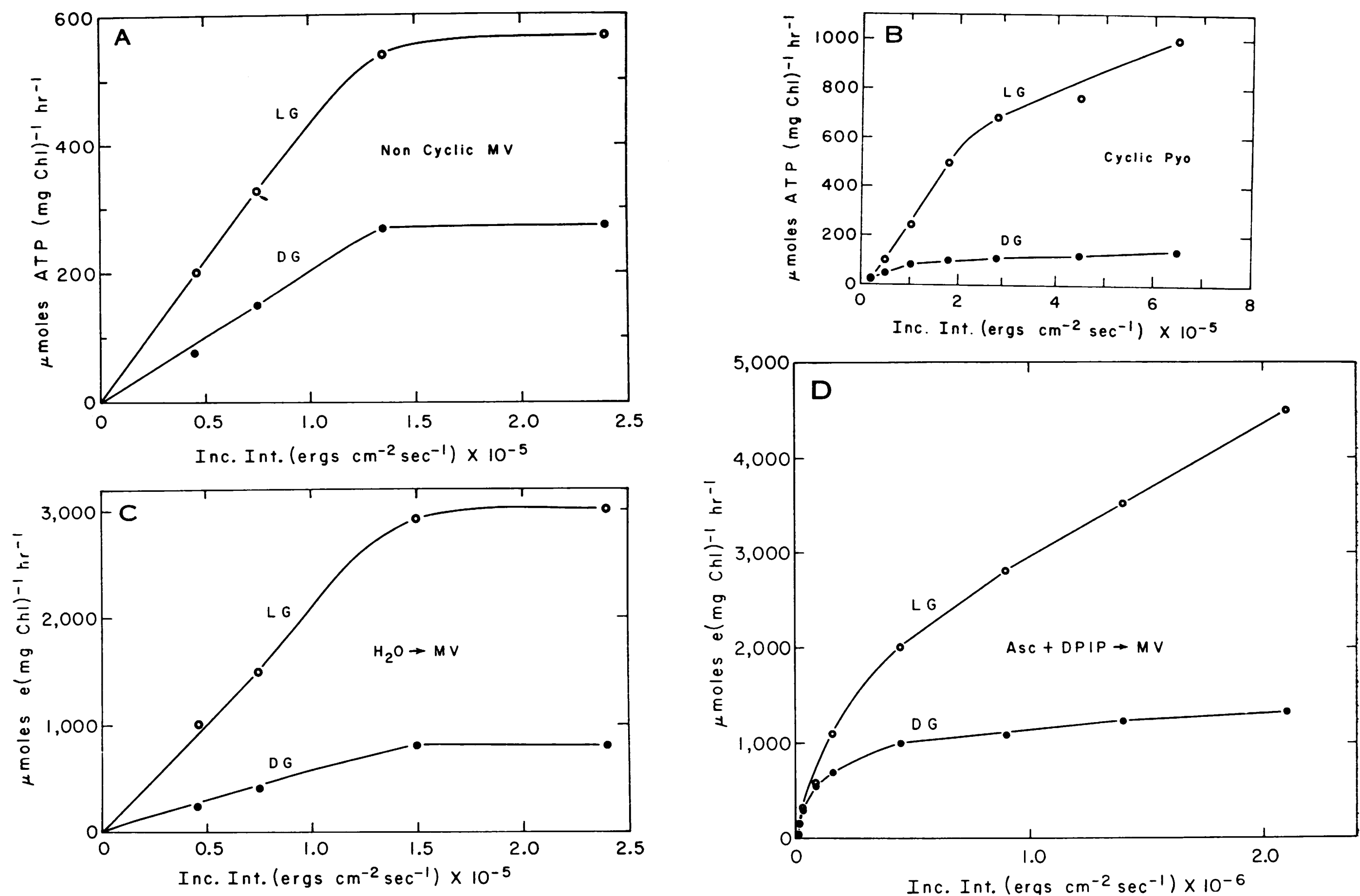

FIG. 1. Plots of incident light intensity *versus* reaction rates of noncyclic photophosphorylation (A), cyclic photophosphorylation (B), PSII plus PSI electron transport (C), and PSI electron transport (D). See "Methods and Materials" for experimental conditions.

urements for an $H_2O \rightarrow$ MV electron transfer reaction. At finite light intensities the DG has a much higher quantum requirement (lower quantum yield) than the LG. The above I/V *versus I* plots were from experiments having equal chlorophyll concentrations in LG and DG reaction mixtures.

Effect of Uncouplers. The effect of some uncouplers and inhibitors of cyclic photophosphorylation in the DG and LG plastids are shown in Table II. The molar concentration of ammonium chloride which induces 50% inhibition was the same in both LG and DG. For the other inhibitors, however, the LG plastids were inhibited 50% only when the concentration was 5 times (nigericin) to 10 times (chlorotri-*n*-butyltin and poly-L-lysine) that of the concentration yielding 50% inhibition in the DG plastids.

CO_2 Uptake. The data from intact terminal leaflet CO_2 uptake are plotted in Figure 3. The data points reveal similar light saturation intensities for LG and DG CO_2 uptake. On a leaf area basis the LG photosynthesized slightly faster when compared to DG. However, on a chlorophyll basis the LG chlorophyll was five times more efficient. The somewhat slower rates at 7,500 and 10,000 ft-c might be due to a higher respiratory rate caused by an increase in leaf temperature. The leaf temperature, however, was not monitored.

Plastoquinone Oxidation-Reduction. Our compositional studies indicated more plastoquinone/chlorophyll in the LG as compared to the DG (13). The oxidation of reduced plastoquinone has been suggested to be the rate-limiting step in photosynthesis (26). We measured the plastoquinone reduction by a flash (100 or 200 msec) of saturating intensity red light superimposed on a continuous far red (720 nm) beam which assured oxidation of the reduced plastoquinone (with methyl viologen as the acceptor) after the red light flash ended. Repetitive flashes (256 nm or 512 nm) were given, and a signal averager summed up the signals and averaged out most of the noise.

In both LG and DG, steady state conditions were attained by the 100-msec saturating actinic light flash (Fig. 4). The LG plastids exhibited a 3- to 5-fold greater change in absorbance (ΔA) per chlorophyll, indicating a 3- to 5-fold larger active plastoquinone pool when compared to DG. Note the different scales for the x and y axes in the two parts of Figure 4. Figure 5 shows light minus dark difference spectra for the LG and DG plastids. The maximal ΔA occurs at about 260 nm with the isosbestic point at about 282 nm. These spectra are consistent with the known ultraviolet absorption for oxidized minus reduced plastoquinone (2) and are similar to an *in vivo* plastoquinone spectrum previously reported (25). The observed difference spectrum is not due to α-tocopherylquinone because the ΔA at 269 nm is much less than would be expected for that compound (2).

Quinone reduction kinetics, induced by the actinic light flash, were similar in both LG and DG plastids. Oxidation of the reduced quinone is faster in the LG than in the DG. Figure 6 shows a semilog plot of quinone oxidation indicating these faster kinetics in the LG. The half-time for the oxidation of reduced quinone estimated from semilog plots averaged about 11 msec in the LG as compared to 25 msec for the DG.

Methyl viologen has an ultraviolet absorption spectrum quite similar to that of plastoquinone, with a peak near 258 nm. Reduction with borohydride results in a decrease in absorbance at

258 nm, an increase around 290 nm, and an isosbestic point near 282 nm, nearly identical to PQ spectrophotometric properties. Under anaerobic conditions reduction of methyl viologen results in semiquinone formation resulting in an increase in absorbance at 390 nm (14) and presumably an absorption decrease in the 260 nm region. Under our aerobic conditions, we could not detect any positive ΔA at 390 nm, in samples which did show the negative ΔA at 260 nm. Hence, the reduced methyl viologen must be oxidized by oxygen so fast as to not attain a detectable level of reduction (as Kok *et al.*, Ref. 14, suggested) and we conclude that the 260 nm negative ΔA is indeed due to PQ and not methyl viologen.

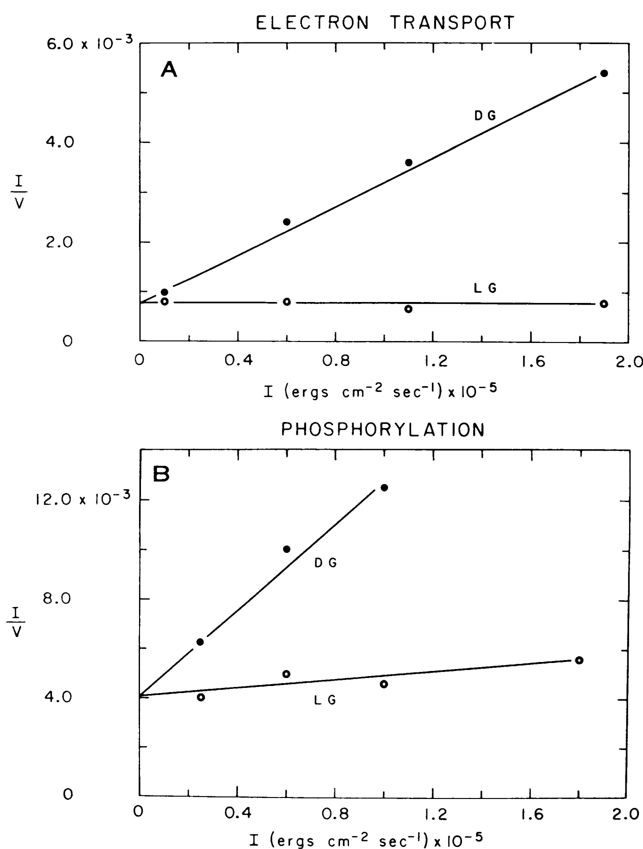

FIG. 2. I/V *versus* I plots of PSII plus PSI electron transport and noncyclic photophosphorylation. I is incident light intensity and V is rate of reactions. See "Methods and Materials" for experimental conditions.

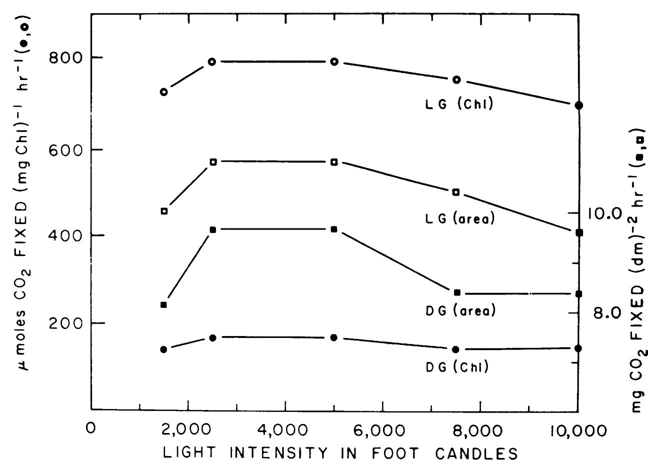

FIG. 3. Light intensity *versus* CO_2 uptake of the terminal leaflet of the first trifoliate leaf. Note CO_2 uptake based on both a chlorophyll and a leaf area basis.

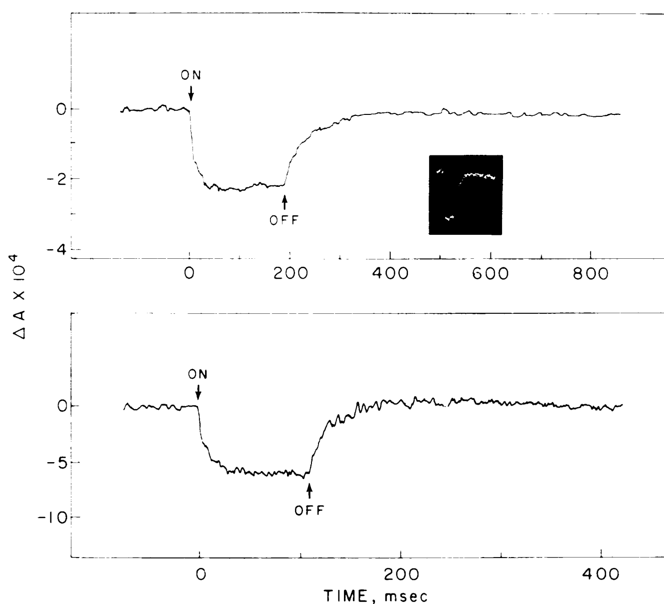

FIG. 4. Typical absorbance change transients at 260 nm for chloroplasts from wild-type and mutant soybean leaves. Inset shows the original oscilloscope display. DG above; LG below. Total number of flashes: 400 for DG, 512 for LG. Chlorophyll for DG, 10 μg/ml; for LG, 7 μg/ml of resuspension medium (see "Methods and Materials"). Background light was 720 nm, about 10^3 ergs/cm²·sec. The actinic light intensity of 10^6 ergs/cm²·sec was saturating.

Table II. *Cyclic Photophosphorylation Inhibition*

Stock inhibitor solutions were added to reaction mixtures as described for cyclic phosphorylation in "Methods and Materials." Light intensity was 4.0×10^5 ergs cm^{-2} sec^{-1}.

Inhibitor	Molar Concentration Yielding 50% Inhibition	
	DG	LG
Ammonium chloride	5.2×10^{-4} M	4.7×10^{-4} M
Nigericin	1.1×10^{-8} M	4.7×10^{-8} M
Chlorotri-*n*-butyltin	2.7×10^{-7} M	1.6×10^{-6} M
Poly-L-lysine (195,000 mw)	2.4×10^{-8} M	2.5×10^{-7} M

DISCUSSION

At high light intensities, viable, chlorophyll-depleted mutants of higher plants exhibit higher photosynthetic rates per chlorophyll than the wild type (1, 8, 9, 22). Intact leaves of the soybean mutant (LG) described above also exhibit a 5-fold greater CO_2 uptake per chlorophyll when compared to the wild type (DG) (Fig. 3). The LG and DG leaves light-saturate for CO_2 uptake at the same intensity (Fig. 3). This observation had been made earlier by Wolf (27), and this result is unlike those for barley, tobacco, and pea mutants (see Introduction).

The partial photosynthetic reaction rates (Table I) from isolated LG plastids are 3 to 5 times those of the DG (on a chlorophyll basis) at high light intensities. On a lamellar protein basis the LG rates are 1.5 to 2.5 times the DG rates (see Ref. 13 for

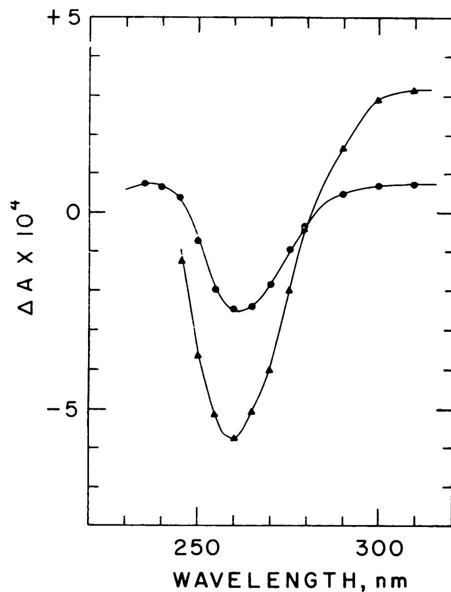

FIG. 5. Plot of the logarithm of the recovery of the 260 nm signal (reoxidation) *versus* time. ●: DG; ▲: LG.

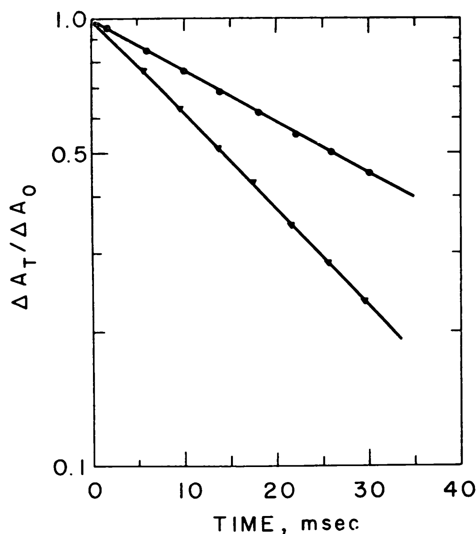

FIG. 6. Light minus dark difference spectra for the DG (●) and LG (▲) soybean chloroplasts.

data on chlorophyll/protein ratios in these chloroplasts). To the extent that our LG and DG lamellar protein assays give a valid estimate of lamellar protein, the approximate 2-fold faster rates in the LG (on a protein basis) suggest that the mutation does result in a release of some rate limitation normally imposed on the wild type plastid electron transport reactions (see discussion of plastoquinone oxidation kinetics below). Isolated plastids of LG light-saturate at the same intensity as DG when PSII plus I electron transport and noncyclic photophosphorylation are assayed (see Fig. 1). Therefore, while the LG shows greater reaction rates per chlorophyll, as other mutants, the LG light-saturates at the same light intensity as the DG, unlike other mutants.

Quantum requirements for O_2 evolution in mutant tobacco were found to be similar to those of the wild type (23). Extrapolating the I/V *versus* I plots (Fig. 2) to zero intensity suggests that the relative quantum requirements are similar in the LG and DG plastids. However, at finite light intensities the DG plastids were much less efficient in quantum conversion. This increased quantum efficiency in the LG may be related to the partial release of the rate-limiting dark step of electron transport at the quinone oxidation level discussed below. Relative quantum requirements are shown for PSII plus I electron transport and noncyclic photophosphorylation, and similar plots were obtained for cyclic photophosphorylation, and PSI electron transport reactions. While the $P/2e$ ratios were not measured directly, a comparison of electron transport and phosphorylation data suggest that the $P/2e$ ratios are similar in LG and DG plastids. This is consistent with the notion that the efficiency of coupling ATP formation to electron transport is similar in the two plastid types.

Because of the similar light saturation intensities and the similar chlorophyll/ P_{700} values (13), we hypothesize similarly sized photosynthetic units in the LG and DG plants.

We have measured plastoquinone pool sizes several times and have found a range of three to five times more photochemically active plastoquinone/chlorophyll in the LG as compared to DG. We showed that the ΔA changes at 260 nm were not due to methyl viologen by checking for its expected change at 390 nm, and not detecting such changes. Witt (26) has suggested that the rate-limiting step in photosynthesis is the oxidation of plastoquinone. His half-time value of about 20 msec for PQ oxidation agrees well with earlier determinations by Emerson and Arnold (6) for the rate-limiting dark step in photosynthesis. While we find variation in the half-time ($t_{1/2}$) for plastoquinone reoxidation, the LG half-time was always shorter than the DG half-time. Published $t_{1/2}$ values for plastoquinone oxidation in spinach plastids are near 20 msec. Representative values for half-times of plastoquinone oxidation in our experiments with soybean range from 7 to 14 msec in the LG plastids and from 18 to 28 msec in DG plastids. Thus the LG chloroplast has a larger active plastoquinone pool per chlorophyll and a faster plastoquinone oxidation rate.

It is thus obvious that the faster electron transport and phosphorylation rates in the LG compared to the DG chloroplasts are related to the faster turnover of PQ in the LG. While the apparent first order rate constant, k_d, for PQ oxidation is about twice as great in the LG, the actual measured electron transfer rates (taken from the slope of the traces as in Fig. 4) for PQ oxidation in a majority of the measurements were three to five times faster in the LG compared to the DG. Such calculated rates of PQ oxidation from a typical experiment were 59 μmoles/mg chlorophyll·hr for the DG and 220 μmoles electron equivalents per chlorophyll·hr for the LG. This agrees well with the three to five times faster rates of electron transport in the LG (measured by oxygen uptake with methyl viologen as the acceptor) and phosphorylation (cyclic and noncyclic). These results are consistent with the prediction based on a cursory examination of a rate equation for PQ oxidation, $d(PQH_2)/dt = k_d[PQH_2]t$.

With this first order rate equation, one would predict a *faster rate* in the LG case either if the rate constant, k_d, were increased or if the total rapidly reducible pool $[PQH_2]$ were larger. Our experimental results show that, in fact, both parameters are increased in the LG plastids. Figure 6 shows that $k_{d_{LG}} \approx 2k_{d_{LG}}$, and Figure 4 shows that in the LG there is about 3-fold more PQ rapidly reduced in the LG plastid. If the pool of reducible PQ were no larger in the LG, and k_d were greater (as shown in Fig. 6), one would expect a lower steady state PQH_2 level in the LG; but a greater steady state was observed (Fig. 4). Hence it is reasonable to explain the 3- to 5-fold greater rates of electron transport by attributing the increase partly to a greater k_d and partly to a larger PQ pool in the LG.

A similar analysis can also be made with a diffusion model for PQ electron transfer. Plastoquinone in chloroplasts, as ubiquinone in mitochondria, is believed to be dissolved in the lipid part of the membrane and diffuse from hydrogen donors to acceptors as a lipid phase electron carrier (16). The larger steady

state PQ pool in the LG indicates a higher concentration of functional PQ in the mutant lamellae. If the "lipid phase shuttle" concept for PQ function is correct, it implies that the diffusion equation may be applicable. One form of the diffusion equation is

$$\frac{dc}{dt} = D \cdot A \cdot \frac{dc}{dx}$$

where dc/dt is the rate of diffusion (here the diffusion of PQH_2 in the lipid phase), D is a diffusion constant, A the area across which diffusion occurs, and dc/dx is the concentration gradient (here the gradient of PQH_2 from donor to acceptor). If such a model is basically correct, it is a logical deduction that a larger pool of reducible PQ would indicate a larger gradient, dc/dx, and diffusion would be faster. This would be analogous to the PQ concentration term in the first order rate equation discussed above.

We have shown less lipid per protein in washed LG lamellae as compared to DG lamellae (13). Therefore, the observed greater k_d for PQ oxidation in the LG could be due to a different lipid environment in the membrane allowing the PQ to diffuse more rapidly from reducing sites to oxidizing sites. Using the diffusion model, one might imagine that an altered lipid environment may well lead to a different diffusion constant of PQ for the LG and thus contribute to the over-all faster electron transfer rates in the LG plastid.

It is apparent that the faster photosynthetic reaction rates of the LG chloroplasts can be readily explained by the altered plastoquinone pool size and kinetics. The underlying reason for the faster PQ kinetics is still obscure. We have no data which discriminate between PQ-A and PQ-C as being responsible for the absorbance changes. Only a small portion of the total PQ-A could be involved since the ΔA (Fig. 4) accounts for only 5% of the total PQ-A [assuming 1 PQ-A per 20 chlorophylls (13)]. In both LG and DG, the observed ΔA would account for about 65% of the total PQ-C present in the preparations. It would be of great interest to know which (or what proportions) of the two major PQ types are involved in the observed ΔA changes.

The greater electron transport rates in the mutant soybean (LG) chloroplast are similar to those of the pea mutant and barley mutants (1, 8, 9) when all are compared on a chlorophyll basis. Since there are more PQ and cytochrome moieties available on a chlorophyll basis in the LG, a DPIP-ascorbate electron feed of PSI would have more potential entry sites/reaction center in the LG, perhaps causing a faster rate of PSI electron transport than in the DG. These results are consistent with other results indicating quinone and cytochrome levels to be determining factors in the photosynthetic reactions of mutants (7, 17, 18, 21, 25).

The suggested mode of action for the phosphorylation inhibitors listed in Table II have been described elsewhere (3, 4, 11, 24). The higher concentration of inhibitors needed for LG phosphorylation inhibition has several possible explanations; among them are the following: more surface area/chlorophyll, more active proton pump, more coupling sites/chlorophyll, or differences in membrane structure and/or composition which affect affinities of the inhibitors.

We have demonstrated, for this chlorophyll-depleted soybean mutant, the correlation of high photosynthetic capacity, the larger pool size of PQ, and the faster oxidation kinetics of PQ. Other chlorophyll-depleted mutants exhibiting high photosynthetic rates may likewise exhibit differences in PQ pool size and/or PQ oxidation rates.

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