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Partial Purification and Characterization of a Blue Light-Sensitive Cytochrome-Flavin Complex from Corn Membranes[1]

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ABSTRACT

A membrane fraction which contains a blue light-sensitive flavin-cytochrome complex (Brain *et al.* 1977 Plant Physiol 59: 948) has been partially purified by sucrose and Renografin gradient centrifugations. Assays for marker enzymes show that this membrane fraction is distinct from endoplasmic reticulum, golgi, and mitochondria. This membrane fraction cosedimented with glucan synthetase II activity, a proposed marker for plasma membrane in higher plants. The purified membrane fraction shows virtually identical light minus dark and dithionite reduced minus oxidized difference spectra with difference bands near 427 and 557 nanometers, suggesting that contamination by other cytochrome-containing membrane fractions is not significant. The photoactivity can be completely solubilized by 0.1% Triton X-100, leaving the bulk of the membrane undissolved. The kinetics for cytochrome photoreduction are not significantly affected by solubilization, indicating that both flavin and cytochrome could be associated with the same protein moiety.

Blue light-inducible reduction of a *b*-type Cyt has been shown in membrane fractions from both *Neurospora* mycelium and etiolated corn coleoptiles (1). In *Neurospora* the photoactivity is shown not to co-sediment with either mitochondria or ER, but rather with an adenosine triphosphatase which is thought to be a plasma membrane marker (1).

The actual photoreceptor supposedly involves a flavin moiety which specifically reduces the *b*-type Cyt (3, 4), on excitation by blue light. This Cyt moiety can also be photoreduced by red light in the presence of methylene blue (3, 16). Biochemical and spectral characteristics in both *Neurospora* and corn coleoptile are sufficiently similar that it has been proposed that the same photoreceptor is involved in both organisms (2).

Subsequent efforts have optimized the conditions for obtaining stable and reproducible light-inducible Cyt reduction in preparations of corn coleoptile membranes either with endogenous flavoprotein or with added free flavin as photosensitizer (6). The conditions include a glucose-glucose oxidase system which lowers O_2 tension, KCN which serves to inhibit contaminating mitochondrial activity, EDTA which may serve as an electron donor (6) though its exact role is not resolved, and assay temperature below 10 C (6). Under these optimized conditions, it was possible to study the kinetics and characteristics of membrane particles from corn showing photoreduction activity.

The present paper reports on further evidence supporting the specificity of this photoreduction and on the partial purification and characterization of the photoreceptor from corn coleoptiles. Evidence that this system may play some physiological role in photoreception has been discussed recently elsewhere (2).

MATERIALS AND METHODS

Plant Material. Corn coleoptiles were harvested and homogenized in Mops[2] buffer (0.1 M Mops, 14 mM 2-mercaptoethanol, 3 mM EDTA, and 0.1 mM $MgCl_2$, adjusted to pH 7.4 with KOH) containing 0.25 M sucrose, as described (6). The homogenate was filtered through fine mesh nylon cloth and the filtrate centrifuged at 2,000*g* for 10 min. The pellet, designated 2KP, containing wall fragments and other cell debris, was discarded. Thus, the starting material for further fractionation studies was the 2,000*g* supernatant, designated 2KS. The 2KS was centrifuged at 9,000*g* for 15 min to give the 9KS and 9KP. The 9KS (30-ml aliquots) was layered onto 8 ml of 32% (w/w) sucrose cushion (buffered with the above Mops solution) and centrifuged in a SS-34 rotor (Sorvall RC-5B centrifuge) at 21,000*g* for 90 min. The pellet collected at the bottom of the centrifuge tube was washed by resuspension in the above Mops buffer containing 0.25 M sucrose, and recentrifuged (21,000*g* for 90 min) to yield a pellet designated 21KP. The 21KS was centrifuged at 50,000*g* for 90 min to give a pellet designated 50KP. Pellets were resuspended in small volumes of Mops buffer of appropriate sucrose concentration, and without 2-mercaptoethanol by repeatedly taking up the material in a syringe and ejecting it through a fine hypodermic needle (Yale, 25G 5.8). All samples were handled under dim green light.

Spectrophotometric Methods. LIAC were monitored with a Perkin-Elmer 356 dual wavelength spectrophotometer on line with a Hewlett-Packard computer as described elsewhere (1, 4–6). For LIAC measurements, KCN, EDTA, glucose, and glucose oxidase were routinely added to the same (6) and the cuvette kept at 4 C. LIAC units are as defined by Goldsmith *et al.* (6). For dose-response studies, a slide projector equipped with a Balzer 462 or 665 nm interference filter was used to provide the blue or red actinic light, respectively. Light intensities were regulated with neutral screen filters and calibrated with a Li-Cor Quantum-photometer (Model LI-185A). Irradiations were sufficiently long (2 min) to assure that photostationary state was achieved. Both fresh and frozen and thawed samples yielded similar results.

Light minus dark and dithionite reduced minus oxidized difference spectra were measured essentially as described elsewhere (6), except for the following modifications and precautions: for light minus dark difference spectra, a light intensity of 2.46 nmol photon m^{-2} s^{-1} with the Balzer 462 nm filter was routinely used. Immediately after 2 min of irradiation, three successive scans (400–500 or 500–600 nm) were recorded. Scanning speed was 480 nm/min. This technique reduced the number of irradiations

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[2] Abbreviations: Mops, *N*-morpholinopropane sulfonic acid; NKP, N thousand × *g* pellet; NKS, N thousand × *g* supernatant; LIAC, light-induced absorbance change; CCO, Cyt *c* oxidase; CCR, antimycin A-insensitive NADH-Cyt *c* reductase; Glu I, glucan synthetase I; Glu II, glucan synthetase II.

required to obtain good difference spectra, thus reducing the extent of photobleaching of the sample. There was no shift in the position of difference maxima from one scan to the next and dark decay was sufficiently slow that a different peak in the third scan was at least 80% as large as in the first. Dark minus dark scans were obtained either before any irradiation or after at least 6 min of darkness following an irradiation. At least 10 scans each (light minus dark, dark minus dark [= oxidized minus oxidized], or dithionite reduced minus oxidized) were averaged before difference spectra were calculated.

Sucrose Fractionation. The 21KP was resuspended in Mops buffer containing 10% (w/w) sucrose and the sample (representing 15 g fresh weight of coleoptile in 5 ml) was then layered onto a 32-ml linear sucrose density gradient of 25 to 45% (w/w) sucrose dissolved in the above Mops-buffer. The sucrose gradients were centrifuged at 100,000*g* in a SW 27 rotor for 16 h. The gradients were fractionated on an ISCO fractionator and 1.7 ml fractions were collected.

Renografin Gradient Fractionation. Membrane particles purified on sucrose gradients (fractions 13–20; corresponding to refractive indices from 1.3937–1.4071) were pooled, diluted with 2 times the volume of sucrose-free Mops buffer, pelleted by centrifugation at 21,000*g* for 50 min and resuspended in 10% (w/w) sucrose-Mops buffer (30 g fresh weight of coleoptile in 5 ml). This suspension is designated "postsucrose gradient" fraction. It was layered onto 32 ml of a Renografin-76 (Squibb) linear density gradient (15–61% Renografin). The original Renografin-76 solutions were diluted with sucrose and 2-mercaptoethanol-free Mops-buffer to the final concentrations (v/v) before use. The Renografin gradients were centrifuged at 100,000*g* in an SW 27 rotor for 60 min.

Assays. Protein was estimated by the method of Lowry *et al.* (11), with BSA as standard. CCO, and antimycin A-insensitive NADH-Cyt *c* reductase activities were measured according to Jesaitis *et al.* (8). Glu I and II activities were measured according to Ray (13). Protein measurements and Glu I and II activities were assayed after removal of Renografin by resuspension of the fractions in Renografin-free Mops buffer and repelleting. Samples were stored frozen at −20 C and were thawed immediately before measurements were made.

RESULTS

Dose-Response Curves. With conditions optimized for measuring the LIAC between 428 and 410 nm, designated $\Delta(A_{428}-A_{410})$, it was possible to obtain stable and reproducible signal sizes even with endogenous photoreceptor in the 21KP. The double-reciprocal plot of $\Delta(A_{428}-A_{410})$ as a function of light intensity gives a straight line (Fig. 1). The maximum A change obtainable at saturating light intensity is designated ΔA_{max} and is given by the intercept of the straight line at the y-axis. The value of the ΔA_{max} is characteristic of the photoreaction and is used as one of the criteria for judging the specificity of the reaction. Triangles are values obtained from an aliquot of membrane preparation in one experiment, whereas circles and squares are data obtained from two other frozen aliquots of the same preparation in another experiment.

Riboflavin at a concentration of 0.37 μM increases the LIAC by a factor of 2 (Fig. 1). The double reciprocal plots of intensity *versus* LIAC for the endogenous photoreceptor and for the exogenously added riboflavin as photoreductant give almost the same ΔA_{max}. The same intercept is obtained when methylene blue is added and red actinic light is used (Fig. 1). LIAC data obtained from frozen aliquots of the same 21KP preparation but used on different days give similar results.

Treatment with Triton X-100. Addition of 0.1% Triton X-100 to the 21KP was efficient in solubilizing the photoreceptor (10). The Triton-solubilized photoreceptor shows almost the same kinetics

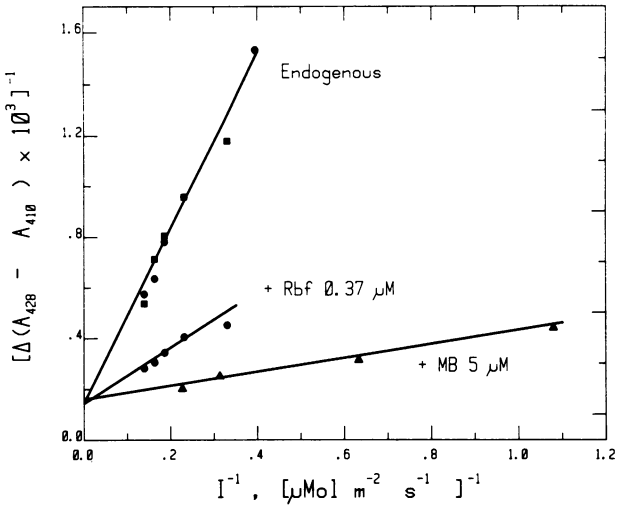

FIG. 1. Double-reciprocal plot of blue light-induced-absorbance change as a function of light intensity for the endogenous photoreceptor alone, with added riboflavin (Rbf), and with methylene blue (MB) as additional photoreductants. Different frozen aliquots of the same 21KP preparation (4.5 mg/ml protein) were used on different days.

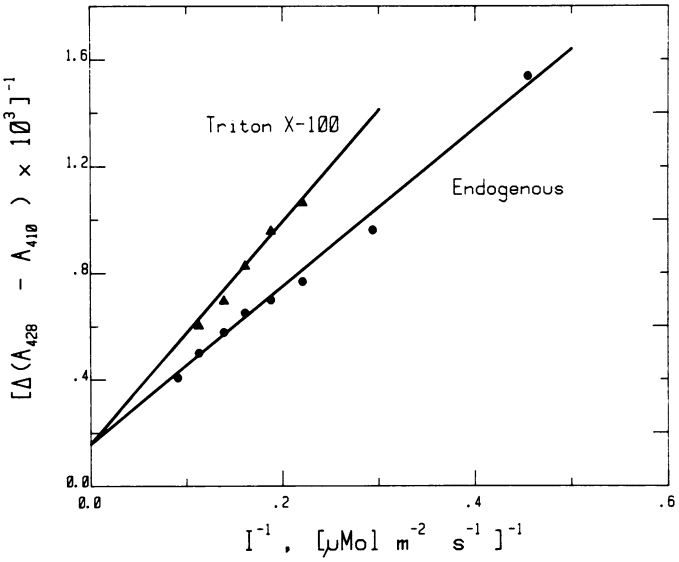

FIG. 2. Double-reciprocal plots of blue light-induced absorbance change as a function of light intensity for the membrane-associated endogenous photoreceptor (5.1 mg/ml protein) and 1% Triton X-100-solubilized preparation.

of the reaction as the membrane fraction from which it was derived (Fig. 2). On double reciprocal plots, the Triton X-100-solubilized photoreceptor gives the same ΔA_{max}. The almost identical kinetics of the two reactions indicate that Triton X-100 has only a relatively small effect on the quantum efficiency for photosensitized transfer of an electron to the Cyt. LIAC specific activity in the solubilized preparation (U/mg protein) was 15.6 (*cf.* Table II).

Fractionation of Membrane Particles. The use of a 32% (w/w) sucrose cushion in the 21,000*g* centrifugation step yields a membrane fraction which contains a relatively high amount of LIAC, has at most 30% of the mitochondria present in the starting material (2KS), and is almost devoid of ER (Table I). However, it is clear that this 21KP is still contaminated to a considerable extent by other membrane particles. It was difficult to calculate the percentage of recovery of activities from the starting 2KS fraction, probably because of contaminating substrates and inhib-

Table I. *Total Activity of Different Fractions*

Fractions	Volume	CLO	CCR	Glu I	Glu II	Protein	LIAC
	ml	$\Delta A_{550}/min$		*cpm*		*mg*	*units* × 10^{-3}
2KS	146.0	33.6	793.8	25,988	44,676	71.5	
9KP	5.2	17.2	88.2	7,779	22,620	32.2	40.6
21KP	16.0	13.3	38.4	16,544	30,912	11.5	70.9
50KP	6.0	8.1	607.2	15,840	34,476	15.3	59.5
Post sucrose gradient fraction	6.0	6.3	4.0	2,472	29,172	4.5	37.6

itors in this fraction. Thus, further purification was undertaken.

The resuspended 21KP was applied onto 25 to 45% (w/w) linear density sucrose gradient and centrifuged at 100,000*g* for 16 h. Under these conditions, ER (as assayed by CCR) stays at the top of the gradient as expected (8), whereas the LIAC activity migrates to almost the same position as the mitochondria (as assayed by CCO) (Fig. 3). Although the photoreceptor was not separated from the mitochondria, it was possible to separate the photoreceptor from the ER and hence from Cyt b_5, a known component of the ER in corn (8). Thus, fractions number 13 through 20 were pooled, diluted with 2 times the volume of sucrose-free Mops buffer, pelleted by centrifugation at 21,000*g* for 50 min (pellet designated as post sucrose gradient fraction) and applied onto the Renografin gradient as described under "Materials and Methods". This post sucrose gradient fraction is virtually devoid of ER: only 0.5% of the total NADH-CCR activity initially present could be detected (Table I).

When the 21KP is layered directly onto a Renografin gradient and centrifuged at 100,000*g* for 60 min, ER, mitochondria, and the LIAC activity migrate to positions quite different from those in a sucrose gradient. In fact, the ER sediments to a position between the mitochondria and the LIAC activity (results not shown). Complete separation of the ER from the LIAC activity in the 21KP on a single Renografin gradient is impossible (results not shown). It is therefore necessary to remove most of the ER and golgi from the 21KP on a sucrose gradient (8) to yield the post sucrose gradient fraction which contains mainly LIAC activity and mitochondria.

When the post sucrose gradient fraction was applied onto Renografin gradients and centrifuged, two sharp bands could be seen in the gradient. (There is also a sharp band at the top of the gradient. The nature of this band is unknown, but it has no LIAC activity and none of the other activities assayed in this study.) Assays for LIAC activity, CCO, CCR, Glu I and II show that the heavier band in the Renografin gradient contains mitochondria and the lighter band has both photoactivity and Glu II activity (Figs. 4 and 5). Glu I, a marker for golgi particles (13), is not detected in the Renografin gradient. The present purification procedure provides evidence that the LIAC activity can be clearly separated from mitochondria and may well be on the plasma membrane (12, 13). Fraction numbers 8 through 12 were pooled, diluted with Renografin-free Mops buffer, centrifuged at 50,000*g* for 90 min, and resuspended in 0.75 M sucrose and Mops-buffer (designated "Renografin purified membrane fraction") for spectral studies. The low level of ER and mitochondrial contamination is indicated in Table II, last line.

Difference Spectra. Using the Perkin-Elmer spectrophotometer in the split-beam mode, on line with the computer system described by Ford and Catanzaro (5), we obtained light minus dark difference spectra from 400 to 500 nm and 500 to 600 nm with Renografin-purified membrane fractions. For the same membrane preparation, dithionite reduced minus oxidized difference spectra over the same wavelength ranges were recorded. Control experiments have shown that the various additives in the system (glucose, glucose oxidase, KCN, EDTA, alone or in combination) have no effect on the difference spectra. No difference peaks of any kind, either light- or dithionite-induced, were obtained unless the mem-

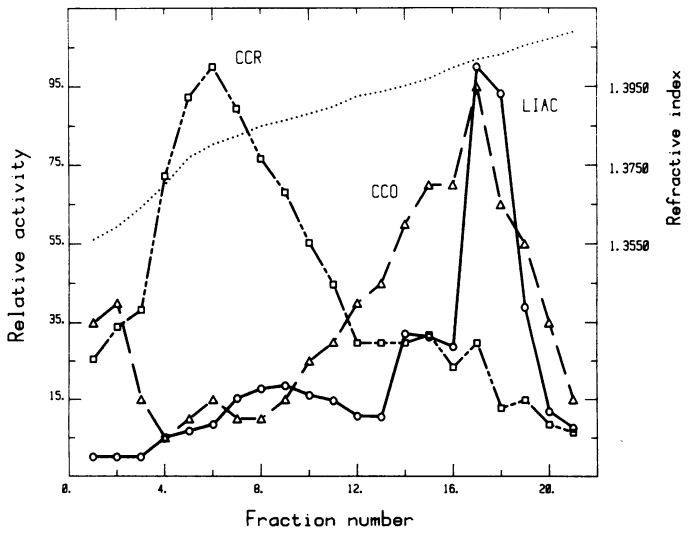

FIG. 3. Fractionation of the 21KP on a sucrose gradient. (△——△, CCO), maximum activity in fraction number 17 is 0.0014 A μg^{-1} protein min^{-1}; (□— -□, CCR), maximum activity in fraction number 6 is 0.0128 A μg^{-1} protein min^{-1}; (○——○, LIAC), maximum ΔA in fraction number 17 is 5.13 units × 10^{-3} mg^{-1} protein; (·····) indicates refractive index of each fraction.

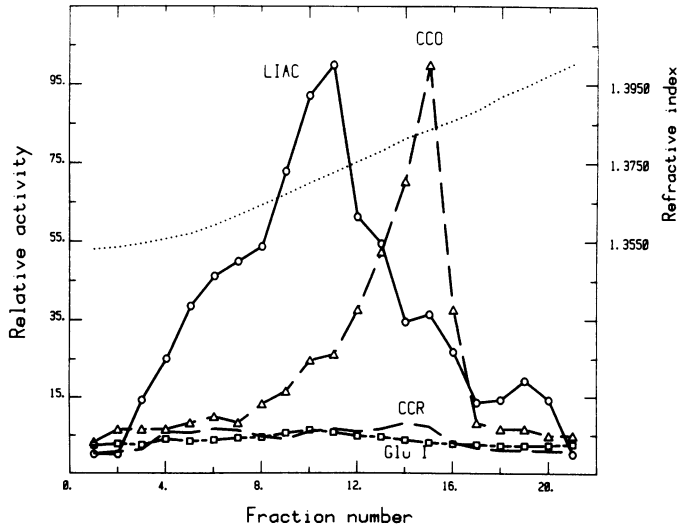

FIG. 4. Fractionation of post sucrose gradient material on Renografin gradient. (□——□, Glu I), maximum activity in fraction number 10 is 1.8 cpm μg^{-1} protein; (— —, CCR), maximum activity in fraction number 14 is 0.00005 A μg^{-1} protein min^{-1}; (△——△, CCO), maximum activity in fraction number 15 is 0.00147 A μg^{-1} protein min^{-1}; (○——○, LIAC), maximum ΔA in fraction number 11 is 18.3 units × 10^{-3} mg $protein^{-1}$; (·····), refractive index of each fraction.

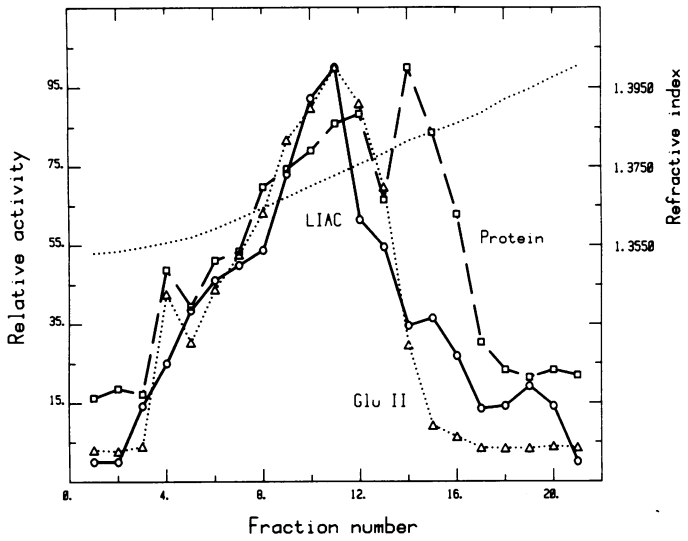

FIG. 5. Fractionation of post sucrose gradient material on Renografin gradient. (△···△, Glu II) maximum activity in fraction number 11 is 27.2 cpm μg^{-1} protein; (□— —□), amount of protein, maximum amount in fraction number 14 is 215 μg protein; (○——○, LIAC), maximum ΔA in fraction number 11 is 18.3 units × 10^{-3} mg^{-1} protein; (·····), indicates refractive index of each fraction.

Table II. *Specific Activity of Different Fractions*

Calculations were per mg protein.

Fractions	CLO	CCR	Glu I	Glu II	LIAC
	$\Delta A_{550}/min$		*cpm*		*units* × 10^{-3}
2KS	0.47	11.10	364	625	
9KP	0.55	2.74	242	703	1.26
21KP	1.15	3.33	1,439	2,688	6.2
50KP	0.53	39.7	1,035	2,253	3.8
Post sucrose gradient fraction	1.4	0.89	549	6,483	8.4
Renografin purified membrane fraction	0.06	0.04	95	27,178	12.8

brane fraction was present.

The spectra were averaged, normalized and smoothed so that the difference peaks have the same relative heights for comparison (Figs. 6 and 7). The dithionite reduced minus oxidized difference curves were subtracted from the light minus dark difference curves and the results are shown in Figures 6 and 7 as well. The light minus dark difference spectrum shows a Soret difference peak at about 427 nm (calibrated against a Balzer interference filter), and the Soret difference peak with dithionite, representing the total reduction of the bulk of the Cyt in the preparation, was also at about 427 nm (Fig. 6). The subtraction result indicates that the membrane preparation may be contaminated no more than 5% by dithionite-reducible but light-insensitive material, yielding the maximum at 431 and minimum at 420 nm.

In the alpha and beta band region, both light minus dark and the dithionite reduced minus oxidized difference spectra show an alpha band difference peak at about 557 nm and a beta band difference peak near 528 nm (Fig. 7). The subtraction curve gives almost a straight line over the wavelength region where the alpha and beta band difference peaks are located. This result indicates that there is probably only one dominant *b*-type Cyt in the purified membrane fraction. The value of A change induced by addition of dithionite was found to be quite close to the ΔA_{max} found in LIAC measurements (Fig. 1).

As to the A difference in the 590 and 600 nm region, it is

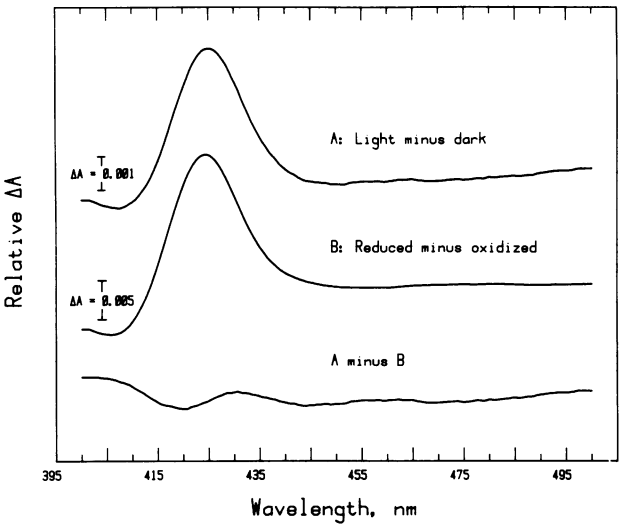

FIG. 6. Comparison of difference spectra induced in the "Renografin-purified membrane fraction" (1.1 mg protein) by actinic light or by dithionite in the Soret region. Ten difference spectra were averaged and normalized to the same relative height for the difference spectrum as described. The actual $\Delta(A_{428}-A_{410})$ were 0.0040 and 0.0174, respectively, for light minus dark and dithionite reduced minus oxidized difference spectra.

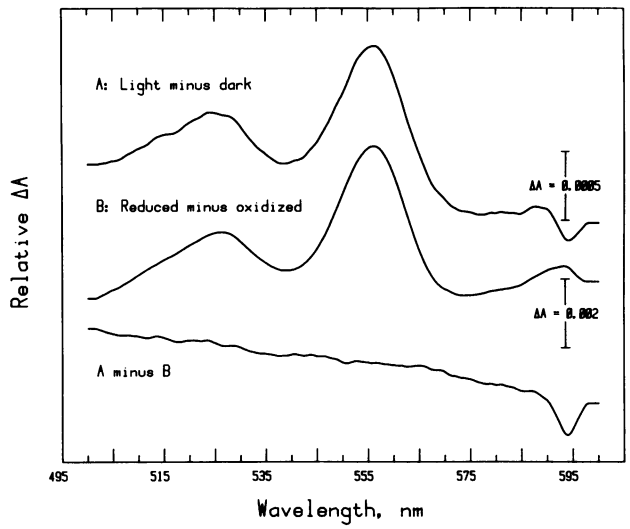

FIG. 7. Comparison of difference spectra induced by actinic light or by dithionite in the alpha band region (details same as in Fig. 6). Actual $\Delta(A_{555}-A_{575})$ were 0.0012 and 0.0044, respectively, for light minus dark and dithionite reduced minus oxidized difference spectra.

possible that the photometer was not entirely stabilized when scans were initiated for light minus dark spectra (scans were recorded from 600 to 500 nm as quickly as possible after turning the photometer circuit on following actinic irradiation). This problem did not occur with the scan from 500 to 400 nm, since it was possible to work at lower instrument sensitivity. In the presence of dithionite, the difference A at around 595 nm could arise from semiquinone A as reported by Vermilion *et al.* (15).

Similar difference spectra were obtained with 0.1% Triton X-100-solubilized Renografin-purified membrane fractions, and show identical Soret, alpha, and beta band difference peaks in both the light minus dark and dithionite reduced minus oxidized difference spectra.

DISCUSSION

The double reciprocal plots used in studying enzyme kinetics can be applied to the blue light-induced reduction of a *b*-type Cyt in this study in that the light intensity is the analog of substrate concentration and the LIAC is the analog of the rate of enzyme reaction. With the endogenous photoreceptor alone or with added riboflavin, or methylene blue as additional photoreductant, the same intercept on the *y*-axis, *i.e.* the same ΔA_{max}, was obtained. These results illustrate not only the stability and reproducibility of the photoreaction but also the limitation of the photoreduction to a particular *b*-type Cyt. It seems highly unlikely that the endogenous photoreceptor, riboflavin and methylene blue would all yield exactly the same ΔA_{max} at most only 20% of the total Cyt present in the 21KP (6), if they photoreduced Cyt indiscriminately.

From Table I, it is clear that the 21KP, with which the dose-response studies were performed, is contaminated by mitochondria and ER, both of which contain other cytochromes as well. The fact that only a particular *b* type Cyt, a relatively minor component among all the other contaminating Cyt, is being reduced by blue light (6) supports the idea that the blue light-induced reduction of *b*-type Cyt is highly specific.

By using the different properties of sucrose and Renografin gradients, it is possible to separate the membrane particles containing the LIAC activity both from the mitochondria and from ER. After removal of ER on the sucrose gradient, the membrane particles enriched in LIAC activity could be separated from mitochondria on a Renografin gradient.

The purification of the LIAC-active membrane particles is shown in Table II in which the specific activity of different enzymes and LIAC in different purification steps are compared. As the specific activity of the LIAC increases during purification, the specific activity of Glu II activity also increases roughly in parallel. Inasmuch as the peak of Glu II activity also coincides with the peak of LIAC activity on the Renografin gradient, we postulated that the LIAC activity is associated with the same membrane particles as those which have Glu II activity. We conclude that this LIAC active fraction is located neither in mitochondria, ER, nor golgi particles.

From the behavior of this fraction in sucrose and Renografin gradients, it is postulated that the LIAC-active fraction in the present study is probably identical to the membrane fraction studied by Jesaitis *et al.* (8), which is characterized to have Glu II activity, binding sites for *N*-naphthylphthalamic acid, NADH oxidase activity which is both antimycin A- and cyanide-insensitive, and a *b*-type Cyt. Quail (12) summarizes arguments that this fraction may be plasma membrane.

It has been reported recently (7) that there are specific binding sites for riboflavin in plasma membrane and ER fractions from corn coleoptile and *Cucurbita* hypocotyls. The riboflavin-binding properties of the purified LIAC-containing fraction still require investigation. The K_m reported by Hertel *et al.* (7) for riboflavin binding in the 21KP is larger by almost a factor of 10 than the K_m reported by Goldsmith *et al.* (6) for flavin sensitization of photoreduction of the *b*-type Cyt. The 21KP assayed by Hertel *et al.* (7) did not measure K_m values on more purified fractions presumably because the amounts of activity recovered were so small.

The nature of the *b*-type Cyt involved in the blue LIAC is still unknown. Comparison of light minus dark and dithionite reduced minus oxidized difference spectra provides a powerful tool in detecting any contaminating Cyt in the membrane preparations. With crude membrane preparations (21KP), it has been shown that the light minus dark difference spectra are different from dithionite reduced minus oxidized difference spectra (3, 6). This result is not unexpected, as the crude membrane preparation still contains mitochondria and ER which have additional Cyt. With membrane fractions purified on a Renografin gradient, we are able to show that both the Soret peak and the alpha and beta band peaks are identical, irrespective of whether the Cyt is reduced by blue light or by dithionite. These results support the previous conclusion that the membrane preparation is probably relatively pure "plasma membrane" and that the blue light specifically reduces only the Cyt in this membrane fraction and not those in other Cyt-containing membranes. The dithionite reduced minus oxidized difference spectrum for the Renografin-purified membrane fraction is similar to that reported for a plasma membrane fraction from the yeast *Candida tropicalis* (14).

Solubilization of the blue LIAC-active fraction with Triton X-100 has only a relatively small effect on the kinetics as well as on the ΔA_{max} of the photoreaction. Furthermore, the 0.1% Triton X-100-solubilized Renografin-purified membrane fraction is shown to have the same difference peaks in both light minus dark and dithionite reduced minus oxidized difference spectra as before solubilization. These results indicate that the flavin and Cyt moieties are probably on the same protein, as is the case in Cyt b_2 from yeast (9), and that both the flavin and the protoheme are probably tightly bound to the protein (17). Since the low concentration (0.1%) of Triton has totally solubilized the flavin-Cyt complex together with only a small portion of the membrane, this solubilization represents an important step forward in photoreceptor purification. With this purified membrane fraction, it should be possible to investigate the redox properties of the system with far greater precision than possible to date.

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LITERATURE CITED

1. BRAIN RD, JA FREEBERG, CV WEISS, WR BRIGGS 1977 Blue light-induced absorbance changes in membrane fractions from corn and *Neurospora*. Plant Physiol 59: 948–952
2. BRIGGS WR 1980 A blue light photoreceptor system in higher plants and fungi. *In* JA DeGreef, ed, Photoreceptors and Plant Development, Antwerpen University Press, 17–28
3. BRITZ SJ, E SCHROTT, S WIDELL, WR BRIGGS 1979 Red light-induced reduction of a particle-associated *b*-type cytochrome from corn in the presence of methylene blue. Photochem Photobiol 29: 359–365
4. CAUBERGS RJ, MHM GOLDSMITH, WR BRIGGS 1978 Effects of inhibitors on light-induced absorbance changes in corn membrane fractions. Carnegie Inst Wash Year Book 77: 356–357
5. FORD GA, B CATANZARO 1978 A centralized laboratory computing system. Carnegie Inst Washington Year Book 77: 305–307
6. GOLDSMITH MHM, RJ CAUBERGS, WR BRIGGS 1980 Light-inducible cytochrome reduction in membrane preparations from corn coleoptiles. I. Stabilization and spectral characterization of the reaction. Plant Physiol 66: 1067–1073
7. HERTEL R, AJ JESAITIS, U DOHRMANN, WR BRIGGS 1980 *In vitro* binding of riboflavin to subcellular particles from maize coleoptiles and *Cucurbita* hypocotyls. Planta 147: 312–319
8. JESAITIS AJ, PR HENERS, R HERTEL, WR BRIGGS 1977 Characterization of a membrane fraction containing a *b*-type cytochrome. Plant Physiol 59: 941–947
9. LEMBERG R, J BARRETT 1973 Cytochrome. Academic Press, London, pp 1–580
10. LEONG T-Y, RJ CAUBERGS, WR BRIGGS 1980 Solubilization of a membrane associated flavin-cytochrome complex from corn. Carnegie Inst Wash Year Book 79: 134–135
11. LOWRY OH, NJ ROSEBROUGH, AL FARR, JR RANDALL 1951 Protein measurements with the Folin phenol reagent. J Biol Chem 193: 265–275
12. QUAIL PH 1979 Plant cell fractionation. Annu Rev Plant Physiol 30: 425–484
13. RAY PM 1977 Auxin-binding sites of maize coleoptiles are localized on membranes of the endoplasmic reticulum. Plant Physiol 59: 594–599
14. SCHNEIDER H, GF FUHRMANN, A FIECHTER 1979 Plasma membrane from *Candida tropicalis* grown on glucose or hexadecane. II. Biochemical properties and substrate-induced alterations. Biochim Biophys Acta 554: 309–322
15. VERMILION J, V MASSEY, MJ COON 1980 Studies with FMN-depleted NADPH-cytochrome P-450 reductase. *In* Yagi K, T Yamano, eds, Flavins and Flavoproteins, Japan Scientific Societies Press, Tokyo, pp 693–702
16. WIDELL S, SJ BRITZ, WR BRIGGS 1980 Characterization of a red light-induced reduction of a particle-associated *b*-type cytochrome from corn in the presence of methylene blue. Photochem Photobiol 32: 679–682
17. YAGI K, T YAMANO 1980 Flavins and Flavoproteins, Japan Scientific Societies Press, Tokyo, pp 1–734