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PHOTOREACTIONS OF CYTOCHROME b_6 IN SYSTEMS USING RESOLVED CHLOROPLAST ELECTRON-TRANSFER COMPLEXES

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Photoreactions of cytochrome b_6 have been studied using resolved chloroplast electron-transfer complexes. In the presence of Photosystem (PS) II and the cytochrome b_6-*f* complex, photoreduction of the cytochrome can be observed. No soluble components are required for this reaction. Cytochrome b_6 photoreduction was found to be inhibited by quinone analogs, which inhibit at the Rieske iron-sulfur center of the cytochrome complex, by the addition of ascorbate and by depletion of the Rieske center and bound plastoquinone from the cytochrome complex. Photoreduction of cytochrome b_6 can also be demonstrated in the presence of the cytochrome complex and PS I. This photoreduction requires plastocyanin and a low-potential electron donor, such as durohydroquinone. Cytochrome b_6 photoreduction in the presence of PS I is inhibited by quinone analogs which interact with the Rieske iron-sulfur center. These results are discussed in terms of a Q-cycle mechanism in which plastosemiquinone serves as the reductant for cytochrome b_6 via an oxidant-induced reductive pathway.

Introduction

Photosynthetic electron transport in chloroplasts involves two different pathways: a noncyclic route, utilizing PS I and PS II, which produces ATP, oxygen and NADPH, and a cyclic route utilizing PS I which produces only ATP [1]. Evidence has been obtained in studies with intact CO_2-fixing chloroplasts that ATP produced from the cyclic and noncyclic pathway is required for CO_2 fixation [2–4].

In recent years, the role of chloroplast cytochromes in noncyclic and cyclic electron transport has been investigated in great detail [5]. This work has involved the use of intact chloroplasts, thylakoid membranes free of stromal proteins, and detergent- or French press-fractionated subchloroplast fragments. In general, this work has led to the conclusion that chloroplast cytochrome *f* has a site of action between PS I and PS II and is a component of both the cyclic and noncyclic pathways [1,5,6]. Other components, such as plastoquinone, plastocyanin and the Rieske iron-sulfur center have been proposed to have a similar mode of action [1,7].

The role and function of chloroplast cytochrome b_6 (*b*-563) remain less clear. Early work assigned a role to cytochrome b_6 as an electron carrier unique to the cyclic electron-transport pathway, since the cytochrome could be shown to undergo photooxidation and photoreduction in

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Abbreviations: DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; UHDBT, 5-(*n*-undecyl)-6-hydroxy-4,7-dioxobenzothiazole; DQH_2, durohydroquinone; DNP-INT, 2-iodo-6-isopropyl-3-methyl-2,4,4′-trinitrodiphenyl ether; DCMU, 3-(3′,4-dichlorophenyl)-1,1-dimethylurea; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; Mes, 4-morpholineethanesulfonic acid; PS, photosystem; Chl, chlorophyll.

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far-red light which is effective in activating PS I [8–11]. However, recent studies have also demonstrated a PS II photoreduction of this cytochrome [12–15], and recent models for its site of function have emphasized the Q-cycle hypothesis [13,16,17] which was originally proposed to describe electron transfer through the cytochrome bc_1 region of the mitochondrial electron-transport chain [18]. In these recent formulations, cytochrome b_6 is considered to undergo a photoreduction which is mediated by reduced plastoquinone [13,17], although various laboratories have not yet arrived at a single model which explains all these observations.

Dutton and co-workers [19,20] have recently described the construction of an in vitro system for the study of electron-transfer reactions in the cytochrome bc_1 region by combining purified mitochondrial Complex III with the photochemical reaction center from *Rhodopseudomonas sphaeroides*. The bacterial reaction center was found to be capable of delivering and accepting electrons from the mitochondrial complex. This work has recently been extended to a study of the interaction of the chloroplast cytochrome b_6-*f* complex with the bacterial reaction center [21], although with this system it has been found that the reaction center can only deliver electrons into the chloroplast cytochrome complex. Our recent work has involved similar techniques except that we have employed a homologous system in that highly resolved electron-transfer complexes from spinach have been used in reconstructing the chloroplast electron-transport pathways. Also, no soluble cytochromes (e.g., cytochrome *c*) are added for electron transfer as was needed in Ref. 21, since spinach plastocyanin serves this function in the homologous system. This allows for a clearer definition of the light-induced reactions of the chloroplast cytochromes. By combining PS I, PS II and the cytochrome b_6-*f* complex in the presence of soluble proteins, a reconstruction of the noncyclic electron-transport pathway from water to NADP has been achieved [22]. The photoreduction of cytochrome *f* using PS II and the cytochrome complex has also been demonstrated [23]. In the present report, we describe studies of the photoreactions of cytochrome b_6 in two different reconstructed systems. The photoreactions of this cytochrome in the cytochrome complex have been characterized in the presence of PS II with the absence of PS I, and in the presence of PS I with the absence of PS II. These results are discussed in terms of current models for the function of cytochrome b_6 in pathways of chloroplast electron transport.

Materials and Methods

Preparation of PS I and PS II and the cytochrome b_6-f complex. The PS I preparation used for the experiments described in the present work was obtained by the method of Mullet et al. [24]. After solubilization of freshly isolated chloroplasts in 0.75% Triton X-100, the supernatant from the subsequent centrifugation (42 000 × *g*, 1/2 h) was loaded onto a sucrose gradient as described previously [24]. The gradients were centrifuged at 100 000 × *g* for 15 h. The lower green band in the gradient after centrifugation was collected and used without further purification. The preparation, in approx. 1.0 M sucrose + 0.02% Triton X-100, was stored at −20°C. As isolated, this fraction contains no detectable cytochromes, and the chlorophyll-to-P-700 ratio is about 200 : 1 as determined by photochemical assay. PS II preparations devoid of P-700, cytochrome *f* and cytochrome b_6 were isolated as described by Berthold et al. [25]. After incubation of freshly isolated chloroplasts with 5% Triton X-100 at 4°C for 30 min, the PS II fragments were collected by centrifugation at 40 000 × *g* for 30 min and then subsequently washed with 1% Triton X-100. The final pellet was then resuspended in 0.3 M sucrose + 20 mM Hepes buffer (pH 7.5) + 5 mM $MgCl_2$ and stored at −20°C until use. This PS II preparation contained about 1 cytochrome *b*-559 per 200 Chl as determined by redox difference spectroscopy in open cuvettes. Using a very sensitive spectrophotometric assay [26], it was found that this preparation contained about 1 Q, the primary acceptor of PS II, per 210 Chl. This preparation routinely gives an oxygen-evolution rate of about 50 μmol O_2/mg Chl per h with potassium ferricyanide as electron acceptor at pH 6.0. The cytochrome b_6-*f* complex [27] and the Rieske-depleted cytochrome complex [28] were obtained by previously described methods. The intact complex from the sucrose gradient had a cytochrome *f* concentration of 20 μM and was in a

buffer containing 30 mM Tris-succinate (pH 6.5) + 30 mM octylglucoside + 0.5% cholate + 0.1% lecithin. This preparation was essentially chlorophyll free and showed no detectable photoreactions in the absence of PS I or PS II.

Photoreaction of cytochrome b_6-f complex with PS I or PS II. The oxidation-reduction kinetics and difference spectra of the cytochrome b_6-f complex were studied using an Aminco DW-2 spectrophotometer modified for side illumination. A Corning 2-64 filter was placed in front of the actinic light source while a Corning 4-96 filter was used to protect the photomultiplier tube from the actinic light. For all studies, 1 cm light-path cuvettes (1 ml volume) were used. For kinetic studies, cytochrome *f* redox changes were monitored at 554–575 or 554–540 nm, while cytochrome b_6 was monitored at 563–575 nm. A slit width of 2.0 nm was routinely used. When fast kinetic changes were studied, the time resolution was limited by the recorder's response time, which is 0.3 s. The cytochrome concentration in the complex was determined by redox difference spectrometry using ascorbate, ferricyanide and dithionite. An extinction coefficient of 18 $mM^{-1} \cdot cm^{-1}$ at 554 nm was used for cytochrome *f* and 20 $mM^{-1} \cdot cm^{-1}$ at 563 nm for cytochrome b_6.

Because of various detergents which were present during the preparation and storage of the respective electron-transfer complexes, some of these detergents were carried over to the reaction mixtures used for light-induced cytochrome assays, but no systematic attempt to define the effects of these detergents was made.

β-Octylglucoside, sodium ascorbate, potassium ferricyanide, sodium cholate, and Triton X-100 were obtained from Sigma Chemical Co. DBMIB was a gift from Dr. A. Trebst, and UHDBT was obtained from Dr. B. Trumpower. Durohydroquinone was obtained from K and K Chemical Co. Plastocyanin was isolated from spinach leaves by R.K. Chain of our laboratory by modification of a standard procedure [29]. All other reagents were of the highest grade available.

Results

Photoreduction of cytochrome b_6 in the cytochrome b_6-f complex in the presence of PS II

The isolated cytochrome b_6-f complex from

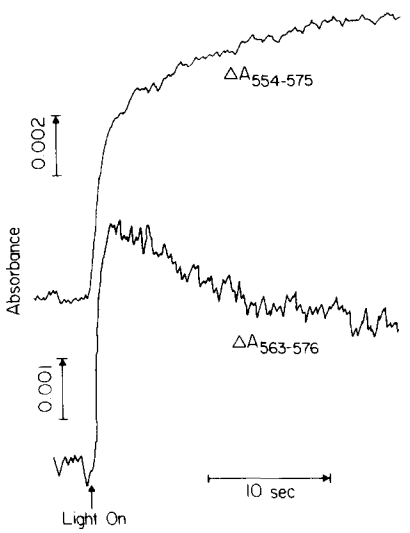

Fig. 1. Photoreduction of cytochrome b_6-f complex by PS II. The cytochrome b_6-f complex was mixed with the PS II preparation in a concentrated form (approx. 45–50 µl in volume) before dilution by the assay buffer. The final mixture contained cytochrome complex (0.64 µM cytochrome *f*), PS II fragments (50 µg Chl/ml) in 15 mM NaCl, 5 mM $MgCl_2$ and 20 mM Mes buffer (pH 6.0).

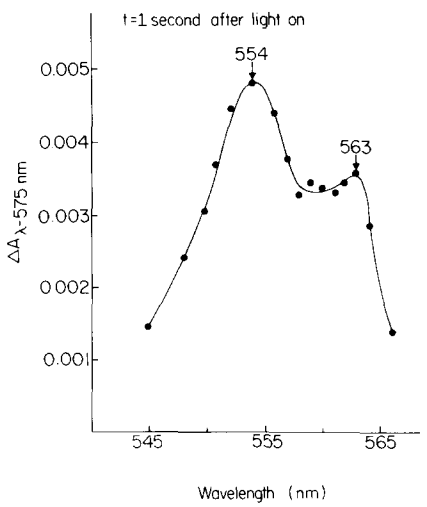

Fig. 2. Light-induced difference spectrum of cytochrome b_6-f complex in the presence of PS II. The reaction mixture was the same as that in Fig. 1 except a 2.5-times faster scan time was used to resolve kinetically the absorbance change at various wavelengths. The absorbance at 575 nm was used as the reference for each assay.

spinach thylakoids has been shown to contain cytochrome b_6, cytochrome *f*, the Rieske iron-sulfur center and plastoquinone in a molar ratio of 2 : 1 : 1 : 1 [27,30]. This preparation is enzymatically active in catalyzing reduced quinone-plastocyanin oxidoreductase activity which is sensitive to inhibitors such as DBMIB, DNP-INT and UHDBT [27]. When mixed with a resolved oxygen-evolving PS II preparation the photoreduction of cytochrome *f* in the complex can be observed by monitoring absorbance changes at 554 nm (Fig. 1, top trace). At this wavelength, biphasic reduction kinetics are observed. When the monitoring wavelength is 563 nm (Fig. 1, bottom trace), the α-band maximum of cytochrome b_6, a different kinetic response is observed: a rapid increase in absorbance is observed in the light, followed by a slow partial decay. The difference in the kinetics at these two wavelengths suggests that an additional component other than cytochrome *f* is undergoing an absorbance change in the 560 nm spectral region.

Since the PS II preparation used in these reconstruction studies contains cytochrome *b*-559 in a ratio of about 1 per 200 Chl molecules, it is possible that the absorbance changes in the 560 nm region could originate from this electron carrier or from cytochrome b_6 in the cytochrome complex. Both these electron carriers are found to be in the oxidized state prior to illumination in their respective complexes, and these absorbance changes would then represent cytochrome photoreduction. A light-minus-dark difference spectrum of the absorbance changes recorded in the 545–565 nm region 1 s after illumination is shown in Fig. 2. The spectrum shows a clear peak at 554 nm (cytochrome *f*) and a second smaller maximum at 563 nm (cytochrome b_6). When the estimated contribution from cytochrome *f* is subtracted from the difference spectrum, an absorbance peak characteristic of cytochrome b_6 is obtained (Fig. 3). The half-width of the subtracted spectrum (approx. 9 nm) shows that the contribution from PS II cytochrome *b*-559 is negligible. Examination of Fig. 3 shows that in the early phase of photoreaction, almost equivalent amounts of cytochrome b_6 and cytochrome *f* are being photoreduced in the reconstructed system with PS II.

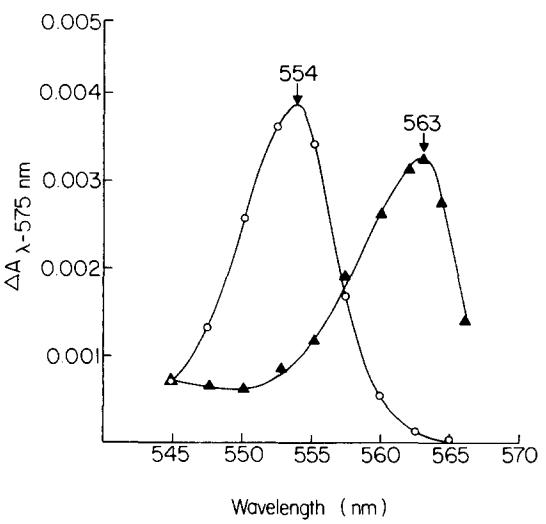

Fig. 3. Resolved difference spectrum of photoreduced cytochromes. The cytochrome *f* absorption curve was calculated from an absorption spectrum (ascorbate minus ferricyanide) taken from a sample of cytochrome b_6-*f* complex (○——○). The difference between this spectrum and the light-induced difference spectrum of Fig. 2 is then calculated (▲——▲).

It was found that mixing of the cytochrome complex with the PS II preparation prior to dilution with the assay buffer greatly enhanced the rate and extent of cytochrome photoreduction. Incubation of the mixture of the two preparations under more dilute conditions for longer periods of time can partly compensate for this effect. These observations suggest that interactions between the cytochrome complex and PS II are more intimate than a simple diffusion process and would be consistent with a specific association between these two complexes. A similar effect has been previously observed in the reconstructed NADP-reducing system [22].

The effects of various inhibitors and treatments on the photoreduction of cytochrome b_6 are shown in Fig. 4. The photoreduction is markedly inhibited by two known inhibitors of chloroplast noncyclic electron transport, DCMU and DBMIB. The low amount of DBMIB used in this experiment (1 μM) inhibited electron transport in the cytochrome b_6-*f* complex with little effect on O_2 evolution catalyzed by the PS II preparation. A small amount of cytochrome b_6 can still be slowly photoreduced in the presence of 1 μM DBMIB while DCMU, which inhibits the oxidation of re-

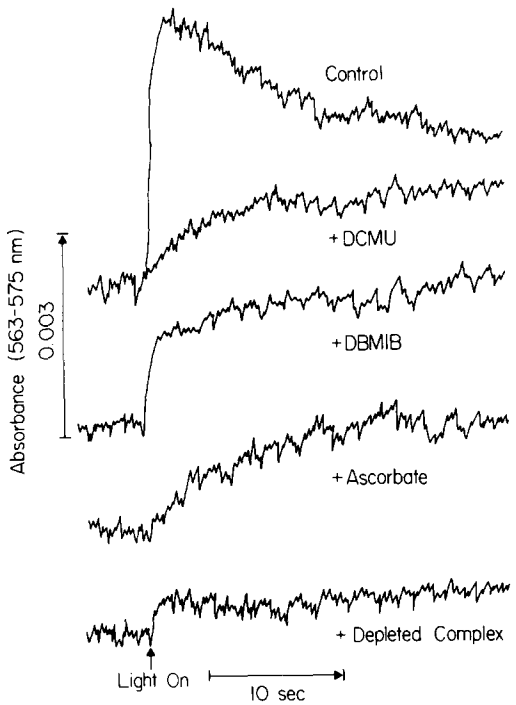

Fig. 4. Effects of inhibitors on photoreduction of cytochrome b_6 by PS II. The reaction mixture was the same as that of Fig. 1. 2 μM DCMU and 1 μM DBMIB were added as indicated. When present, a few grains of sodium ascorbate were added to reduce cytochrome *f* and the Rieske iron-sulfur protein. For the depleted complex, a concentration of 0.81 μM cytochrome *f* was used in place of 0.64 μM cytochrome *f* for the native complex. Due to the preparation procedure, 0.005% Triton X-100 was also present in the final assay mixture for the depleted complex.

duced Q, appears to be a more effective inhibitor of the photoreduction. The addition of ascorbate prior to illumination also severely inhibits cytochrome photoreduction. This effect is not related to a chemical reduction of the cytochrome by ascorbate, since it has been found that cytochrome b_6 in the complex (with $E_m = -110$ mV) [31] is not ascorbate reducible.

It has been possible to resolve the Rieske iron-sulfur center and the bound plastoquinone from the cytochrome complex by hydroxyapatite chromatography in the presence of Triton X-100. The depleted complex retains cytochrome *f* and cytochrome b_6, but cytochrome *f* photoreduction driven by PS II was drastically inhibited in the depleted complex [23]. Shown in Fig. 4 are similar results of an experiment attempting to photoreduce the cytochrome b_6 of the depleted complex. Essentially no cytochrome b_6 reduction can be detected after depletion of the Rieske center and plastoquinone from the cytochrome complex.

Photoreduction of cytochrome b_6 in the cytochrome b_6-f complex in the presence of PS I

Preliminary studies of cytochrome b_6 photoreactions in the presence of PS I have been reported by Hauska and co-workers [32]. We have carried out a systematic study of this system using a PS I subchloroplast fragment which had been previously demonstrated to be reconstitutively active in reconstructing NADP photoreduction activity with water as the electron donor [22]. This PS I preparation lacks any detectable cytochromes.

The results of a study in which absorbance

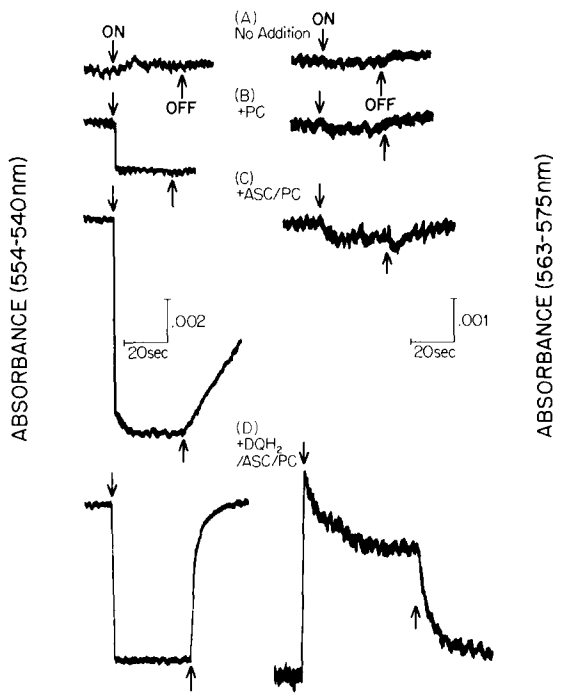

Fig. 5. Photoinduced changes of cytochrome b_6-*f* complex in the presence of PS I. The assay system contained cytochrome complex (0.64 μM cytochrome *f*), PS I fragments (22 μg Chl/ml) 5 mM $MgCl_2$, 15 mM NaCl and 20 mM Mes, pH 6.0. In A, no additions were made. In B, 0.6 μM plastocyanin (PC) was added while in C, a further addition of 0.5 mM sodium ascorbate (ASC) was made. In D, 60 μM durohydroquinone was added to C.

changes of both cytochrome *f* and b_6 of the cytochrome complex have been monitored in the presence of PS I are shown in Fig. 5. The right-hand portion of the figure depicts absorbance changes at 563–575 nm, indicative of cytochrome b_6, while the left-hand portion indicates cytochrome *f* absorbance changes (554–540 nm). With no additions (Fig. 5A), no photoinduced changes in either cytochrome are observed, while after the addition of plastocyanin (Fig. 5B), a small irreversible photooxidation of cytochrome *f* is observed. The addition of ascorbate to this reaction mixture results in a large photooxidation of cytochrome *f* (Fig. 5C), and this absorbance change is slowly reversed in the dark after illumination. Under the above conditions, no absorbance changes at 563 nm, indicative of cytochrombe b_6, are detected. If durohydroquinone, an electron donor for the cytochrome complex, is next added to the same reaction mixture, cytochrome *f* photooxidation still occurs, but the extent is smaller than that observed in the presence of ascorbate only, and a rapid rereduction occurs after illumination (Fig. 5D, left). In the presence of this electron donor, an absorbance increase at 563 nm is observed which decreased in the light to a steady-state level that persists during illumination. After illumination, the absorbance change at 563 nm rapidly decreases to the baseline (Fig. 5D, right).

The absorbance spectra shown in Fig. 6A confirm that the assignments discussed in the previous figure are correct. In the presence of ascorbate and

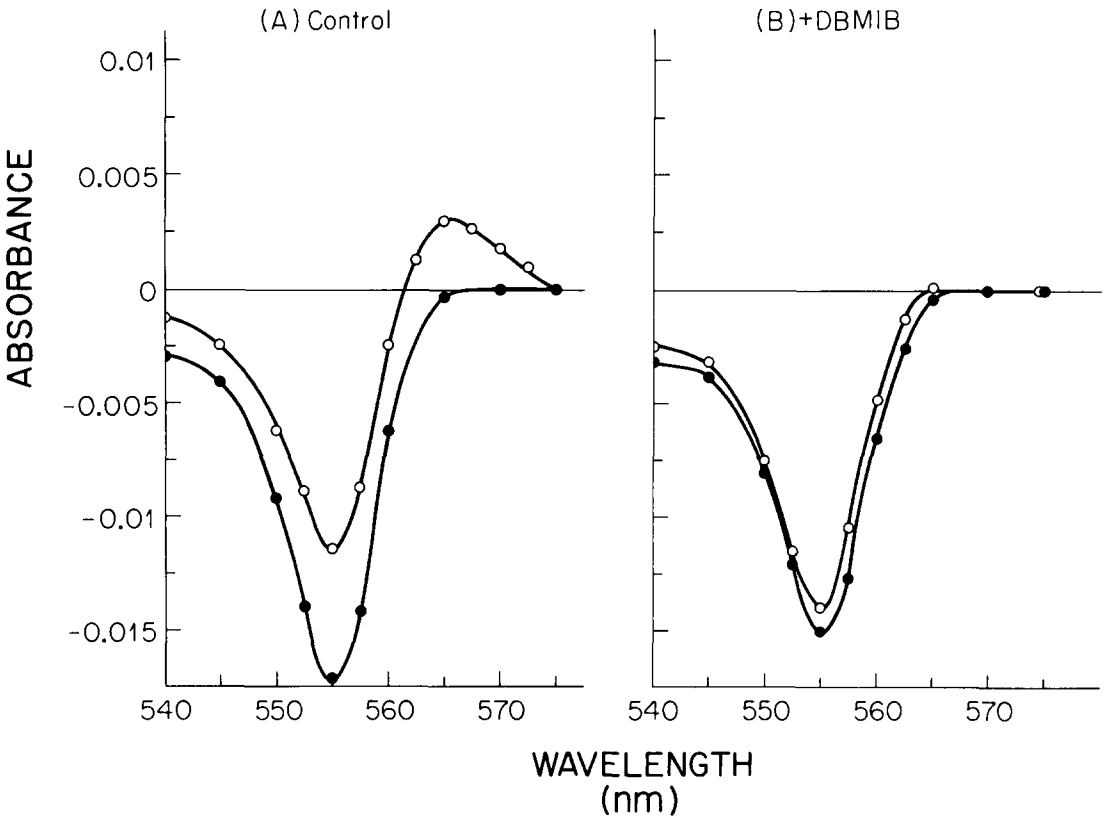

Fig. 6. Photoinduced difference spectra of the cytochrome complex in the presence of PS I – Effects of DBMIB. The assay mixture contained PS I fragments (23 μg Chl/ml), cytochrome complex (0.64 μM cytochrome *f*), 5 mM $MgCl_2$, 15 mM NaCl, and 20 mM Mes, pH 6.0. 0.5 mM sodium ascorbate was present in both sample and reference cuvettes. The sample cuvette also contained 0.6 μM plastocyanin. The absorption spectrum in the dark and in the light were then obtained between 540 and 580 nm. When present, 60 μM durohydroquinone was added to the sample cuvette. (A) No inhibitor. (B) 1.0 μM DBMIB was added to the sample cuvette prior to illumination. (●———●) Ascorbate, (○———○) ascorbate plus durohydroquinone.

durohydroquinone, an absorbance decrease centered at approx. 555 nm, indicative of cytochrome *f* photooxidation, is apparent, while an absorbance increase centered at approx. 563 nm, indicative of cytochrome b_6 photoreduction, is observed only after the addition of durohydroquinone. These observed changes are also totally dependent on the addition of plastocyanin. In steady-state illumination, only one-third as much cytochrome b_6 is reduced as compared with the amount of cytochrome *f* being photooxidized.

The effect of the plastoquinone analog, DBMIB, on the observed photoreactions is shown in Fig. 6B. DBMIB has been found to be an effective inhibitor of electron transport from reduced quinones to plastocyanin catalyzed by the cytochrome complex [27], and recent EPR studies have demonstrated an interaction between this inhibitor and the Rieske iron-sulfur center in both unfractionated chloroplast membranes [33] and the isolated cytochrome complex [30]. The addition of 1 μM DBMIB has a small effect on the extent of cytochrome *f* photooxidation in the presence of either ascorbate or durohydroquinone but completely eliminates the cytochrome b_6 photoreduction in the presence of the latter electron donor (Fig. 6B, cf. Fig. 6A). Similar effects are observed

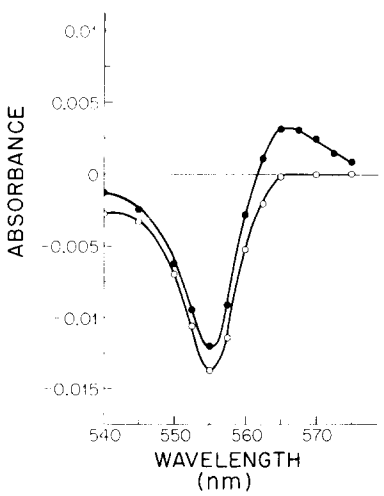

Fig. 7. Replacement of ascorbate by durohydroquinone. The assay conditions were identical to those of Fig. 6 except ascorbate was omitted from the sample cuvette. (●———●) Durohydroquinone, (○———○) durohydroquinone plus 1 μM DBMIB.

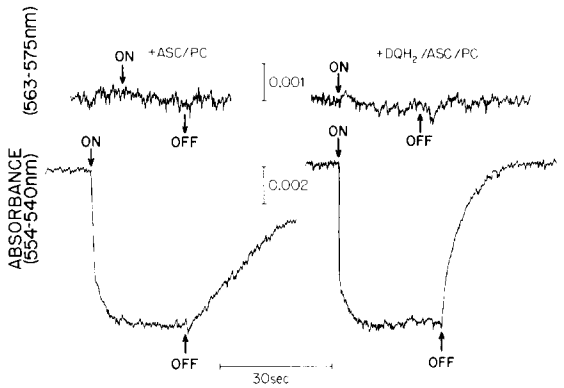

Fig. 8. Effect of Rieske center depletion on photoreduction of cytochrome b_6 in the presence of PS I. The assay mixture contained PS I fragments (18 μg Chl/ml) and depleted cytochrome complex, (0.67 μM cytochrome *f*). All other conditions identical to that of Fig. 5.

when durohydroquinone alone in the absence of ascorbate is present as the electron donor (Fig. 7).

A study of the effect of UHDBT, a second quinone analog which inhibits electron transfer through the cytochrome complex [27], gave similar results to those obtained with DBMIB. A small effect on the extent of cytochrome *f* photooxidation was observed after the addition of 1 μM UHDBT, while cytochrome b_6 photoreduction in the presence of durohydroquinone was inhibited about 80% (data not shown).

It was also possible to inhibit cytochrome b_6 reduction without affecting cytochrome *f* photooxidation by using the depleted cytochrome complex previously described. As shown in Fig. 8, cytochrome *f* photooxidation in the presence of ascorbate or ascorbate plus durohydroquinone did not require the presence of either the Rieske center or the bound plastoquinone of the cytochrome complex while cytochrome b_6 photoreduction was eliminated after the depletion of these carriers.

Discussion

Studies of reactions in the cytochrome bc_1 region of photosynthetic systems have recently utilized isolated cytochrome complexes in conjunction with various photochemical reaction centers which can be used to photoactivate electron-transfer reactions [19–23,32]. In the present work, we have used the chloroplast cytochrome complex in

conjunction with either resolved PS I or PS II complexes in an attempt to define the photoreactions of chloroplast cytochrome b_6.

It has been possible to demonstrate a PS II-dependent photoreduction of cytochrome b_6 which occurs in the absence of PS I. Inhibition of water oxidation by DCMU results in inhibition of cytochrome b_6 reduction as well as cytochrome *f* reduction [23]. One important characteristic of this reaction is that it is substantially inhibited by the plastoquinone analog, DBMIB. At low concentrations this inhibitor is known to prevent oxidation of plastohydroquinone by interaction with the Rieske iron-sulfur center which is present in the cytochrome complex [30,33]. Thus, under the conditions of this study, when electron transfer from plastohydroquinone to the Rieske center and cytochrome *f* is prevented by DBMIB, cytochrome b_6 reduction was severely inhibited.

A small, but significant, photoreduction of cytochrome b_6 occurred in the presence of DBMIB. This indicates that a DBMIB-insensitive pathway of reduction is present, and such a pathway could involve a direct reduction of the cytochrome from the PS II electron acceptor, Q, or the two-electron gate, B. Studies of the flash dependency of the DBMIB-insensitive reduction are required to differentiate between these two alternatives, but the small extent of the reaction raises questions about this being a major reductive pathway.

A requirement for the high-potential electron acceptors of the cytochrome complex in cytochrome b_6 photoreduction is also based on the finding that the addition of ascorbate inhibits this reaction. Similar results have been observed in the mitochondrial cytochrome *bc* region with succinate as the electron donor [34] and in the reconstructed system utilizing the bacterial reaction center [21]. These results can be interpreted as indicating that the 'removal,' by reduction, of high-potential electron carriers which act as electron acceptors for plastohydroquinone eliminates cytochrome b_6 reduction. A similar argument can be put forth for the inhibition of cytochrome b_6 reduction in the Rieske iron-sulfur center-depleted cytochrome complex.

Our results on cytochrome b_6 reduction dependent on PS II are consistent with plastohydroquinone serving as the source of electrons for the cytochrome as described in the equations below:

$$PQH_2 + RFeS(ox) \rightarrow PQH^{\cdot} + H^+ + RFeS(red) \quad (1)$$

$$PQH^{\cdot} + \text{cyt } b_6(ox) \rightarrow PQ + H^+ + \text{cyt } b_6(red) \quad (2)$$

The oxidation of reduced plastohydroquinone (PQH_2) by the oxidized Rieske iron-sulfur center (RFeS) yields a plastosemiquinone species ($PQH^{\cdot}$) which serves as the reductant for cytochrome b_6 in a subsequent reaction. This mechanism is essentially an 'oxidant-dependent reduction' of cytochrome b_6, similar to that proposed in 'Q-cycle' mechanisms for mitochondrial *b*-type cytochromes [18]. According to this formulation, any treatment (DBMIB or ascorbate addition or Rieske center removal) which prevents reaction 1 from occurring will result in cytochrome b_6 reduction being inhibited (reaction 2). The function of the Rieske iron-sulfur center in this formulation is similar to that proposed by Trumpower [35] for the iron-sulfur protein in the cytochrome bc_1 region of the mitochondrial respiratory chain.

It was also possible to demonstrate a photoreduction of cytochrome b_6 in the cytochrome complex which occurred in the presence of PS I. This photoreduction required the presence of a moderately low-potential electron donor, such as durohydroquinone, since ascorbate could not serve as the donor for this reaction. No soluble low-potential protein components, such as ferredoxin or ferredoxin-NADP reductase, were required. The PS I-dependent photoreduction of cytochrome b_6 was inhibited by quinone analogs such as DBMIB and UHDBT. These observations rule out the possibility that cytochrome b_6 was reduced directly by way of the low-potential acceptors of PS I. Since these quinone analogs interact with the Rieske iron-sulfur center of the cytochrome complex [30], a requirement for the iron-sulfur center in the PS I reduction of cytochrome b_6 was also apparent. This was also demonstrated with the complex from which the Rieske center had been removed. In the latter cases, cytochrome *f* photooxidation still occurred, but was not linked to cytochrome b_6 photoreduction. The reactions of cytochrome b_6 in the presence of PS I can also be explained on the basis of an oxidant-induced reduction where the photosystem, in the light, is the

source of the oxidant (P^+-700) and generates in a subsequent dark reaction the oxidized Rieske iron-sulfur center. The oxidized iron-sulfur center would then accept an electron from plastohydroquinone as shown above in reaction 1 and cytochrome b_6 would be reduced by reaction 2. Although Hauska et al. [32] did not report a requirement for an electron donor in their initial reconstruction studies, we find an absolute dependence for durohydroquinone in cytochrome b_6 photoreduction. This requirement according to the above formulation, would be to generate plastohydroquinone from plastoquinone, although the possibility that durohydroquinone itself actually serves as the reductant for cytochrome b_6 cannot be excluded from the present results.

While this manuscript was in preparation, a report by Hurt and Hauska [36] appeared which described the interaction of the cytochrome complex with PS I. The results in that report are in substantial agreement with those reported in this work and have been interpreted as an indication of an oxidant-induced reduction of cytochrome b_6.

Our results indicate it is possible to use resolved electron-transfer complexes of chloroplast membranes for the study of cytochrome b_6 photoreactions. These reconstructed systems have provided evidence that plastosemiquinone, generated by the oxidation of plastohydroquinone by the Rieske iron-sulfur center, serves as the most probable reductant for cytochrome b_6 when the cytochrome complex is linked to either PS I or PS II. Further studies with these reconstructed systems may prove useful in characterizing the pathway of cytochrome b_6 oxidation following photoreduction.

Acknowledgements

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