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A REDOX STUDY OF THE ELECTRON TRANSPORT PATHWAY RESPONSIBLE FOR GENERATION OF THE SLOW ELECTROCHROMIC PHASE IN CHLOROPLASTS

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The amplitude of the slow phase of the electrochromic bandshift and the dark redox state of cytochrome b_6, as well as its flash-induced turnover, have been measured as a function of ambient redox potential between +200 and −200 mV. Formation of a quinol-like donor with an $E_{m,7} = +100 \pm 10$ mV is required for generation of the slow phase. 80–100% of the amplitude of this signal with a $t_{1/2} = 3$–4 ms is observed at −200 mV where cytochrome b_6 was almost fully reduced ($E_{m,7}$ of dark and flash-induced photoreduction was −30 mV and −75 mV, respectively). The change in the photoreduction of cytochrome b_6 above 0 mV had an $E_{m,7}$ of +50 mV, about 50 mV more negative than the midpoint at this pH for the onset of the slow electrochromic change. At potentials below −140 mV the amplitude of b_6 photoreduction becomes small or negligible. The nature of the cytochrome b_6 photoresponse is changed at potentials below −140 mV from a net photoreduction with a $t_{1/2} = \lesssim 1$ ms to a photooxidation with a $t_{1/2} = 15$–20 ms that is substantially slower than the electrochromic band-shift with a $t_{1/2} = 3$–4 ms. It is concluded that the slow electrochromic phase probably does not arise from a mechanism involving a turnover of cytochrome b_6. From consideration of the possible flash-induced electron-transfer steps and alternative mechanisms for generation of the slow phase, it is suggested that it may arise from a redox-linked H^+ pump involving the high potential iron-sulfur protein.

Introduction

The slow (millisecond) component of the electrochromic bandshift was shown by Joliot and Delosme [1] to be associated with electron transfer in Photosystem I. H^+/e^- ratios greater than 1 and sometimes approaching 2 in the intersystem electron-transport chain have been measured under conditions of limited $\Delta\tilde{\mu}_{H^+}$ (Refs. 2–6; and see exceptions, Refs. 7–9). This H^+/e^- stoichiometry larger than 1 would require an electrogenic step that could in principle involve a Q cycle mechanism [10] or an H^+ pump coupled to the electron-transport chain [58,11,12]. The characteristic time of a few milliseconds for the rise of the slow phase implies that it is, in either case, coupled to electron transfer from the plastoquinol pool to the Photosystem I donors. In the Q cycle mechanism, the

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Abbreviations: $E_{m,7}$ midpoint potential at pH 7; E_h, measured redox potential; PS, Photosystem; Chl, chlorophyll; H^+/e^-, stoichiometry of protons translocated to electrons transferred; $t_{1/2}$, time required for half-maximum change of absorbance; $\tilde{\mu}_{H^+}$, proton electrochemical potential; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; HQNO, 2-heptyl-4-hydroxy-quinoline-*N*-oxide; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Mes, 4-morpholineethanesulfonic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

slow phase is proposed to arise from one of the two electrons released upon PQH_2 oxidation recrossing the membrane and generating a field in a cycle translocating $2H^+/e^-$ [13–21]. This path of transmembrane electron transfer is thought to include cytochrome b_6 as an acceptor of electrons from plastosemiquinone and a component in the transmembrane network. For generation of the slow phase, one would then need reduced donor, oxidized acceptor, and a hole in the Rieske FeS/cytochrome *f* region of the chain generated by PS I turnover. We undertook a redox study of the slow phase to characterize the donor and acceptor of this proposed cycle.

Materials and Methods

Chloroplast preparation

Chloroplasts were prepared from spinach grown in a controlled climate chamber essentially according to the procedure of Ort and Izawa [22]. The chloroplasts were suspended in 0.2 M sucrose, 5 mM Hepes, 2 mM $MgCl_2$, and 0.05% (w/v) bovine serum albumin, at pH 7.5. The assay medium consisted of 0.1 M sucrose, 10 mM KCl, 2 mM $MgCl_2$ and 30 mM of either Mes (pH 6.0), Hepes (pH 7.0) or Tricine (pH 8.0). Chlorophyll concentrations were determined by the method of Arnon [23]. The chlorophyll concentration was 45 μg/ml except where noted.

Spectrophotometry

Light-induced absorbance changes were measured using a single beam spectrophotometer with the amplified output from the photomultiplier tube (EMI 9524B) coupled to a signal averager (Tracor Northern NS-570). The time constant of the apparatus was 0.3 ms. The intensity of the measuring beam was either $1 \cdot 10^{-1}$ or $2.2 \cdot 10^{-2}$ $J \cdot m^{-2} \cdot s^{-1}$ both giving identical results. Further decrease of the measuring light intensity was also found to have no effect. Single-turnover actinic flashes were provided by a dye laser (Phase-R DL-1100; flash width at half height, 0.4 μs) using Rhodamine 640 as the dye. Flashes were saturating and reduction of their intensity by 50% had no effect on the amplitude of the absorbance changes. The cytochrome b_6 redox titration measured in the dark utilized a modified Aminco-Chance dual wavelength spectrophotometer described previously [24].

The absorbance changes due to the slow phase of the electrochromic shift were measured by subtracting the signals recorded at 515 nm in the presence of 4 μM DBMIB (fast phase only) from the fast and slow phase recorded in its absence [17]. Cytochrome b_6 turnover was measured by subtracting traces recorded at 570 nm from those recorded at 563 nm. Final data for all flash-induced absorbance changes were the average of ten signals at each wavelength obtained at a frequency of 0.1 Hz except where noted.

Redox titrations

For the flash-induced absorbance change measurements at defined redox potentials, the following mediators (midpoint potentials from Refs. 25 and 26) were used: 20 μM 1,2-naphthoquinone ($E_{m,7} = +135$ mV), 40 μM 1,4-naphthoquinone ($E_{m7} = +65$ mV), 20 μM menadione ($E_{m,7} = 0$ mV), 20 μM 2,5-dihydroxy-1,4-benzoquinone ($E_{m,7} = -60$ mV), 20 μM anthraquinone-2,6-disulfonate ($E_{m,7} = -185$ mV) and 20 μM anthraquinone-2-sulfonate ($E_{m,7} = -225$ mV). For the titration of flash-induced cytochrome b_6 turnover, 20 μM indigo-tetrasulfonate ($E_{m,7} = -38$mV) was also included. Its presence did not alter the extent or kinetics of the slow phase of the 515 nm change at high or low potential. Several redox mediators were found to be unsuitable for these experiments. Phenazine methosulfate (PMS) and phenazine ethosulfate (PES) increased the decay of the flash induced field, making detection of the slow phase more difficult. The reduced form of 2-hydroxy-1,4-naphthoquinone ($E_{m,7} = -140$ mV) inhibited the slow phase and, when included as a mediator, the slow phase disappeared at low potentials with an $E_{m,7}$ equal to that of this mediator. 3 μM DCMU and 0.1 mM methyl viologen were present in all samples [17]. Ambient redox potentials were measured using a Metrohm AG9100 Pt:Ag|AgCl redox electrode and a Corning Digital III millivolt meter. The potentials were adjusted by injection of small volumes of sodium dithionite or potassium ferricyanide as reductant or oxidant. Anaerobic conditions were maintained by flushing the cuvette with water-saturated argon (Chemetron Corp., Chicago, IL). The system was calibrated at high

potentials with saturated quinhydrone [25] and its accuracy checked at negative potentials by titration of a 20 μM FMN solution [27], which had a midpoint of -205 mV and a slope corresponding to $n = 2$ in the Nernst equation.

Results

Measurement of the slow electrochromic phase at different redox potentials

The individual and difference traces obtained at high ($+160$ mV), intermediate (0 mV), and low (-175 mV) ambient redox potential are shown in Fig. 1. It can be seen that the slow electrochromic phase is absent in the positive potential region (Fig. 1C, left) and has a similar amplitude and time course ($t_{1/2} = 3.1$ and 3.3 ms, respectively) at intermediate and low potentials (Fig. 1C, center and right). The spectrum of the slow phase obtained in the low-potential region (Fig. 2) is similar to that of the fast phase measured aerobically in the absence of defined redox conditions [28].

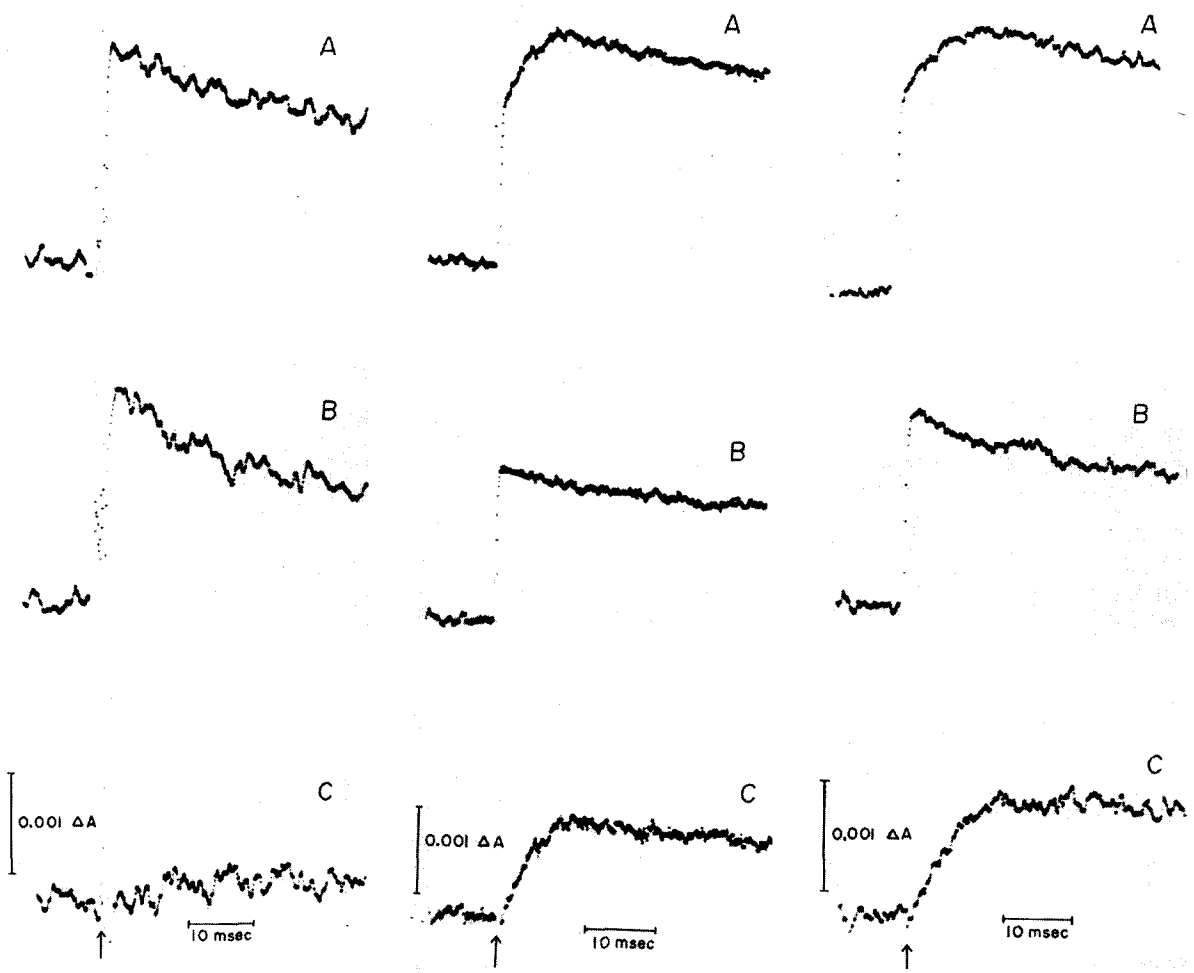

Fig. 1. Measurement of the flash-induced 515 nm absorbance change at ambient potentials of $+160$ mV (left), 0 mV (center), and -175 mV (right). 3 μM DCMU and 0.1 mM methyl viologen present. (A) No other additions; (B) addition of 4 μM DBMIB; (C) difference of (A) and (B). Amplitude determined as an average of ten traces. Mediators: 20 μM 1,2-naphthoquinone, 40 μM 1,4-naphthoquinone, 20 μM each of menadione, anthraquinone-2,6-disulfonate, and anthraquinone-2-sulfonate. Including 20 μM each of 2,5-dihydroxy-benzoquinone and indigo-tetrasulfonate had no effect on the amplitude of the slow phase at high or low potential.

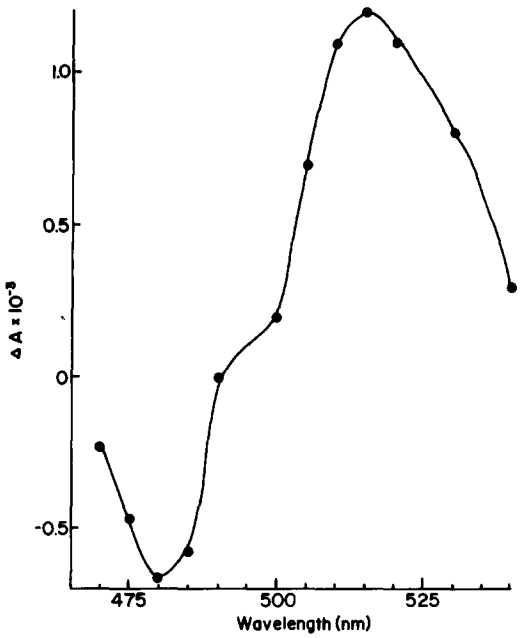

Fig. 2. Spectrum of the slow absorbance change at an ambient potential of −130 mV. Conditions otherwise as in Fig. 1.

Titration of a quinol-like electron donor for the slow phase

An extended titration of the amplitude of the slow phase over the range −200 to +200 mV showed that reduction of a component with an $E_{m,7} = +100$ mV (±10 mV for five titrations) is required for generation of the slow phase (Fig. 3). This measurement has been reported in preliminary communications [29,30]. The relatively steep $n = 2$ slope shown in Fig. 3 was always observed. The midpoint is similar to that of the pool plastoquinone A [31] and the $n = 2$ slope characteristic of the quinol-quinone couple. A pH dependence of the midpoint potential expected for a quinol-quinone couple ($\Delta E_m/\Delta pH = -60$ mV) is shown in Fig. 4, similar to that reported by Giorgi et al. [32], with values of $E_{m,6}$, $E_{m,7}$, and $E_{m,8}$ equal to +160, +105 and +25 mV, respectively. As found previously by Bouges-Bocquet [14], the amplitude of the slow phase decreased markedly with decreasing pH so that the ratio of the maximum absorbance values measured at pH 6, 7 and 8 was 0.5 : 0.8 : 1.0. The rise of the slow phase was previously reported to have an $E_{m,8.1} = -55$ mV [33], a value substantially lower than the value reported here, in Refs. 29 and 30, and recently as well in Ref. 34. The value of $E_{m,7} = +100 \pm 10$ mV is close to that expected for plastoquinone in the pool [31,35] or interacting with the high-potential PS I donors [15]. It is concluded from Figs. 3 and 4 that a quinol-like component must be in the reduced state for subsequent generation of the slow phase and that this component is the donor of the electrons and possibly the protons as well, needed for the initial electron transfer and subsequent charge separation events assoicated with the slow electrochromic phase.

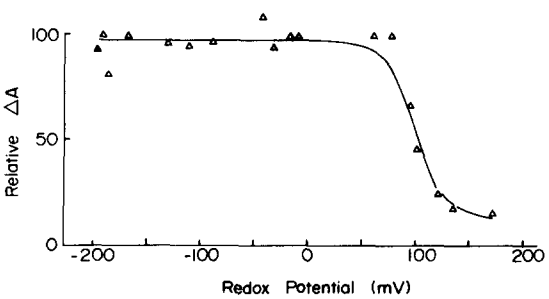

Fig. 3. Amplitude of the slow electrochromic phase as a function of ambient redox potential. Conditions and mediators as in Fig. 1.

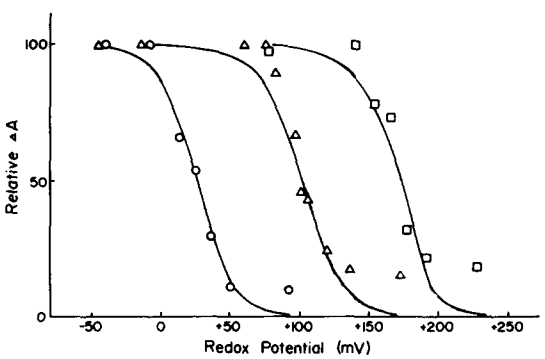

Fig. 4. Redox titration in the high potential region of the amplitude of the slow electrochromic phase as a function of pH at pH values of 6(□), 7(△) and 8(○). Amplitudes are normalized to the maximum at pH 8.0. The absorbance changes at the maximum found at pH values of 6, 7 and 8 were $9 \cdot 10^{-4}$, $1.4 \cdot 10^{-3}$ and $1.8 \cdot 10^{-3}$, respectively, with the respective ratios of amplitudes of slow/fast phase 0.36, 0.50, and 0.90.

Titration of possible electron acceptors for the slow phase – the question of the involvement of cytochrome b_6

The amplitude of the slow phase was found to be approximately independent of redox potential over the range −200 mV to 0 mV (Fig. 3). Reversibility was checked by decreasing the potential from +100 to +150 mV to −50 mV, then raising it to +140 mV, and showing that the pattern of the slow phase was unchanged by this transient decrease of potential. In order to be sure that the independence of slow phase amplitude on E_h was not a consequence of slow and incomplete equilibration with the redox buffers between flashes, two controls were done. (i) The amplitude and time-course of the slow phase generated in the first flash was compared to that of later flashes and found to be the same (data not shown). (ii) The time interval between flashes, 10 s for all measurements discussed thus far, was extended to 60 s. The amplitude and time-course ($t_{1/2}$ = 3.1 and 3.4 ms, respectively) of the slow phase was found to be very similar at E_h = −195 mV (Fig. 5A–C) and −15 mV (Fig. 5D–F). Measurements are not reported below −200 mV because the redox buffering was found to be less stable at the lower potentials. Preliminary measurements made below −200 mV indicated that the amplitude of the slow electrochromic phase decreased to a value of 75% of the maximum at a potential of −250 mV (data not shown).

After incubation at an ambient potential of −200 mV, all electron transport components with midpoints more positive than this value will tend to be reduced before the light flash. For example, a hypothetical one-electron component with an E_m = −140 or −80 mV would be 90% and 99% reduced, respectively, at an ambient potential of −200 mV. Electron-transport components which are completely or mostly reduced in the dark state before the flash would not be available to accept an electron from the quinol donor after the flash unless they were oxidized by the flash more rapidly than the quinol. There is some disagreement in the literature as to the precise midpoint potential of cytochrome b_6 in situ, with the different measurements ranging from +5 mV [26,36] to −110 mV [37]. However, the cytochrome should be completely or almost completely reduced at an E_h of

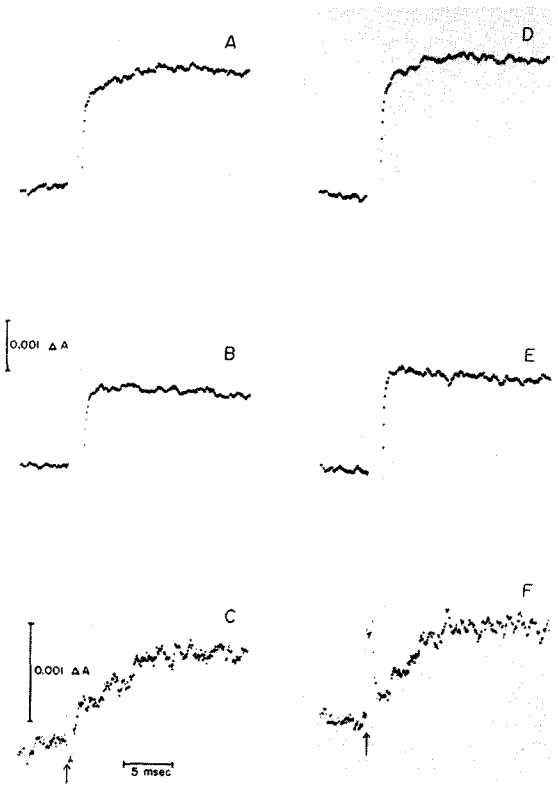

Fig. 5A–C. Measurement of the slow 515 nm absorbance change at lower flash frequency (A–C) at −195 mV and (D–F) at −15 mV. Traces are averages of five flashes with 60 s between the flashes. Conditions otherwise as in Figs. 1–3. (A,D) No other addition; (B,E) addition of 4μM DBMIB; (C,F) difference of (A,B) and (D,E). $t_{1/2}$ of slow change is 3.1 ms in (C) and 3.4 ms in (F).

−200 mV using either of the above estimates for its midpoint.

Given the range in reported midpoint potentials of cytochrome b_6, it appeared important to document the values operating under the experimental conditions utilized in the current work. A titration of cytochrome b_6 in the dark shows an E_{m7} = −30 mV and a redox change corresponding to 2.0 cytochrome b_6/600 Chl (Fig. 6) using the extinction coefficient derived [38] from the cytochrome b_6 preparation of Stuart and Wasserman [39]. The variability in literature values cited above for the b_6 midpoint [26,36,37] is reflected in that of our measurements. The b_6 midpoint is more variable than that of the electrochromic phase reported in the present work. In the last year we have mea-

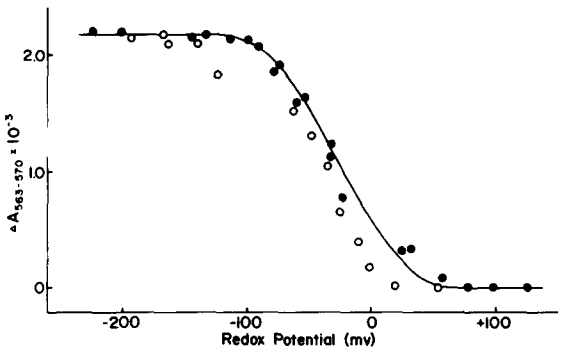

Fig. 6. Redox titration of cytochrome b_6 in the dark. Redox conditions as in Fig. 1, including dihydroxy-benzoquinone, but not indigo-tetrasulfonate in the set of redox mediators. (●) Reductive titration; (○) oxidative titration. The solid curve is fit to the Nernst equation with a one-electron slope and a midpoint ($E_{m,7}$) = −30 mV. Full reduction of the cytochrome corresponds to 2.0 molecules/600 Chl using a reduced-oxidized millimolar extinction coefficient at 563–570 nm of 14 [38,39].

sured $E_{m,7}$ values of b_b ranging between zero and −80 mV. The midpoint, $E_{m,7}$ = −30 mV, of the dark titration shown in Fig. 6 is somewhat more positive than that, $E_{m,7}$ = −85 mV, measured for the decrease in amplitude of the single-turnover flash-induced photoreduction of cytochrome b_6 (Fig. 7). The change in amplitude of b_6 photoreduction at higher potentials titrates with $E_{m,7}$ = +50 mV. This midpoint is lower than expected if the source of electrons for b_6 reduction were the same quinol-like component shown in Fig. 3 that titrated with an $E_{m,7}$ = +100 mV for the onset of the slow phase. The +50 mV component may be an acceptor for cytochrome b_6 which must be reduced to observe an appreciable amplitude of cytochrome b_6 photoreduction.

At an ambient potential of 0 mV, the photoreduction of cytochrome b_6 occurs with a half-time less than approx. 1 ms (Fig. 8A) and corresponds to reduction of 0.3–0.4 molecules of cytochrome b_6/600 Chl (Figs. 8A and 9). It is of interest as well to note that the in situ titrations (Figs. 6 and 7) are fit reasonably well by a single E_m value, unlike the titrations in vitro of isolated cytochrome b_6 [40] or b_6 in the b_6-f complex [40–42],

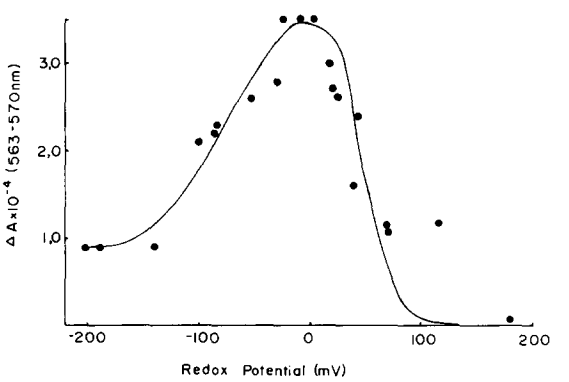

Fig. 7. Flash-induced cytochrome b_6 reduction as a function of redox potential. Cytochrome reduction was measured as the difference between ten traces at 563 nm and ten at 570 nm. Conditions were otherwise the same as in Fig. 1, except that 20 μM each of 2,5-dihydroxy-benzoquinone and indigo-tetrasulfonate were present.

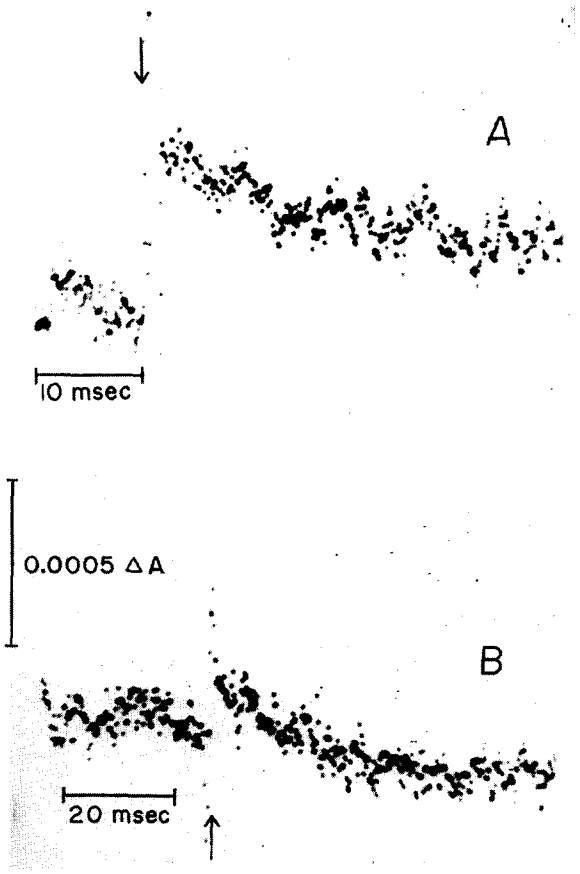

Fig. 8. Cytochrome b_6 turnover at +10 (A) and −190 mV (B) Conditions as in Fig. 3. At +10 mV, 0.4 cytochrome b_t molecules/600 Chl are photoreduced with a $t_{1/2}$ < 1 ms. At −190 mV, 0.1 cytochrome b_6/600 Chl are oxidized with a $t_{1/2}$ of approx. 20 ms after a flash.

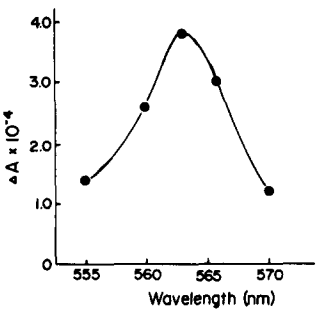

Fig. 9. Spectrum of the transient reductive change observed at +25 mV. The change corresponds to reduction of 0.3 cytochrome b_6/600 Chl.

which are fit best by two widely separated E_m values. Although the amplitude of the slow phase remains at an approximately constant level down to potentials of −200 mV (Fig. 3), the amplitude of the cytochrome b_6 photoreduction decreases in this potential region with its $E_m = -85$ mV to a small amplitude below $E_h = -140$ mV (Fig. 7).

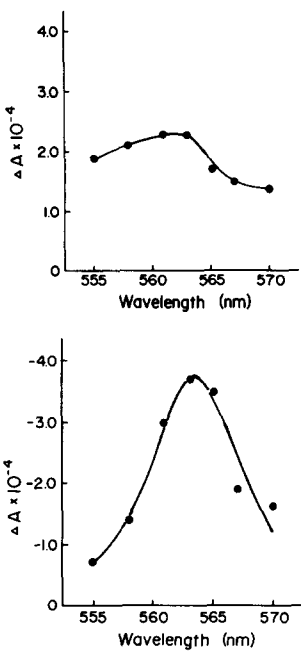

Fig. 10. Light-dark difference spectra of the transient reductive (left) and of the oxidative (right) absorbance changes obtained at −195 mV. The amplitude of the transient reduction corresponds to 0.06 cytochrome b_6/600 Chl and that of the oxidation to 0.2 cytochrome b_6/600 Chl.

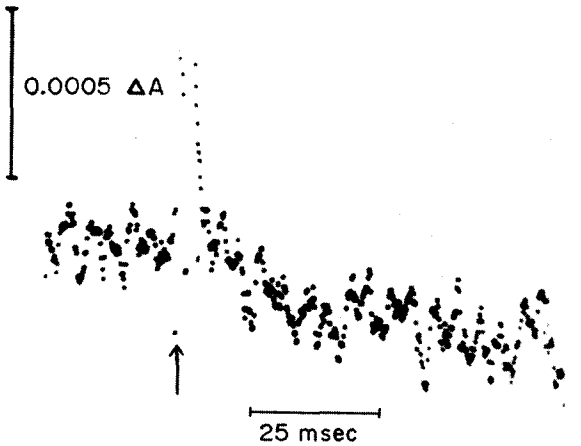

Fig. 11. Cytochrome b_6 turnover at an ambient potential of −195 mV at a flash frequency of 0.017 Hz and an average of five flashes. The amplitude of the oxidative absorbance change corresponds to oxidation of 0.35 cytochrome b_6/600 Chl occurring with a $t_{1/2} = 17$ ms.

The photoreduction at E_h values below −140 mV is transient, generally of smaller amplitude than indicated in Fig. 7, and sometimes not observable. A spectrum of the small transient photoreduction obtained at a potential of −195 mV is shown in Fig. 10A. The recording of the absorbance changes in the potential range below −140 mV is, in fact, dominated by a relatively slow ($t_{1/2} = 15$–20 ms) absorbance change of opposite sign (Fig. 8B obtained at −190 mV) whose spectrum shows that it arises from oxidation of cytochrome b_6 (Fig. 10B). The variability in amplitude of the transient photoreduction seen in Fig. 8B appears to arise from occasional lack of equilibration of the redox dyes with cytochrome b_6 during the 10 s time interval between flashes, resulting in an increasing accumulation with successive flashes of the cytochrome in the oxidized state. A larger amplitude of transient photoreduction can be seen if the time between flashes was decreased to 2 s (data not shown; Ref. 34), but negligible photoreduction is observed when the time interval was increased to 60 s (Fig. 11). The 10 s interval between flashes, corresponding to a 0.1 Hz flash frequency, appears to be just sufficient to allow reduction of the cytochrome after the slow oxidation shown in Fig. 8B that occurs at potentials below −140 mV.

Discussion

The slow electrochromic phase associated with PS I has kinetics approximately equal to those of the rate-limiting step of electron donation to the PS I donors. The amplitude of the slow phase at pH 8 is approximately the same as the fast phase that is attributed to primary charge transfer across the membrane dielectric. These data imply that the slow phase also arises from transmembrane charge transfer, an outwardly directed transfer of an electron, or inward transfer of a proton across the hydrophobic membrane phase. The titration data presented here provide documentation for a plastoquinol-like component ($E_{m,7} = +100$ mV and $\Delta E_m/\Delta pH = -60$ mV being the donor of the electrons, and possibly the protons as well, needed to generate the slow electrochromic phase. The full amplitude of the slow phase of the electrochromic shift persisted at low ambient redox potentials near -200 mV, where titration of cytochrome b_6 in the dark and after a flash indicated it and any higher potential acceptor should be essentially fully reduced. At these potentials the single-turnover flash-induced change of cytochrome b_6, an oxidation with a half-time 4–6 times that of the slow phase (15–20 ms vs. 3–4 ms), is very different from the photoreduction ($t_{1/2} \lesssim 1$ ms) measured at higher potentials, where the quinone pool is initially reduced in the dark and the cytochrome oxidized. The $t_{1/2} = 15$–20 ms photoxidation of cytochrome b_6 at low ambient potentials is too slow to be responsible for the electrogenic step. These data argue that the slow electrogenic step can occur in the absence of cytochrome b_6 turnover. The difficult question raised by the titration data of the slow phase, and of the cytochrome b_6 turnover at lower potentials, is the nature of the pathway and mechanism by which the slow phase is generated. In particular, the question is raised as to whether turnover of cytochrome b_6 is involved in this electrogenic step. The existing set of data on cytochrome b_6 turnover in the context of a Q cycle mechanism is complex: the oxidation of cytochrome b_6 initially poised in a predominantly reduced state (Fig. 8B) is slower than expected, and the reduction of initially oxidized b_6 faster (Fig. 8A above; Refs. 17 and 43) than predicted by simple models for the Q cycle mechanism. In addition, assuming that there are two hemes present in the cytochrome b_6 polypeptide [41], the amplitude of light-induced turnover from an initially fully reduced or fully oxidized redox state is always smaller than expected, even allowing some uncertainty in the extinction coefficient of the cytochrome in situ.

Other mechanisms proposed in the literature for the slow electrochromic phase are that it arises from a localized electric field associated with proton release in a restricted proton-binding domain near the cytochrome b_6-f complex [44], or lateral transfer of a proton from the PS I to the PS II region of the lamellae [45]. The Q loop model for the mechanism of electrogenic H^+ movement has received a large amount of attention and support through studies on the analogous b-c_1 region of the electron-transport chains of mitochondria (e.g., Refs. 12, 46 and 47) and chromatophores [48–50]. It is not possible to critically discuss in this space the data that bear on the Q loop concept in all of these energy-transducing membrane systems. A major point in support of the Q loop model in mitochondria and chromatophores is that the inhibitor antimycin A is thought to block specifically cytochrome b oxidation [49,51]. Antimycin A does not appear to affect the turnover of cytochrome b_6 in chloroplasts (Ref. 51; but see Ref. 52). The inhibitor HQNO appears to have antimycin-like effects in chloroplasts [17,19,34], although it may be less specific here since it inhibits the rate of cytochrome f reduction [17] and accelerates the decay of the 515 nm absorbance change (data not shown).

Redox titrations obtained by other workers bear on the results presented here. Diner and Delosme titrated a 515 nm absorbance change associated with PS I that should have included both the fast and slow components, since the signal was measured at either 3 or 13 ms after the far-red flash. They found that lowering the potential from 0 mV to -200 mV did not cause a decrease in this signal [53]. On the other hand, titration data of Giorgi et al. [32] disagree with ours in several aspects and, in particular, show the slow phase decreasing monotonically in amplitude to zero as the ambient potential is decreased from 0 to -200 mV. Hind et al. [34] have obtained a result for titration of the slow phase similar to ours, with its amplitude

independent of potential over the range 0 to −300 mV. However, the amplitude of b_6 turnover in that work was also found to be large at the negative potentials, leading to a conclusion that the redox dyes did not equilibrate under the conditions of those experiments (flash frequency, 0.5 Hz) and that these results did not contradict a Q cycle mechanism [34]. We have confirmed that equilibration is uncertain at 0.5 Hz flash frequency (data not shown), and have therefore conducted our titrations at flash frequencies of 0.1 and 0.017 Hz.

Since a number of the experiments presented here argue against electron transfer through cytochrome b_6 as the mechanism of generation of the slow phase, an alternative mechanism that would explain the observation of H^+/e^- ratios larger than 1 in the b_6-f region of the chain would be that it arises from H^+ translocation [11,12,54] through a redox-linked H^+ pump. Under the conditions of the present experiments, the redox event triggering operation of the pump would seem, by process of elimination, to be the reduction by plastoquinol of the Rieske high-potential iron-sulfur center oxidized in the flash. A similar role for a redox-controlled H^+ pump involving the high-potential iron-sulfur protein of the b-c_1 complex in mitochondria was proposed by Papa [11,12] in order to explain the fact that the pH dependence of the H^+/e ratio associated with mitochondrial complex III correlated better with the pK of the iron-sulfur protein than with that of the mitochondrial b cytochromes.

It was surprising to find that cytochrome b_6 turnover was apparently not needed for the slow electrogenic phase, since much data exist that are consistent with a Q cycle in chloroplasts. Such data include: (i) the ability of plastosemiquinone to reduce oxidized cytochrome b_6 [55]; (ii) the labeling of cytochrome b_6 by a plastoquinone photoaffinity probe [56]; (iii) the electron donor for the slow electrochromic phase being a quinol-like molecule (Figs. 3 and 4 above); and (iv) the two hemes of the cytochrome b_6 protein spanning the membrane dielectric [57]. An obvious problem with the present work is the reopening of the question of the function of cytochrome b_6.

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