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OXIDATION-REDUCTION PROPERTIES OF THE ELECTRON ACCEPTORS OF PHOTOSYSTEM II

I. REDOX TITRATION OF THE FLASH-INDUCED CAROTENOID BAND SHIFT, OF C550 AND OF THE VARIABLE FLUORESCENCE YIELD IN SPINACH CHLOROPLASTS

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Redox titration of the electrochromic carotenoid band shift, detected at 50 μs after a saturating actinic flash, in spinach chloroplasts, shows that only one electron acceptor in Photosystem II participates in a transmembrane primary electron transfer. This species, the primary quinone acceptor, Q, shows only one midpoint potential ($E_{m,7.5}$) of approx. 0 V and is undoubtedly equivalent to the fluorescence quencher, Q_H. A second titration wave is observed at low potential ($E_{m,7.5} \simeq -240$ mV) and at greater than 3 ms after a saturating actinic flash. This wave has an action spectrum different from that of Photosystem II centers containing Q and could arise from a secondary but not primary electron transfer. A low-potential fluorescence quencher is observed in chloroplasts which largely disappears in a single saturating flash at −185 mV and which does not participate in a transmembrane electron transfer. This low-potential quencher (probably equivalent to fluorescence quencher, Q_L) and Q are altogether different species. Redox titration of C550 shows that if electron acceptor Q_β is indeed characterized by an $E_{m,7}$ of +120 mV, then this acceptor does not give rise to a C550 signal upon reduction and does not participate in a transmembrane electron transfer. This titration also shows that C550 is not associated with Q_L.

Introduction

In recent years several laboratories [1–4] have reported the redox titration of two fluorescence quenchers, Q_H and Q_L, associated with PS II and having midpoint potentials at pH 7 ($E_{m,7}$) of approx. 0 and −250 mV in chloroplasts. Erixon and Butler [5] have reported (detection at 77 K) an $E_{m,7}$ of close to 0 V for C550, an indicator pigment of the redox state of the primary quinone acceptor, Q, of this photosytem. These authors as well as Knaff [6] have observed a similar $E_{m,7}$ for cytochrome *b*-559 photooxidation at 77 K which they attributed to the redox titration of Q. Q_H and Q are very likely the same species in that both are quenchers of fluorescence and show approximately the same midpoint potentials.

Eckert and Renger [7] and Joliot and Joliot [8] have reported the detection of a second primary electron acceptor (X_a and Q_2, respectively) in PS II. This acceptor, shown by the latter authors to be

Abbreviations: C550, an absorbance shift which acts as a linear indicator of Q^-; DCMU, diuron, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Mes, 4-morpholineethanesulfonic acid; P-680, primary electron donor chlorophyll of PS II; PS, photosystem; Chl, chlorophyll.

a quencher of fluorescence, was shown by both groups [7,10] to be a molecule other than a plastoquinone. Its photoreduction does not give rise to a carotenoid band shift, implying that it is located on the same side of the thylakoid membrane as the primary electron donor, P-680. In light of these similar properties, X_a and Q_2 are probably equivalent.

While it is tempting to attribute Q_2 (X_a) to Q_L, some experimental evidence appears to oppose such an association. Reduction of Q in the primary photoreaction of PS II leads to the generation of a transmembrane electric field [11,12]. Malkin [13] has reported a two-wave titration of the carotenoid band shift, a linear indicator of the field [14] with the same E_m as Q_H and Q_L. This titration could be interpreted to mean that Q_L is involved in a transmembrane electron transfer. As the photoreduction of Q_2 (X_a) appears not to involve generation of a transmembrane electric field [7.9], it would seem that Q_L cannot be equivalent to Q_2 (X_a).

Both Golbeck and Kok [4] and Thielen and Van Gorkom [15] have argued that Q_H could correspond to the reduction of half of the Q via the doubly reduced secondary acceptor, B^{2-} ($E_{m,7}(B/B^{2-}) \simeq 0$ V) according to the equation $Q^-B^- \rightleftharpoons QB^{2-}$ with equilibrium constant, $K = 1$, considerably lower than that reported by Diner [16]. They further suggested that Q_L would correspond to the redox couple QB^{2-} (Q^-B^-)/Q^-B^{2-}, i.e., the reduction of the second half of the Q. This model is, however, unlikely based on the experimental evidence of this and the accompanying paper [17] as well as on purely thermodynamic considerations. A low equilibrium constant ($K = 1$) for $Q^-B^- \rightleftarrows QB^{2-}$ implies that redox couples Q/Q^- and B^-/B^{2-} have the same E_m (−300 mV according to Ref. 15). As $QB^-/Q^-B \approx 20$ [16], the E_m of B/B^- is also close to that of Q/Q^- ($E_m(B/B^-) - E_m(Q/Q^-) \simeq 80$ mV). Thermodynamics requires that $E_m(B/B^{2-})$ also be approx. −300 mV which is inconsistent with an $E_{m,7}$ of +30 to +100 mV reported for this redox couple [4,15].

In this paper redox titrations of the light-induced transmembrane electric field arising from the primary charge separation of PS II in spinach chloroplasts show that Q_H and Q_L arise from different species altogether. These data and those of the following paper strengthen the assignment of Q_H alone to Q. These data also show that Q_L and Q_2 (X_a) share several properties in common and are probably the same species.

Materials and Methods

Osmotically shocked chloroplasts were prepared by grinding in a blendor 50 g of spinach leaves in 200 ml of 0.02 M Tricine-KOH, 0.4 M sorbitol and 0.01 M ascorbate. The chloroplasts were filtered through eight layers of cheesecloth and collected by centrifugation at $1000 \times g$ for 5 min. The chloroplasts were resuspended in the same solution without ascorbate at 1–2 mg Chl/ml and then diluted 10–20-fold with degassed distilled water (final concentration approx. 80 μg Chl/ml). After 5 min this suspension was diluted 2-fold with either degassed 0.04 M Tricine-KOH, pH 7.4, 0.66 M sorbitol or 0.1 M Hepes-KOH, pH 7.5, 0.66 M sorbitol, 4 mM EDTA and 10 mM $MgCl_2$.

Freeze-thawed chloroplasts were prepared from market spinach according to the method of Avron [18] and stored at −70°C in 0.05 M Tris-HCl, pH 7.5, 0.4 M sucrose, 0.01 M NaCl and 5% dimethyl sulfoxide at a chlorophyll concentration of 1–2 mg/ml. The chloroplasts were thawed just before use and suspended in degassed 0.05 M Hepes-KOH, pH 7.5, 0.33 M sorbitol with or without 2 mM EDTA and 5 mM $MgCl_2$. Osmotically shocked and freeze-thawed chloroplasts were used to make the electron acceptors more accessible to the redox mediators.

Redox titrations were performed using a flash-detection spectrophotometer as described by Joliot et al. [19]. The xenon actinic flashes were normally filtered by a broad-band red Schott RG2 filter. These flashes were saturating for both photosystems and lasted for 1 μs at half height. In one case (Fig. 2c), nonsaturating far-red actinic flashes (filtered by a Schott RG2 plus a Wratten 97 filter, $\lambda > 705$ nm) were used to excite selectively PS I.

A reservoir containing 40 ml of suspended chloroplasts was connected to the spectrophotomer measuring cuvette such that when a measurement was not being made both the reservoir and the cuvette were flushed with high-purity argon gas (less than 5 ppm O_2) passed through two scrubbers;

the first containing 250 ml of 50 mM $KCr(SO_4)_2$ reduced to Cr(II) with 0.1 M H_2SO_4 and 2 g powdered Zn and the second containing 250 ml of water. By drawing on a syringe located downstream, a sample was loaded into the cuvette under anaerobic conditions through a long needle immersed into the chloroplast suspension in the reservoir. This suspension was stirred by a magnetic stirring bar in the reservoir and flushed with argon gas at all times. The potential in the reservoir was monitored by platinum and calomel electrodes (Radiometer models P101 and K401, respectively) connected to a Radiometer pH 4 pH meter. The electrodes were calibrated with respect to quinhydrone at pH 7.5. After a spectroscopic measurement, the flashed sample (approx. 10% of the reservoir volume) was reinjected into the reservoir. Filling and emptying the cuvette did not usually displace the potential in the reservoir except at very low potentials in regions of poor redox buffering where we occasionally noted displacements of up to several millivolts in a positive direction. The potential was allowed to stabilize for 10–15 min before a measurement was made during reductive titrations and for approx. 20 min during oxidative titrations. Measurements at +200 to +300 mV were usually made before and after a titration to verify the activity of the chloroplasts. The reductive and oxidative titrants were degassed solutions of 40 mM $Na_2S_2O_4$ in 0.1 M Tris-HCl, pH 9.0, and 0.1 M $K_3Fe(CN)_6$, respectively. All redox potentials are reported relative to the standard hydrogen electrode.

The flash-detection spectrophotometer was also used for measurements of the variable fluorescence yield. In this case, the measuring photodiode was replaced by a photomultiplier (EMI 9558 QB). Continuous exciting light was filtered by a Corning 4-96 blue filter. Complementary filters mounted on the photomultiplier were a Schott KV550, two Ulano Rubylith, and two Kodak Wratten 70 ($\lambda >$ 670 nm). Fluorescence induction curves were stored using a Nascom computer (Model II).

Two groups of redox mediators were used, designated 'mediators A and B'. The first number in parentheses indicates the concentrations of that mediator used for group A, the second for group B. The third number is the $E_{m,7}$ of the mediator: 1,2-Naphthoquinone (20 μM, 10 μM, +143 mV), phenazine methosulfate (5 μM, 1.3 μM, +80 mV), 1,4-naphthoquinone (20 μM, 10 μM, +36 mV), duroquinone (20 μM, not used, +5 mV), pyocyanin (5 μM, not used, −34 mV), 2,5-dihydroxybenzoquinone (not used, 10 μM, −60 mV), 2-hydroxy-1,4-naphthoquinone (20 μM, 10 μM, −139 mV), and anthraquinone-2-sulfonate (20 μM, 10 μM, −250 mV).

All absorbance change measurements are expressed in terms of $\Delta I/I$ for a 16 mm light path. All measurements were made at ambient temperature, approx. 22°C.

Results

Redox titration of the light-induced electrochromic carotenoid band shift

Witt and Zickler [14] have shown that the electrochromic carotenoid band shift [20,21] which may be detected at 520 nm is a linear indicator of the light-induced transmembrane electric field. We consequently used the $\Delta I/I$(520 nm) as a linear indicator of the number of PS II centers undergoing flash-induced transmembrane charge separation as a function of redox potential. In order to be sure to titrate the primary electron acceptor participating in this charge separation, we detected the flash-induced $\Delta I/I$(520 nm) at 50 μs following an actinic flash, an interval considerably shorter than the reoxidation time of Q^- ($t_{1/2} \approx 500$ μs) [22,23]. Fig. 1a and b shows the amplitude of the saturating flash-induced $\Delta I/I$(520 nm) as a function of redox potential. Detection at 50 μs indicates a single reversible wave closely approximating a Nernst theoretical curve (dashed lines) with $n = 1$ and a midpoint potential of approx. 0 V (−20 mV for the reductive titration, 0 V for the oxidative titration).

Detection at 10 ms in the same experiment shows two waves at approx. 0 and −240 mV. For this time interval, but not at 50 μs, the curve is distorted by a gradually increasing rate of relaxation of the field because of increased membrane permeability with time. This increased permeability gives rise to a slight positive slope in the reductive titration (Fig. 1a and b) and a slight negative slope in the oxidative titration (Fig. 1c). Fig. 1c shows that the more negative titration wave is also reversible. This observation of two waves at

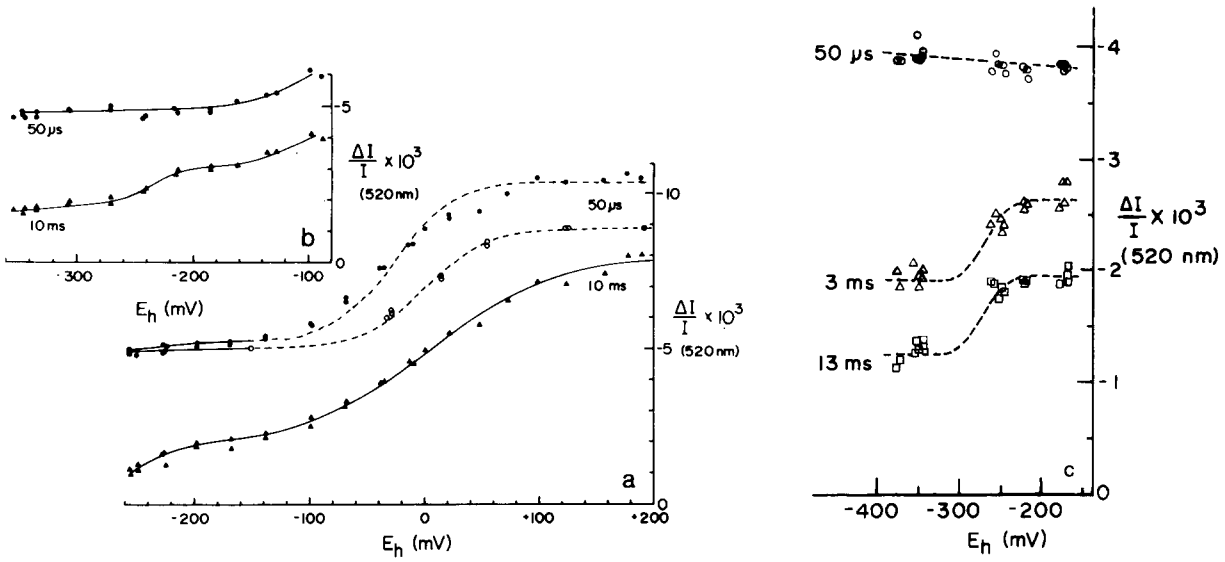

Fig. 1. Redox titration in spinach chloroplasts of the flash-induced transmembrane electric field ($\Delta I/I$(520 nm)) detected at the indicated times after a saturating 1 μs flash. Freeze-thawed chloroplasts (a and b) suspended in 50 mM Hepes, pH 7.5, 0.33 M sorbitol at 40 μg Chl/ml. Osmotically shocked chloroplasts (c) suspended in 20 mM Tricine, pH 7.4, with 0.33 M sorbitol. Reductive titration, filled symbols; oxidative titration, open symbols. Group A redox mediators used.

0 and −240 mV for detection times greater than 3 ms is similar to that observed by Malkin [13] for a detection time probably greater than 1 ms (Malkin, R. personal communication). There is absolutely no indication of a low-potential wave (approx. −240 mV) upon detection at 50 μs. The signals detected at 50 μs, 3 and 13 ms were measured between 500 and 535 nm and gave spectra characteristic of the electrochemic carotenoid band shift [20] (not shown). As Q is implicated in transmembrane electron transfer [11,12] and as neither pheophytin [24] nor any PS I primary components titrate in this region, the 0 V wave must arise from Q.

In order to determine the origin of the low-potential wave detected at greater than 3 ms we performed reductive titrations under a variety of conditions (Fig. 2). These were performed in the presence of Mg^{2+} which stabilizes somewhat the membrane conductivity during the titration. The addition of the ionophore gramicidin D at 10^{-6} M in the presence of 25 mM K^+ (50 mM Hepes-KOH, pH 7.5) (Fig. 2b) greatly diminishes the amplitude of $\Delta I/I$(520 nm) at greater than 1 ms and abolishes the −240 mV wave. This observation confirms that the −240 mV wave arises from a titration of the transmembrane electric field and not from another light-induced absorbance change superimposed on the carotenoid band shift.

The flash-induced $\Delta I/I$(520 nm) was also titrated using far-red flashes exciting approx. 40% of the PS I reaction centers (Fig. 2c). Practically no PS II centers (less than 4%) were excited by these flashes as evidenced by the fact that the 0 V wave represents less than 10% of the $\Delta I/I$(520 nm) detected at 50 μs (cf. Fig. 1a). The low-potential wave, however, is still plainly visible despite the absence of PS II excitation. In fact, the ratio of the amplitude of the low-potential wave at 3 ms to that of the $\Delta I/I$(520 nm) detected at 50 μs is the same in Fig. 2c for far-red flashes as in Fig. 2a for saturating flashes which excite both photosystems. As at potentials below −150 mV the $\Delta I/I$(520 nm) detected at 50 μs arises from PS I alone in both titrations, this constant ratio implies that PS II makes no contribution to the low-potential wave of Fig. 2a. In other words, this experiment implies that the low-potential wave detected at greater than 3 ms probably arises from excitation of PS I alone.

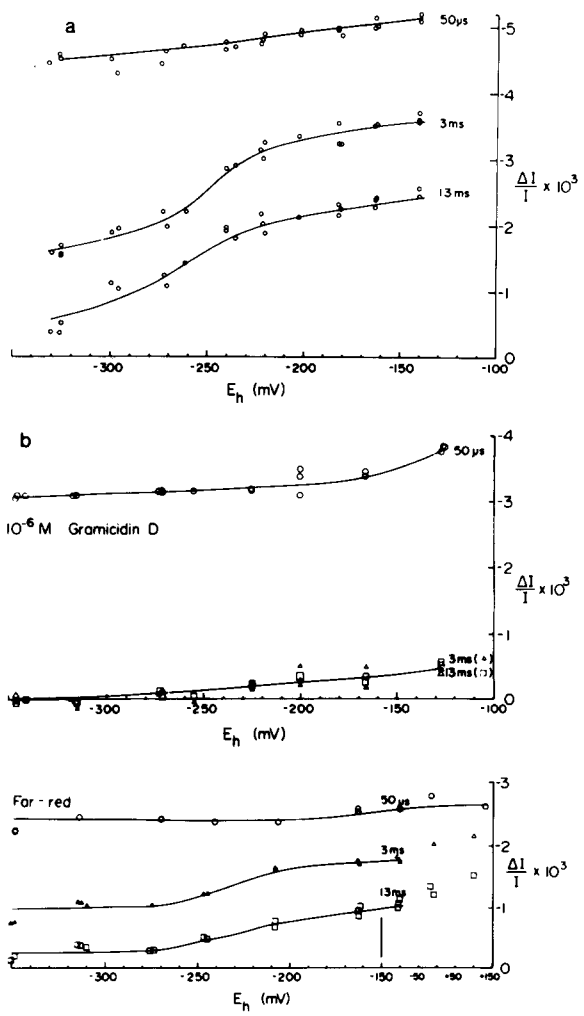

Fig. 2. (a) Low-potential redox titration in osmotically shocked spinach chloroplasts of flash-induced transmembrane electric field detected at the indicated times after a 1 μs flash saturating both photosystems. Chloroplasts suspended in 50 mM Hepes-KOH, pH 7.5, 0.33 M sorbitol, 2 mM EDTA and 5 mM $MgCl_2$ at 40 μg Chl/ml. (b) Same as a plus 10^{-6} M gramicidin D. (c) Same as a except that the saturating actinic flashes were replaced by far-red actinic flashes exciting 40% of PS I centers and less than 4% of the PS II centers. All of the titrations are reductive. Group A redox mediators used.

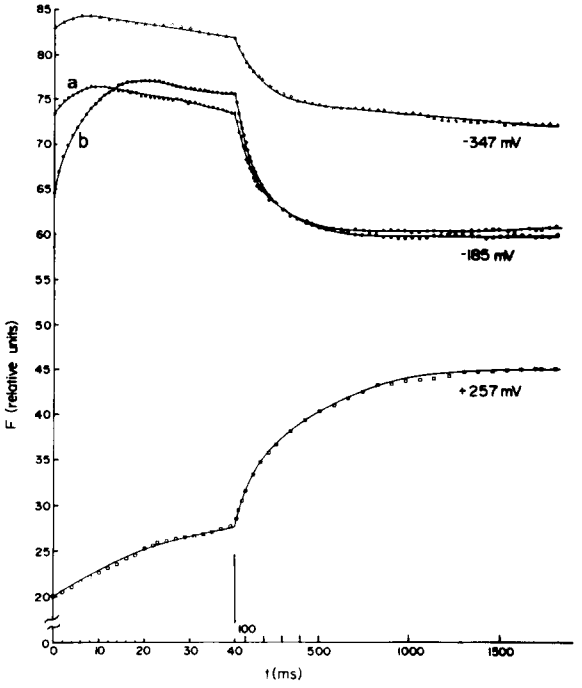

Fig. 3. Variation of the fluorescence yield with time in continuous light for freeze-thawed spinach chloroplasts at the indicated potentials. Chloroplasts suspended in 50 mM Hepes-KOH, pH 7.5, 0.33 M sorbitol, 2 mM EDTA, 5 mM $MgCl_2$ at 30 μg Chl/ml. All samples were dark adapted except for that at −185 mV (curve a) where the continuous light was preceded, 200 ms before, by a 1 μs saturating flash. The equilibrium time at each potential was approx. 20 min. Group B redox mediators used.

Dependence of the variable fluorescence yield on redox potential

The variation of the fluorescence yield with time in continuous light (fluorescence induction) was examined at various redox potentials (Fig. 3) to be sure that under our experimental conditions there were indeed two observable fluorescence quenchers. Broad-band blue light (approx. 1 photon/reaction center per 14 ms) induced a slow reduction of the plastoquinone pool and of Q at +257 mV. At −185 mV where Q is fully reduced there is a variable fluorescence yield (curve b) which is largely eliminated by preillumination with a saturating flash given 200 ms before continuous illumination (curve a). At −347 mV there is practically no variable fluorescence yield. The maxium fluorescence yields increase with decreasing potential. This phenomenon is in large part due to the chemical reduction of redox mediators, most of which are fluorescence quenchers in their oxidized states.

These results show that lowering the potential from −185 to −347 mV eliminates a fluorescence quencher which also disappears upon illumination

TABLE I

FLASH INDUCED $\Delta I/I$(520 nm) IN FREEZE-THAWED SPINACH CHLOROPLASTS DETECTED 50 μs AFTER EACH OF TWO SATURATING FLASHES GIVEN 200 MS APART AT −185 AND −350 mV

Same conditions as Fig. 3 except at 36 μg Chl/ml.

E_h (mV)	$\Delta I/I$(520 nm)	($\times 10^3$)
−185	−5.24	1st flash
	−5.01	2nd flash
−350	−5.12	1st flash
	−4.65	2nd flash

at −185 mV. As between these potentials (Figs. 1 and 2) no difference is observed in the flash-induced transmembrane electric field detected at 50 μs, we conclude that the low-potential quencher (probably Q_L) is not involved in a transmembrane electron transfer. This conclusion is further confirmed by detecting the $\Delta I/I$(520 nm) at 50 μs after each of two saturating flashes given 200 ms apart at −185 mV. This experiment was performed under the same conditions as in Fig. 3 including 20 min of incubation at −185 mV. The amplitude of the two spectroscopic signals is practically the same (Table I) despite the observation that at this potential the fluorescence quencher is largely eliminated by a single saturating flash (Fig. 3). The flash-induced disappearance of the quencher is thus not linked to a transmembrane charge separation.

At −185 and −347 mV a fluorescence quenching reappears at long times of continuous illumination. This quenching appears with too great a quantum yield to be due to the photoreduction of pheophytin. A more likely explanation is the photoreduction of a mediator to produce a quenching species.

Redox titration of C550

Erixon and Butler [5] have shown that signal C550 is a linear indicator of [Q^-]. The redox titration of the light-induced formation of C550 compared to that of the carotenoid band shift should indicate whether or not there are centers that give rise to a C550 signal which do not participate in a transmembrane electron transfer. This redox titration was performed by taking the difference $\Delta I/I$(550 nm) $-\Delta I/I$(540 nm) detected 50 μs after a saturating actinic flash (Fig. 4). This differences also includes a contribution of the electrochromic effect, as shown by the signal remaining below −150 mV which arises from the charge separation in PS I. A separate spectroscopy experiment (not shown) was performed in the presence of the same medium as in Fig. 4 plus $1.5 \cdot 10^{-5}$ M DCMU, 10^{-6} M gramidicin D and 3 mM $K_3Fe(CN)_6$ to determine the $\Delta I/I$(550 nm) $-\Delta I/I$(540 nm) attributable to C550 alone. C550 was detected at 10 ms after each of a series of five saturating actinic flashes at 8.3 Hz. This flash regime was used to fully reduce Q. As a result of this calibration we attribute 60% of the wave of Fig. 4 to C550 and 40% to the electrochromic effect arising from charge separation in PS II. This latter contribution is of the same amplitude as that arising from charge separation in PS I (at below −150 mV) in agreement with Fig. 1. In a separate experiment we verified that the relative contributions of PS I and PS II to the carotenoid band shift were independent of the presence or absence of 2 mM EDTA, 5 mM $MgCl_2$. The data of Fig. 4 show as good a fit to an $n = 1$ Nernst curve as

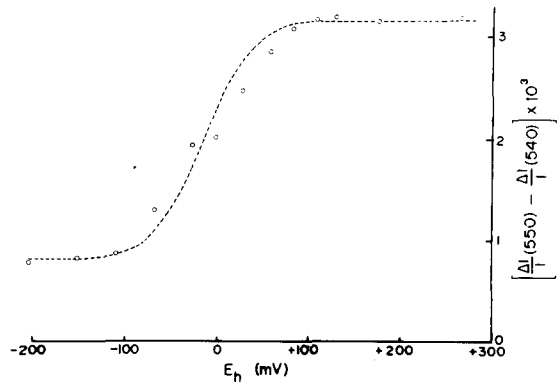

Fig. 4. Redox titration in freeze-thawed spinach chloroplasts of the difference in the flash-induced absorbance changes at 550 and 540 nm, detected 50 μs after a saturating 1 μs flash. Chloroplasts suspended in 50 mM Hepes-KOH, pH 7.5, 0.33 M sorbitol, 2 mM EDTA and 5 mM $MgCl_2$ at 40 μg Chl/ml. Group A redox mediators used except for anthraquinone-2-sulfonate. 60% of the titration wave is contributed by C550, 40% by the electrochromic carotenoid band shift. The dashed line corresponds to an $n = 1$ theoretical Nernst curve with an E_m of −15 mV.

those of Fig. 1, indicating that C550 and the light-induced transmembrane electric field titrate together with the same midpoint potential.

Discussion

The origin of Q_H and Q_L

The flash-induced transmembrane electric field arising from PS II and detected at 50 μs titrates as a single wave ($E_{m,7.5} \simeq 0$ V, $n = 1$) as also observed for Q_H [1–4] and for C550 detected at 22°C (Fig. 4) and at 77 K [5]. As Q and P-680 are know to be located on opposite sides of the thylakoid membrane and as C550 is a linear indicator of Q^- [5], these observations identify the 0 V wave of the $\Delta I/I$(520 nm) with Q/Q^-. As Q_H and Q are both quenchers of fluorescence and have the same midpoint potential, they are almost certainly the same species. Furthermore, as all of C550 titrates at 0 V (Fig. 4) and as only a single wave is observed in the titration of $\Delta I/I$(520 nm) (detection at 50 μs, Fig. 1) we conclude that Q titrates with only one midpoint potential.

A fluorescence quencher which is present and presumably oxidized at -185 mV, and absent and presumably reduced at -347 mV, disappears upon illumination at -185 mV in an efficient photoreaction (Fig. 3). This quencher is observed under conditions where Q is already reduced. The photoreduction that we observe at -185 mV does not give rise to a carotenoid band shift detected at 50 μs, indicating that the electron responsible for this reduction does not cross the photosynthetic membrane. As all the PS I-bound Fe-S electron acceptors are known to be at considerably lower potential (below -500 mv) [26] it is most likely that this quencher is associated with PS II. Numerous authors have described a fluorescence quencher, Q_L, having an $E_{m,7}$ of -250 mV. This species is probably the low-potential quencher that we observe. However, the fluorescence quenching observed at -185 mV gradually decreases with time at this potential, suggesting that the apparent potential-dependent behavior of this low potential quencher may also contain a kinetic component. We conclude that as no low-potential wave is observed for the light-induced $\Delta I/I$(520 nm) detected at 50 μs (Fig. 1) and as all of C550 titrates at 0 V (Fig. 4), Q_L cannot be a redox component of Q/Q^- as has been previously suggested [4,15]. Furthermore, the redox couple Q_L/Q_L^- cannot be attributed to Q^-/Q^{2-} as photoreduction of Q^- would also give rise to a transmembrane electric field.

The PS II electron acceptor Q_2 [8] or X_a [7] which is not a plastoquinone is also a fluorescence quencher [8] which is photoreduced in a reaction that does not give rise to a transmembrane electric field [9]. These similar properties would suggest that the low-potential quenchers Q_L and Q_2 (X_a) are proabably the same species.

The low-potential wave (E_m -240 mV) for the flash-induced $\Delta I/I$(520 nm) detected at greater than 3 ms is probably the same wave as that observed by Malkin [13]. While the time resolution was not specified in Malkin's paper, our results can be reconciled with his as his measurements were apparently made on the millisecond time scale (Malkin, R., personal communication). That this component is observed at greater than 3 ms and not at 50 μs indicates that it cannot arise from a primary electrogenic photoreaction. We find that the low-potential wave is fully apparent upon excitation with far-red flashes (Fig. 2c) which excite 40% of PS I centers and less than 4% of the PS II centers (note the absence of the 0 V wave). It is most likely that the low-potential wave arises either from an electrogenic secondary electron transfer associated with PS I of from membrane conductivity modulated by the redox potential. This conclusion disagrees with that of Malkin who attributed the low-potential wave to PS II based on its apparent sensitivity to DCMU. Our data indicate that Q_L and the low-potential wave of the flash-induced $\Delta I/I$ are not directly related.

A heterogeneity has been reported in the fluorescence behavior of PS II in chloroplasts and attributed to what have become known as α and β reaction centers. Both are reported to contain plastoquinone as electron acceptor (Q_α and Q_β) and to show absorbance changes associated with C550. They differ in that β-centers have a 3-fold smaller [26], red-shifted [30] antenna. Q_α is reported to have two redox components, one at approx. 0 V (Q_H) and the other at -250 mV (Q_L) [15,31]. A single E_m is attributed to Q_β at $+120$ mV [15,31].

The chloroplasts used in these experiments show

a slow phase in the fluorescence induction curves in the presence of 1 and 20 μM DCMU at potentials above +120 mV. This slow phase (not shown), which is characteristic of Q_β, decreases at potentials close to those reported for the E_m of Q_β (approx. +120 mV), suggesting that the α, β type of heterogeneity is also present in our chloroplasts.

As a far-red action spectrum has been reported [30] for the excitation of β-centers, we cannot completely exclude these centers as being responsible for the low-potential wave of the light-induced $\Delta I/I$(520 nm). Such a hypothesis is, however, contrary to the redox potential reported for Q_β/Q_β^-.

C550, $\Delta I/I$(520 nm) and Q_β

Melis and Schreiber [29] have argued in favor of associating C550 with Q_β based on a parallel evolution of the C550 signal and the area bound by the variable and maximum fluorescence yields during fluorescence induction of chloroplasts in the presence of DCMU. A consequence of this association would be that C550 should show a midpoint potential at +120 mV (the $E_{m,7}$ of Q_β/Q_β^-) as well as at 0 V. The titrations of C550 in spinach chloroplasts and membrane fragments have been, however, ambiguous on this point with Erixon and Butler [5] and Knaff [32] reporting an $E_{m,7}$ of approx. 0 V at 77 K and +75 mV at ambient temperature, respectively.

We observe only a 0 V wave and no sign of a +120 mV wave (Fig. 4) for the titration of C550 in chloroplasts in which Q_β is apparently present. We conclude that if indeed Q_β is characterized by an $E_{m,7}$ of +120 mV, then a C550 signal cannot be associated with its reduction. By the same reasoning, the absence of a titration wave at 120 mV in Fig. 1 would also indicate that Q_β does not participate in a transmembrane electron transfer.

Melis and Duysens [28] have reported that in spinach chloroplasts inhibited by 15 μM DCMU only 70% of Q_β is reduced in a saturating flash that reduces 98% of Q_α. Joliot and Joliot [33] have shown that in the same material, in the presence of 10 μM DCMU, C550 is formed completely during the first of a series of saturating flashes. The maximum fluorescence yield was attained only after several flashes. These results would suggest that C550 is not an indicator of Q_β.

There are two ways to reconcile our results and those of Joliot and Joliot [33] with those of Melis and Schreiber [29]. Either (a) the E_m attributed to Q_β is correct in which case we must conclude that C550 is not an indicator of Q_β. In this case we would also have to conclude that the comparison of the slow phase of C550 and fluorescence induction [29] is subject to considerable uncertainty, particularly as concerns the position of the asymptote corresponding to the maximum fluorescence yield; or (b) the primary acceptor Q_β does not exist and the E_m attributed to Q_β/Q_β^- arises from some other source (e.g., titration of a secondary acceptor, the redox state of which modifies DCMU binding [34]).

Acknowledgements

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