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TWO ELECTROGENIC MECHANISMS CONTRIBUTING TO THE 560 nm ABSORPTION CHANGES IN INTACT *BRYOPSIS* CHLOROPLASTS

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Summary

Light -induced absorbance changes at 560 nm in dark-adapted intact chloroplasts of the green alga, *Bryopsis maxima* were studied in the time range of 200 ms. The initial rise of the 560 nm signals constists of two major components which are both electrochromic absorbance changes of the carotenoids, sipnonein and/or siphonaxanthin, but different in mechanisms of the field formation.

The first component (component S) is related to electron transport since it was sensitive to 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB) and showed a light-intensity dependence similar to that of electron transport in chloroplasts. In the presence of DCMU, component S could be restored on addition of proton-transporting electron donors such as reduced 2,6-dichlorophenol indophenol and phenazine methosulfate, but not on addition of *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine which does not carry protons with electrons (Trebst, A. (1974) Annu. Rev. Plant Physiol. 25, 423—458). We propose that component S is due to the electric field set up by the proton translocation across the thylakoid membrane.

The second component (component R) was resistant to DCMU and DBMIB. The light-intensity dependency of component R was similar to that of cytochrome *f* photooxidation which showed saturation at a relatively low light intensity. The magnitude of component R was markedly reduced by phenylmercuric acetate, suggesting the participation of ferredoxin and ferredoxin-NADP oxidoreductase in the mechanism of the field formation responsible for this component. In the presence of DCMU and phenylmercuric acetate, time

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCIP, 2,6-dichlorophenol indophenol; TMPD, *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine; Hepes, *N*-2-hydroxyethylpiperazine-*N′*-2-ethanesulfonic acid.

courses of the 560 nm changes paralleled those of cytochrome *f* changes. These results indicate that component R is due to the electric field formed between oxidized cytochrome *f* and other intersystem electron carriers located in the inner part of the thylakoid membrane and reduced electron acceptors of Photosystem I situated on the membrane surface.

The complex natures of the 560 nm changes, as well as the contributions of Photosystems I and II to the absorbance changes, are explained in terms of the two electrogenic mechanisms.

Introduction

Light-induced absorbance changes in the wavelength region from 515 to 525 nm have been observed in various higher plants and algae [1—18] and are currently interpreted to be the electrochromic absorbance changes of photosynthetic pigments responding to the electric field formed across the thylakoid membrane [9,16,18]. Large light-induced absorbance changes centering around 560 nm, which have been found by Nakayama et al. [14] in a green alga, *Bryopsis maxima*, and subsequently shown by Kunifuji et al. [19] to occur in a number of green algae, are also absorption changes indicating the electric field generated across the thylakoid membrane [14,20]. The pigment responsible for the 560 nm changes is either or both of siphonaxanthin and siphonein, carotenoids which exhibit in situ absorption bands between 520 and 560 nm [19].

For the study of the electrochromic absorbance changes, *B. maxima* offers unique advantages that the magnitude of the 560 nm changes is several-fold larger than that of the 515 nm changes [14] and that dark-adapted intact chloroplasts isolated from the alga exhibit two induction phenomena of which transient features could be well correlated to the complex time course of the 560 nm changes. *Bryopsis* chloroplasts were the first cell-free system in which all the transient features of the chlorophyll fluorescence induction were demonstrated [21]. Satoh et al. [22] also found that illumination of dark-adapted chloroplasts induced sequential changes in the redox state of cytochrome *f*. Evidence indicates that the early transients in the fluorescence yield and redox changes of cytochrome *f* are clearly interrelated with each other; they are different manifestations of light-regulated changes in electron transport at the reducing side of Photosystem I [22]. More recently, we have shown that time course of the 560 nm changes also paralleled that of cytochrome *f* at least for the first 2 s of illumination [20]. Inhibitors and redox reagents which selectively affected different transient features of cytochrome *f* changes photooxidation also specifically influenced corresponding transient features of the 560 nm changes. We proposed, therefore, that the carotenoids would respond to the electric field between oxidized cytochrome *f* and other intersystem electron carriers located in the inner part of the thylakoid membrane and reduced electron acceptors situated on the outer membrane surface [20].

It was noted, however, that the 560 nm changes were only partly variable and any treatments which kept cytochrome *f* in the fully reduced state failed to eliminate the 560 nm changes completely [20]. These observations could not be explained by the above mechanism alone. In the present work, therefore,

we have studied whether or not another mechanism of the field formation contributes to the early transients of the 560 nm changes. The absorbance changes at 560 nm in dark-adapted *Bryopsis* chloroplasts were studied in a short time range where the light-scattering changes of chloroplasts were negligible. The initial rise of the 560 nm changes was found to consist of two components different in their mechanisms of the field formation. The results indicate that, in addition to the electric field formed between oxidized electron donors and reduced acceptors of Photosystem I, the field set up by proton translocation from the stroma to the intrathylakoid space significantly contributes to the absorbance changes. The characteristic transient time course and other complex features of the 560 nm changes, as well as the contributions of Photosystems I and II to the absorbance changes, were explained in terms of the two electrogenic mechanisms.

Materials and Methods

Preparation of intact chloroplasts from the marine green alga, *B. maxima*, was described previously [21]. Chloroplasts were kept in the dark for 2 h or more before use.

Light-induced absorbance changes were measured with a single-beam spectrophotometer as described previously [22]. The time resolution of the apparatus was 0.2 ms. Samples were illuminated by a red light ($3.2 \cdot 10^5$ ergs $\cdot$ cm^{-2} $\cdot$ s^{-1}) obtained by passing the light beam from a 650 W Ushio halogen lamp through a Toshiba cut-off filter VR-65 and a water layer of 10 cm thickness. The photomultiplier was protected against the actinic light with a Corning band-pass filter 4-96. All measurements were carried out at room temperature. The basal reaction mixture contained, in a final volume of 2 ml, 1.0 M sorbitol, 2 mM EDTA, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM $NaNO_3$, 50 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonate (Hepes) (pH 7.5) and intact *Bryopsis* chloroplasts containing indicated amounts of chlorophyll. Additions are indicated in the figure legends.

Methyl viologen photoreduction was determined with a Clark-type oxygen electrode at 25°C. The illumination system and the reaction mixture were the same as those employed in the spectrophotometric experiments, except that 2 mM methyl viologen and 0.5 mM KCN were included in the reaction mixture.

DBMIB was kindly provided by Dr. A. Trebst.

Results

Fig. 1 shows time courses of light-induced absorbance changes at 560 nm in dark-adapted intact *Bryopsis* chloroplasts determined in a time range of 200 ms. On turning the continuous light on, the absorbance at 560 nm increased rapidly and reached the maximum E_D at about 50 ms after the onset of illumination, then followed by a gradual decrease to the minimum E_P (see ref. 20 for the nomenclature of the transient features of the 560 nm change). In a previous paper, we have shown that the decline from E_D to E_P was suppressed in the presence of 0.15 μM DCMU accompanied by an inhibition of the transient reduction of cytochrome *f* [20]. In the present work in which a faster record-

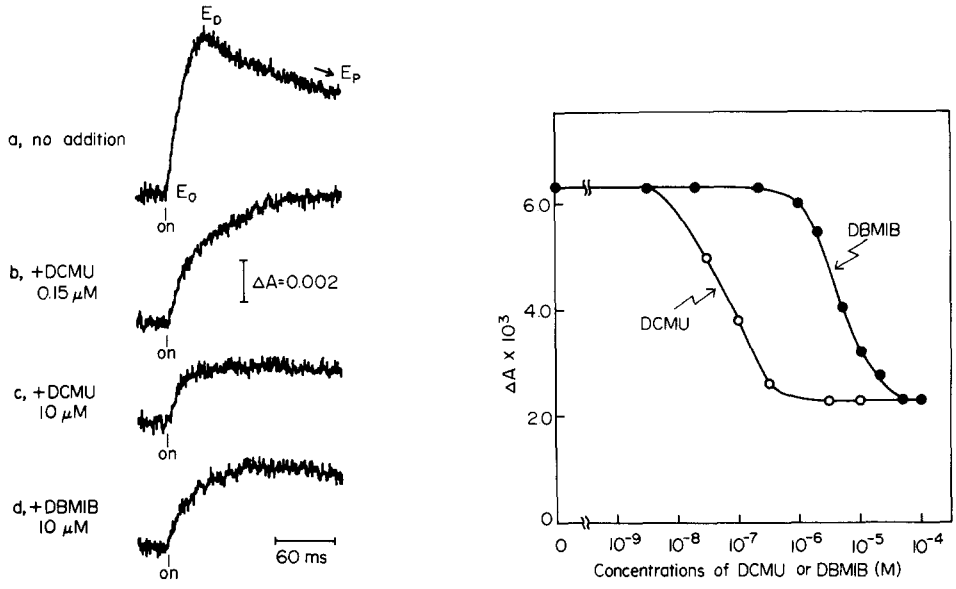

Fig. 1. Effects of DCMU and DBMIB on the initial rises of absorbance changes at 560 nm. The reaction mixture contained, in a final volume of 2 ml, 1.0 M sorbitol, 2 mM EDTA, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM $NaNO_3$, 50 mM Hepes (pH 7.5) and dark-adapted intact *Bryopsis* chloroplasts equivalent to 42.4 μg chlorophyll/ml. The sample was illuminated with red light (longer than 650 nm, $3.2 \cdot 10^5$ ergs $\cdot$ $cm^{-2} \cdot s^{-1}$).

Fig. 2. Magnitudes of the light-induced absorbance changes at 560 nm in the presence of various concentrations of DCMU and DBMIB. The reaction conditions were the same as in Fig. 1 except that the concentrations of DCMU and DBMIB were varied. Chlorophyll concentration, 32.1 μg/ml.

ing time was used, the E_O-E_D rise was also found to be affected by 0.15 μM DCMU so that the rise kinetics was separated into a fast and a slow component (trace b). The slow component was completely eliminated in the presence of 10 μM DCMU (trace c). Nakayama et al. [14] have previously reported that the initial spike of the 560 nm changes was markedly reduced by high concentrations of DCMU. Another electron transport inhibitor, DBMIB [23], also diminished the magnitude of the E_O-E_D rise.

Fig. 2 summarizes the effects of different concentrations of DCMU and DBMIB on magnitudes of the 560 nm signals. About 60% of the E_O-E_D rise was sensitive to the inhibitors and disappeared in the presence of 2 μM DCMU or 20 μM DBMIB, whereas the remaining part of the absorbance changes was highly resistant to the inhibitors. For the convenience, we shall designate a part of the E_O-E_D rise that is resistant to DCMU and DBMIB as component R and another part that is sensitive to the inhibitors as component S, respectively.

Difference spectra of components R and S determined between 490 and 580 nm are depicted in Fig. 3. In this wavelength region, a prominent peak at 560 nm and a minor peak at 515 nm were observed in the absence of DCMU (open circles, see also ref. 20). Addition of DMCU affected magnitudes of the absorbance increases to a similar extent at all wavelength tested. Consequently, the difference spectrum of component R, which was determined in the

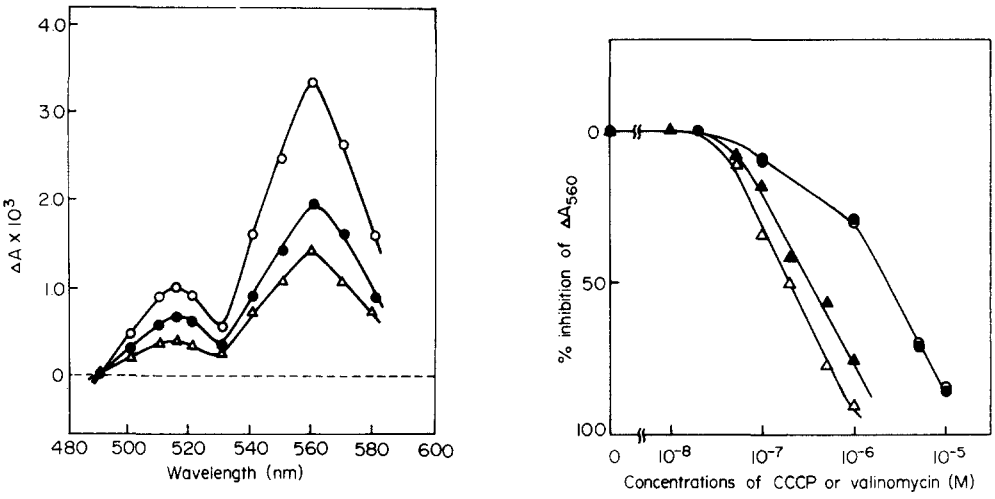

Fig. 3. Difference spectra of components R and S. Magnitudes of the initial rises of light-induced absorbance changes were determined between 490 and 580 nm. Chlorophyll concentration, 20.9 μg/ml. Other reaction conditions were the same as in Fig. 1. ○, no addition. △, spectrum of component R determined in the presence of 10 μM DCMU. ●, spectrum of component S obtained by plotting parts of the absorbance changes which had been diminished by DCMU (open circles minus triangles) against wavelengths.

Fig. 4. Effects of CCCP and valinomycin on the magnitudes of components R and S. Magnitudes of components R and S were determined as in Fig. 3. Chlorophyll concentration, 25.6 μg/ml. Other reaction conditions were the same as in Fig. 1, except that 20 mM KCl was added in experiments where effects of valinomycin were studied. Open and solid symbols indicate components R and S, respectively. ○, ●, CCCP. △, ▲, valinomycin.

presence of DCMU, was similar in shape to that of component S which was obtained by subtracting the spectrum of component R from that determined in the absence of DCMU. This clearly indicates that pigments responsible for the absorbance changes are the same with the two components. Kunifuji et al. [19] have shown that the 560 nm peak is due to the absorbance changes of carotenoids, siphonaxanthin and/or siphonein.

The 560 nm changes were diminished or abolished in the presence of uncouplers of photophosphorylation or ionophores which increase permeability of the thylakoid membrane to cations [14,20]. Fig. 4 compares sensitivities of components R and S to CCCP, a proton carrier [24], and valinomycin, a K^+-specific ionophore [25]. Magnitudes of the two components decreased in parallel with increasing concentrations of CCCP and valinomycin, indicating that they are similarly sensitive to the redistribution of H^+ or K^+ between the outer and inner spaces of the thylakoid membrane. Thus the results support the view that components R and S are both due to the electric field formed across the thylakoid membrane.

Sensitivity of component S to DCMU and DBMIB suggests that this part of the absorbance change is related to electron flow from Photosystem II to Photosystem I. Electron transport in intact *Bryopsis* chloroplasts, which was monitored with an oxygen electrode in the presence of methyl viologen as electron acceptor, was completely suppressed by DCMU and DBMIB at concentra-

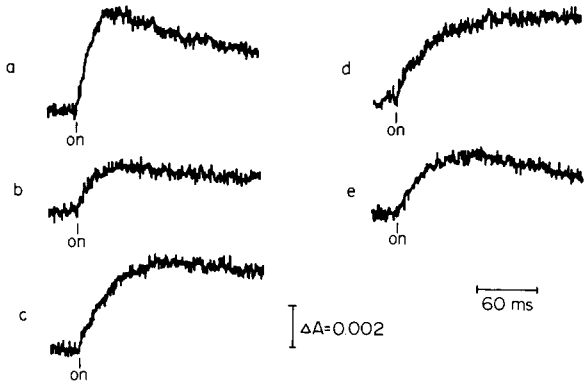

Fig. 5. Effects of electron donors on the absorbance changes at 560 nm determined in the presence of DCMU. a, no addition; b, 10 μM DCMU; c, b plus 0.1 mM DCIP and 5 mM ascorbate; d, b plus 0.1 mM phenazine methosulfate and 5 mM ascorbate; e, b plus 0.1 mM TMPD and 5 mM ascorbate. Chlorophyll concentration, 39.0 μg/ml. Other conditions were the same as in Fig. 1.

tions where component S was totally abolished. However, substantial rates of methyl viologen photoreduction could be restored on addition of artificial electron donors of Photosystem I such as the DCIP- or TMPD-ascorbate couple (data not shown). It is of interest, therefore, to examine effects of the electron donors on the 560 nm changes in the presence of DCMU. Fig. 5 shows that, in the presence of DCMU, the signal size of the 560 nm change was markedly increased on addition of the DCIP-ascorbate couple (trace c). Phenazine methosulfate was also effective in enhancing the absorbance change in the presence of DCMU (trace d). In contrast, the TMPD-ascorbate couple was less effective in this respect (trace e, see also Fig. 6), although the couple served as a better electron donor for methyl viologen photoreduction than the DCIP-ascorbate couple (data not shown). This concurs with the observation of Larkum and Bonner [12] that reduced DCIP, but not reduced TMPD, restored the DCMU-inhibited 518 nm signal in intact spinach chloroplasts.

According to the mechanism of energy conservation with artificial electron donors which has been explored by Trebst and his associates [26], the proton gradient across the thylakoid membrane can be formed with reduced DCIP and phenazine methosulfate, but not with reduced TMPD, since DCIP and phenazine methosulfate bring protons, with electrons, across the thylakoid membrane and, on oxidation, release protons into the intra-thylakoid space, whereas TMPD serves as a carrier of electrons but not of protons. We propose, therefore, that component S is due to the electric field formed by translocation of protons across the thylakoid membrane. The proton influx is coupled with electron transport to Photosystem I either from water in the absence of DCMU or from reduced DCIP or phenazine methosulfate in the presence of the inhibitor.

The two components of the 560 nm changes could be distinguished from each other with respect to their dependencies on light intensity. As shown in Fig. 6A, the light intensity curve of the 560 nm changes was biphasic; it shows a steep rise with increasing light intensity up to $3.2 \cdot 10^4$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1} and a

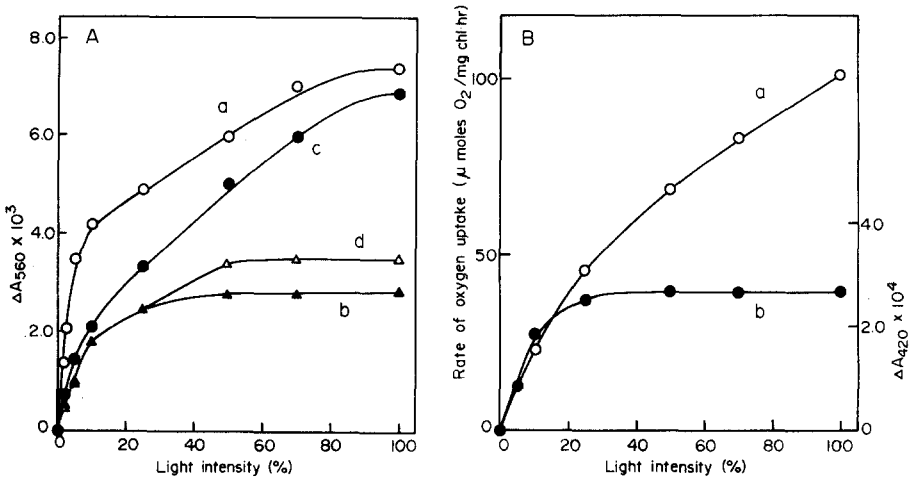

Fig. 6. (A) Effects of light intensity on magnitudes of the absorbance changes at 560 nm. a, no addition; b, 10 μM DCMU; c, b plus 0.1 mM DCIP and 5 mM ascorbate; d, b plus 0.1 mM TMPD and 5 mM ascorbate. Chlorophyll concentration, 41.8 μg/ml. Other conditions were the same as in Fig. 1. (B) Effects of light intensity on rates of methyl viologen photoreduction and on magnitudes of cytochrome *f* photooxidation. a, rates of methyl viologen photoreduction; b, magnitudes of cytochrome *f* oxidation determined at 420 nm. The intensity of red actinic light was $3.2 \cdot 10^5$ ergs $\cdot$ cm^{-2} $\cdot$ s^{-1} at 100% and varied with neutral density filters.

gradual rise at higher light intensities. DCMU selectively eliminated the gradual rise at high light intensities, leaving the steep rise at low light intensities in a diminished extent. This strongly suggests that the gradual rise at high intensities is due to component S. If this assumption is correct, the gradual rise at high intensities should be regenerated on addition of the DCIP-ascorbate couple, but not on addition of the TMPD-ascorbate couple, to DCMU-poisoned chloroplasts. Traces c and d of Fig. 6A clearly show that this is indeed the case.

For the comparison, rates of methyl viologen photoreduction and extents of cytochrome *f* photooxidation were determined as a function of light intensity (Fig. 6B). It is apparent that the light intensity dependence of component S is similar to that of methyl viologen photoreduction. This is compatible with the notion that component S depends upon a process linked with electron transport. On the other hand, the light intensity profile of component R is similar to that of cytochrome *f* oxidation. Extents of component R and cytochrome *f* oxidation were both saturated at a relatively low light intensity. This strongly indicates that component R is a portion of the absorbance change which responds to the electric field between oxidized cytochrome *f*, or adjacent electron carriers, inside the thylakoid membrane and reduced electron acceptors of Photosystem I on the outer membrane surface [20].

Fig. 6A also suggests that the maximum extent of component R is attained only in the absence of DCMU, since magnitudes of the steep rise at low intensities were much larger in the absence (trace a) than in the presence of the inhibitor (traces b and c). Although this point was not further studied in the present work, the observation implys a possible contribution of the charge separation among electron carriers associated with Photosystem II to component R.

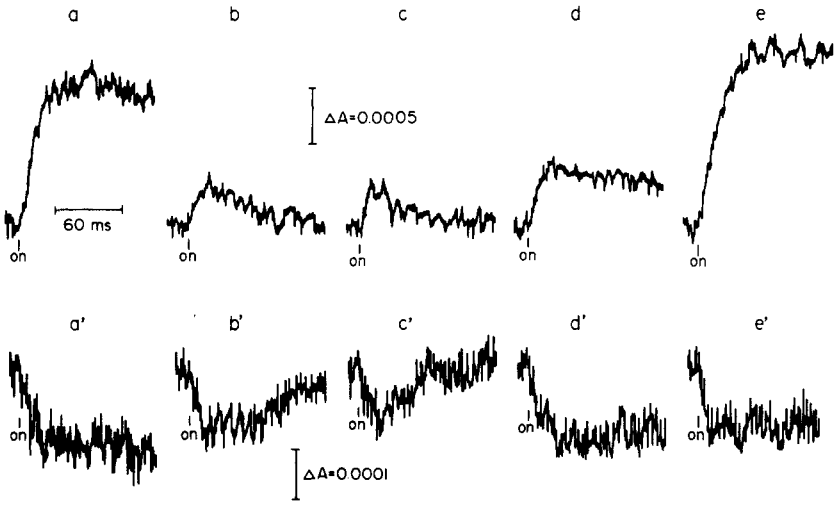

Fig. 7. Effects of phenylmercuric acetate and an electron donor or acceptor of Photosystem I on the magnitudes of component R and cytochrome *f* oxidation. a—e, absorbance changes at 560 nm; a'—e', cytochrome *f* oxidation determined at 420 nm. a,a', 10 μM DCMU; b,b', 10 μM DCMU and 50 μM phenylmercuric acetate; c,c', 10 μM DCMU, 50 μM phenylmercuric acetate, 0.1 mM DCIP and 5 mM ascorbate; d,d', 10 μM DCMU, 50 μM phenylmercuric acetate and 2 mM methyl viologen; e,e', 10 μM DCMU, 50 μM phenylmercuric acetate, 2 mM methyl viologen, 0.1 mM DCIP and 5 mM ascorbate. Chlorophyll concentration, 32.5 μg/ml. Other conditions were the same as in Fig. 1.

In accord to the above assumption that component R determined in the presence of DCMU is due to the charge separation through Photosystem I, magnitudes of the component were largely diminished on addition of 50 μM phenylmercuric acetate (Fig. 7, trace b). Since the inhibitor inactivates ferredoxin and ferredoxin-NADP oxidoreductase [27], the results indicate the participation of these redox proteins in the mechanism of the field formation responsible for component R.

Fig. 7 further provides a strong evidence for the above hypothesis, by showing that changes of the 560 nm signals were in parallel with those of cytochrome *f*. Note that the signal decayed during illumination in the presence of DCMU and phenylmercuric acetate (trace b). Under the same experimental conditions, cytochrome *f* also underwent a transient oxidation (trace b'). Thus, the decay of the 560 nm signal can be ascribed to a disappearance of the electric field due to reduction of oxidized cytochrome *f* and other electron carriers. This explanation was further substantiated by the following observations. Reduction of cytochrome *f* was accelerated by the addition of DCIP-ascorbate couple (trace c') but suppressed in the presence of methyl viologen, which accepts electrons from Photosystem I by bypassing the phenylmercuric acetate-inhibition site (trace d'). Correspondingly, the decay of the 560 nm signal was accelerated by reduced DCIP and suppressed by methyl viologen (traces c and d).

Another point to be noted is that the DCIP-ascorbate couple failed to regenerate component S in the presence of DCMU and phenylmercuric acetate (trace d). In contrast, we have seen above that the couple restored component

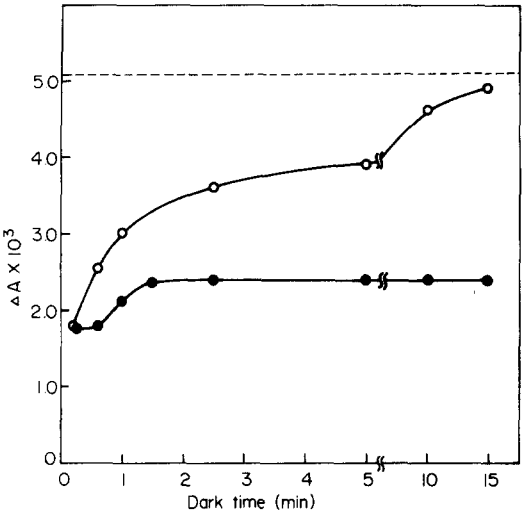

Fig. 8. Magnitudes of absorbance changes at 560 nm during the dark incubation of preilluminated chloroplasts. Chloroplasts were preilluminated for 10 s with red actinic light (longer than 650 nm, $3.2 \cdot 10^5$ ergs $\cdot$ cm^{-2} $\cdot$ s^{-1}) and incubated in the dark for indicated periods of time. ○, no addition. ●, 10 μM DCMU was added after the preillumination and before the measurement. Chlorophyll concentration, 27.9 μg/ml. Other conditions were the same as in Fig. 1.

S in the presence of DCMU alone (Fig. 5). Thus the failure of the couple in regenerating component S should be ascribed to a situation that the size of the electron acceptor pool of Photosystem I was greatly reduced in the presence of phenylmercuric acetate. In fact, further addition of methyl viologen induced a large absorbance increase comparable in size to component S (trace e). This again supports the view that electron transport is a prerequisite for the appearance of component S.

Finally, we have studied the effect of preillumination of chloroplasts on components R and S. It has been described previously that the complex time course of the 560 nm changes was observed only in the dark-adapted chloroplasts [20]. The preillumination of chloroplasts resulted in an elimination of the transient features accompanied by a marked diminition in the magnitude of the absorbance changes. In the experiments shown in Fig. 8, chloroplasts were exposed to the actinic light for 10 s and, after varied periods of the dark intervals, the initial E_O-E_D rises of the 560 nm changes were measured. The magnitude of the absorbance increase determined 15 s after the cessation of preillumination was less than a half of the magnitude of the E_O-E_D rise in the dark-adapted chloroplasts. The signal size was not affected by the addition of DCMU just prior to the second measuring illumination, indicating that the remaining absorbance change is due to component R. As the dark incubation proceeded, the signal size increased biphasically, rapidly for the first 1 min, then more slowly, and attained nearly, but not completely, the original magnitude of the absorbance change after 15 min of the dark incubation. Note that the magnitude of absorbance changes determined in the presence of DCMU remained constant, except a slight increase at the early stage of the dark incubation. It

is apparent, therefore, that a portion of the absorbance change which was eliminated by the preillumination and restored during the dark incubation corresponds to component S.

The results suggest a large difference in the dark relaxation times between the two electrogenic reactions contributing to the 560 nm changes. The appearance of component R after a short dark interval is compatible with the previous observations that photooxidized cytochrome *f* was rapidly reduced (within 1 s after the cessation of illumination) and therefore fully reoxidized by the subsequent illumination [22]. The small increase in magnitudes of component R observed at the early dark period might be due to the dark relaxation of other electron carriers. On the other hand, the slow and biphasic kinetics of the dark recovery of component S implys complex mechanisms underlying the relaxation of the component, which may be related to redistribution of ions between the inner and outer spaces of the thylakoid, the disappearance of light-induced conformational changes of the thylakoid membrane, or an inactivation of electron transport beyond Photosystem I in the dark [22].

Discussion

The results obtained in the present work indicate that the initial rise of the 560 nm changes in dark-adapted *Bryopsis* chloroplasts consists of two components, R and S, which are both electrochromic absorbance changes of siphonaxanthin and/or siphonein but depend upon the electric fields across the thylakoid membrane generated by different mechanisms.

Several lines of evidence indicate that component R is due to the electric field formed between positive and negative charges in electron carriers of Photosystem I which are located asymmetrically in the thylakoid membrane. Witt and coworkers [2,9,16,18] have shown that the electric field invoked by a single turnover flash was generated by the primary charge separations in the reaction centers of photosynthesis. However, component R cannot be explained by this fast mechanism alone, since the magnitude of the 560 nm change induced by a short saturating flash was less than a half of the magnitude of component R. In addition, the flash-induced signal was not affected by phenylmercuric acetate (data not presented).

Electron transport is a prerequisite for the appearance of another component S. Experiments with artificial electron donors suggest that this component is related to the proton uptake by the thylakoid membranes. The proton uptake will affect the electrochromic shift in two different mechanisms; first, the membrane potential will be set up by the proton translocation across the thylakoid membrane or, second, the protonation of the thylakoid membrane will affect the membrane potential by altering the membrane thickness [28] and thus capacitance.

It has been shown previously that electron transport is limited by a block beyond Photosystem I in the dark-adapted *Bryopsis* chloroplasts [22]. Thus only a limited amount of proton must have been taken up by the thylakoid in the time range studied here. In fact, in this time range, any significant light-scattering change of chloroplasts, an indicative of the protonation of the thylakoid membrane [29], was not detected and absorbance changes at 560 nm

were totally abolished in the presence of valinomycin which dissipates the membrane potential but does not collapse the proton gradient across by thylakoid membrane (Fig. 4; see also ref. 20). Yamagishi et al. [30] have recently shown that 7—10 s illumination were needed to generate a significant extent of the proton gradient across the thylakoid membrane in the dark-adapted *Bryopsis* chloroplasts. On the other hand, a substantial magnitude of the membrane potential can be generated by the influx of a small amount of proton into the intramembrane space from the stroma. We conclude, therefore, that component S is due to the first mechanism, although the second mechanism might be important under prolonged illumination.

The complex time course of the 560 nm changes can now be explained in terms of the two electrogenic mechanisms. On illumination with continuous light, the absorbance at 560 nm varied successively showing two transient peaks E_D and E_{S_1}, with a minimum E_P in between, then followed by a gradual decrease to a low steady state [20]. The early transients can be ascribed to variations of component R, which varies with redox changes of electron carriers associated with Photosystem I. Component S also contributes significantly to the early absorbance changes, and the minimum E_P is explained by this component alone. After the second peak E_{S_1}, the magnitude of the 560 nm changes will be diminished as electrophoretic efflux of cations or influx of anions and proton efflux through the reversible ATPase, compensate the membrane potential. It should be born in mind that the long-term absorbance change at 560 nm is complicated by the superposition of the light-scattering changes of chloroplasts [13,31].

The present work also provides a reasonable answer to a long-disputed question; what are the roles of the two light reactions in the electrochromic absorbance changes [3—8,11,12,14,15,17]. Photosystem I is needed for the two electrogenic reactions. It mediates the transmembrane charge separation responsible for component R and also supports, by promoting electron transport, the build up of the proton gradient across the thylakoid membrane which gives rise to component S. On the other hand, the effect of Photosystem II light is complex, since the electron flow from Photosystem II has the two and opposing actions. Component S depends upon electron transport from Photosystem II to I, whereas component R is diminished by reduction of cytochrome *f* and other intersystem electron carriers with electrons provided from Photosystem II. This would be one of the main reasons for notable controversies reported in the literature [3—8,12,14,17] concerning with the contribution of Photosystem II to the electrochromic absorbance changes. In addition, there is a possibility that charge separation between donors and acceptors of Photosystem II also contributes to the 560 nm changes. This has been suggested from the observation that the maximum magnitudes of component R was attained only in the presence of DCMU (Fig. 6A). Experiments along this line are in progress.

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