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MEMBRANE POTENTIAL-DEPENDENT REDUCTION OF CYTOCHROME *b*-6 IN AN ALGAL MUTANT LACKING PHOTOSYSTEM I CENTERS

JÉRÔME LAVERGNE

Institut de Biologie Physico-Chimique, 13, rue Pierre et Marie Curie, 75005 Paris (France)

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Absorption changes induced by illumination of a double mutant strain of the green alga *Chlorella sorokinia*, lacking Photosystem (PS) I centers and deficient in light-harvesting complex, were investigated. A reduction of cytochrome *b*-6 was observed, which appeared to be correlated to the membrane potential: the dark reoxidation of cytochrome *b*-6 followed linearly the decay of the field, and the light-induced reduction was inhibited by an ionophore collapsing the membrane potential. This field-dependent reduction was also observed after one flash in the presence of a saturating concentration of 3-(3,4-dichlorophenyl)-1,1-dimethylurea, blocking the electron injection from PS II. This suggests that the reduction of cytochrome *b*-6 is controlled by a membrane potential-dependent redox equilibrium with an unknown carrier, 'G', which is already reduced in the dark-adapted state. When benzoquinone-treated algae were used, G appeared to be oxidized in the dark so that reductants from PS II were required to observe the field-driven reduction of cytochrome *b*-6. Analysis of the spectra allowed the ascription of an absorption increase around 420 nm to the reduction of G. The midpoint potential of G, its location within the membrane and its possible role in the electron-transfer chain are discussed.

Introduction

In chloroplasts, cytochrome *b*-6 is found associated with a complex (cytochrome b_6-*f* complex), which is homologous to the cytochrome *b*-*c* complexes of mitochondria or bacteria. The complex contains one Rieske Fe-S protein, one cytochrome *f*, and two *b*-6 cytochromes [1,2]. It was shown to mediate electron transport from PS II to PS I, oxidizing plastoquinol and reducing plastocyanin. Whereas cytochrome *f*, and, probably, the Rieske protein are involved in the direct electron-transfer pathway, the role of cytochrome *b*-6, with its midpoint potential around −100 mV [3], i.e., lower than that of the PQ/PQH_2 couple, is less clear. It has been suggested by Velthuys [4] that this cytochrome is involved in the electrogenic loop of a modified Mitchellian 'Q-cycle' [5]. Recent work by Selak and Whitmarsh [6] has provided support for this hypothesis (see, however, Refs. 7 and 8 for other Q-cycle schemes with different roles proposed for cytochrome *b*-6). The reoxidation of plastoquinol is thought to yield in a concerted way a high-potential electron towards cytochrome *f* (probably via the Rieske Fe-S protein [9]), plastocyanin, and, ultimately, P-700, and a low-potential electron which reduces cytochrome *b*-6. Two protons are concomitantly released into the thylakoid lumen. The reoxidation of cytochrome *b*-6 would involve an electrogenic transfer towards the external surface of the membrane, which causes the slow phase (phase b) of the electrochromic absorption change [10]. Through a process which may

Abbreviations: PS, photosystem; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PQ, plastoquinone;

involve charge accumulation or the cooperation of two parallel pathways, a plastoquinone molecule would be reduced, with the uptake of two protons from the external medium. According to this scheme, the reduction of cytochrome *b*-6 is coupled to that of cytochrome *f* (through the Rieske Fe-S protein), so that the whole process is triggered by the prior oxidation of cytochrome *f* by PS I. A very convincing demonstration of these mechanisms in vitro using the isolated cytochrome b_6-*f* complex has been reported by Hurt and Hauska [9].

In this paper, we report on light-induced cytochrome *b*-6 reduction in a mutant strain which lacks PS I centers. This reduction is shown to proceed through a field-dependent equilibrium involving an unidentified species denoted G.

Materials and Methods

The material used in this work consists in whole cells of a double mutant strain (denoted S44) of the green alga *Chlorella sorokinia*. This strain, which was kindly provided by Dr. P. Bennoun, is very deficient in light-harvesting complex and lacks PS I centers. Details about *sorokinia* mutants and their use in spectroscopic experiments will be given elsewhere (Bennoun, P., et al., unpublished data). Due to the small size of *C. sorokinia* cells (3 μm), optical measurements are improved compared to other organisms: slower settling rate, smaller diffusion and flattening of the spectra. The use of a mutant with a lowered pigment content offers two advantages for the present study. First, the lower absorbance in the chlorophyll bands allows a better signal-to-noise ratio for detecting absorption changes in these spectral regions. The second advantage is that the relative magnitude of the field-indicating absorption change is smaller (with a shift of the positive peak from 518 to 511 nm). Such a property was previously reported by Renger and Schmidt [11] in pigment-deficient mutants from another organism.

The spectrophotometer used in this study is an improved version of that described in Ref. 12. The absorbance of the photosynthetic material is sampled by weak monochromatic flashes, with a simultaneous detection of the measuring and reference beams. Actinic illumination is provided by either a xenon flash, or continuous illumination from a xenon lamp. The experiments were run at room temperature.

The root-mean-square noise in the sampling of $\Delta I/I$ was about $2 \cdot 10^{-5}$. The spectra were obtained by averaging five to ten measurements at each wavelength.

When indicated, algae were treated by adding $4 \cdot 10^{-4}$ M benzoquinone to the algal suspension immediately before centrifugation. The pellet was then suspended in a medium containing 50 mM phosphate buffer (pH 6.5) and 0.1 M sucrose.

Results

We have investigated the light-induced absorption changes in mutant algae devoid of PS I. The spectra shown in Fig. 1A were measured at various times following a 1 s continuous illumination. In this experiment, the algae have been treated with benzoquinone, as described in Materials and Methods. As can be seen, the light-induced absorbance change measured in the 400–440 nm range at the end of the illumination or during the first hundred milliseconds of darkness is close to the spectrum of reduced-minus-oxidized cytochrome *b*-6 (shown in the inset) The presence of reduced cytochrome *b*-6 is confirmed by the appearance of a peak at 563 nm. It is, however, more sensitively detected in the blue part of the spectrum, because the 563 nm peak has a lower extinction coefficient, and is distorted by the C-550 and electrochromic absorption changes. At later times after the illumination, the spectra show the progressive decay of reduced cytochrome *b*-6, and the appearance of an absorption increase with a peak around 420 nm. Both phenomena seem to be correlated, as indicated by the occurrence of isobestic points around 425 and 441nm. A simple way of accounting for this correlation is to assume that the oxidizing partner involved in cytochrome *b*-6 reoxidation is responsible for the peak at 420 nm. Denoting this carrier by G, and assuming that its reduction causes an absorption increase around 420 nm, the succession of spectra drawn in Fig. 1A may be interpreted by the scheme (cyt, cytochrome): $G_{ox}cyt_{ox} \xrightarrow{light} G_{ox}cyt_{red} \xrightarrow{dark} G_{red}cyt_{ox}$. The figure gives also, on a smaller vertical scale, the

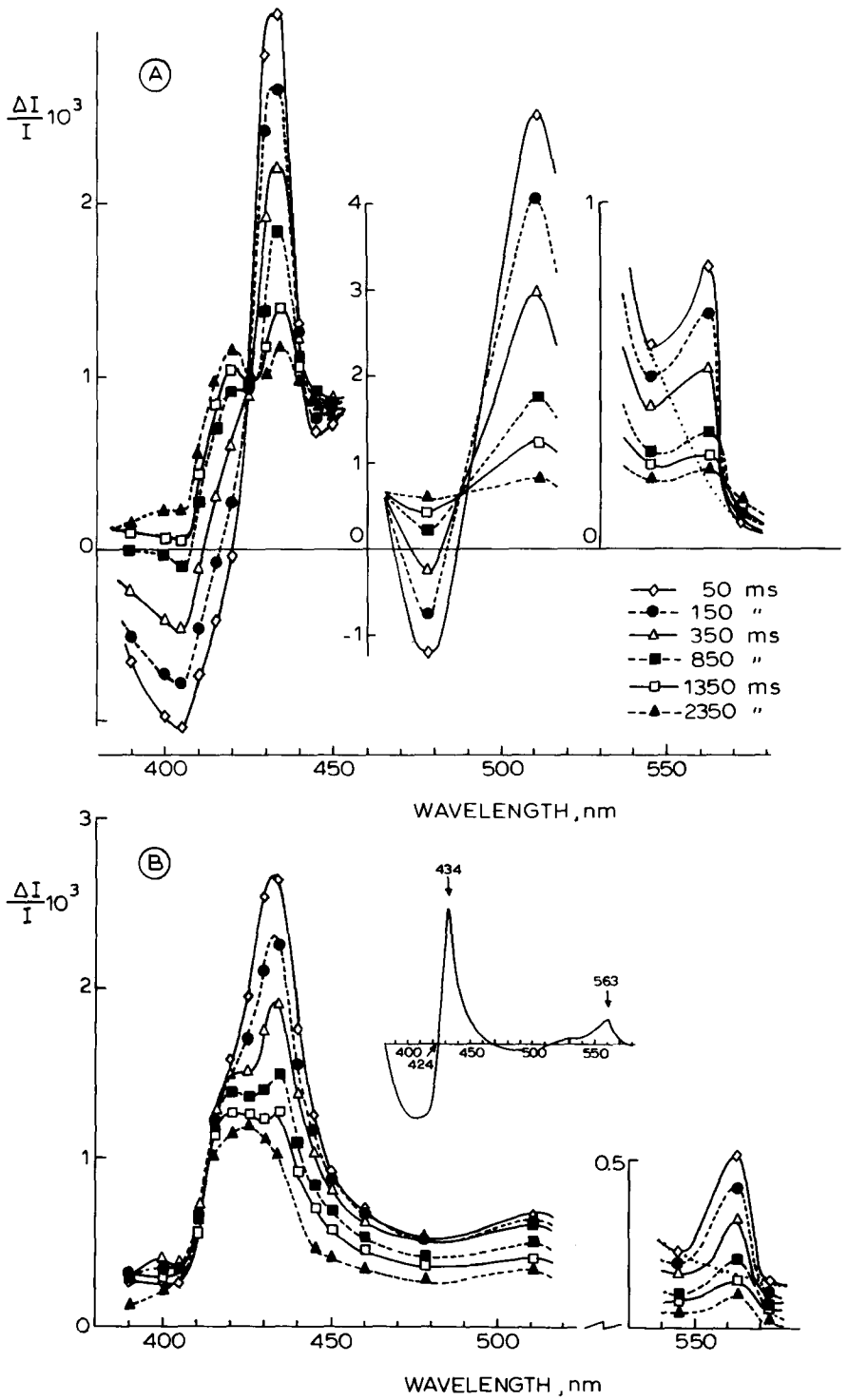

Fig. 1. Spectra of the absorption changes measured at various times after a 1 s continuous illumination, using benzoquinone-treated algae. (A) No addition, (B) same batch of algae + 10^{-4} M dicyclohexyl-18-crown-6. Symbols for the various dark times after putting off the light are indicated in panel A. Notice the different scales used for $\Delta I/I$ (positive with respect to an absorption increase). The spectra in the 480–511 and 545–572 nm regions are drawn schematically. A dotted line from 545 to 572 is an interpolation of the field-indicating change used as a baseline for measuring cytochrome *b*-6 at 563 nm. Inset (in panel B): spectrum of reduced vs. oxidized cytochrome *b*-6 according to Ref. 19.

absorbance changes at 480 and 511 nm, which are the negative and positive peaks, respectively, of the electrochromic change in this particular strain (whereas the positive peak in plants chloroplasts is around 518 nm). During the illumination, a large membrane potential was built up which amounts to about 4-times that induced by one saturating single-turnover flash. As may be noticed, the dark reoxidation of cytochrome *b*-6 and the decay of the potential take place in the same time range.

Fig. 1B shows a family of spectra obtained under identical conditions, but in the presence of an ionophore, dicyclohexyl-18-crown-6 (Diner, B., unpublished data and Ref. 13). This substance causes a fast relaxation of the light-induced membrane potential, and the flat spectra obtained in the 480–511 nm region confirm that no substantial potential could build up during the illumination in its presence. More surprising is the dramatic change caused by dicyclohexyl-18-crown-6 in the blue region where the contribution of the field-indicating spectrum itself is expected to be small. Only little reduction of cytochrome *b*-6 is now observed, whereas the 420 nm peak has been present since the end of the illumination period (instead of appearing slowly during the dark relaxation). The successive spectra indicate a decay of the small amount of reduced cytochrome *b*-6 present, and a slower decrease of the 420 nm band. It seems therefore that the presence of reduced cytochrome *b*-6 is linked to the membrane potential: in Fig. 1A, the reoxidation of cytochrome *b*-6 accompanies the field decay, whereas the addition of dicyclohexyl-18-crown-6 (Fig. 1B) causes a suppression both of the potential and of most cytochrome *b*-6 reduction.

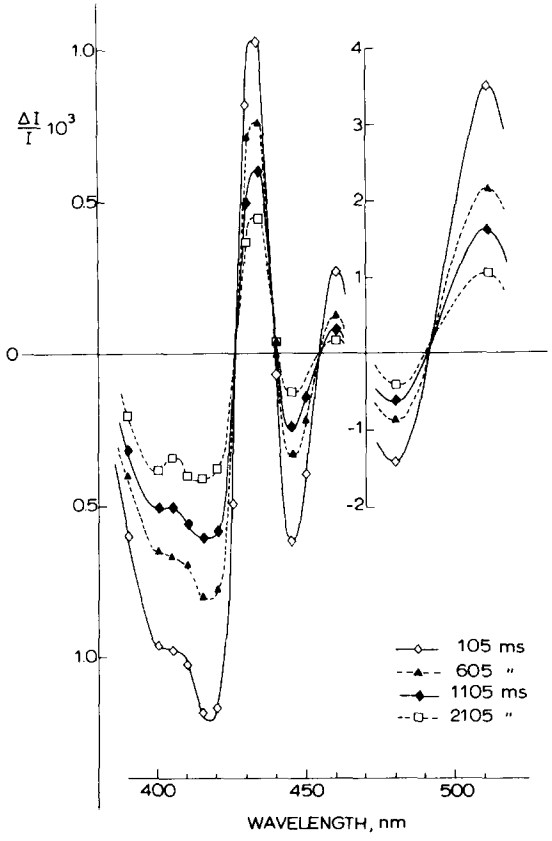

Fig. 2. Spectra of the absorption changes measured at various times after a 1 s continuous illumination, using untreated algae.

The results shown in Fig. 1 were obtained with quinone-treated algae. Fig. 2 shows a similar experiment using untreated material. The characteristic spectrum of reduced-minus-oxidized cytochrome *b*-6 appears again at short times after switching off the illumination, but it is distorted towards short wavelengths by an absorption decrease which makes the spectrum more symmetrical. The change of the spectra at increasing dark times is also different: the whole spectrum decreases almost homogeneously with isobestic points at 426 and 440 nm close to the baseline. It seems that the 420 nm peak which was progressively built up in the experiment of Fig. 1 is now present as an absorption decrease at short times after the illumination, and disappears concomitantly with the reoxidation of cytochrome *b*-6. This may be accounted for by the scheme proposed earlier, assuming that in untreated algae, G is already in its reduced form in the dark-adapted state. One then has the reactions: $G_{red}cyt_{ox} \underset{dark}{\overset{light}{\rightleftarrows}} G_{ox}cyt_{red}$. The effect of light is then mostly to promote a membrane potential which shifts the above equilibrium towards the right. In the experiment of Fig. 2, the reoxidation of the cytochrome is again correlated to the field decay, and an inhibitory effect of dicyclohexyl-18-crown-6 was found (not shown).

If the above assumptions are correct, i.e., that in untreated algae the reduction of cytochrome *b*-6 proceeds via a membrane potential-dependent redox equilibrium with a carrier G already reduced

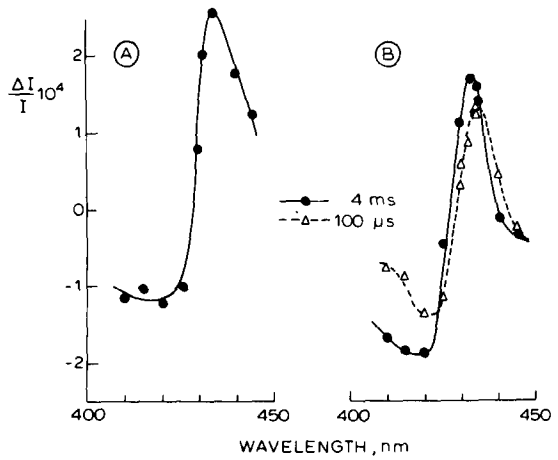

Fig. 3. Difference spectra showing the effect of the membrane potential after a saturating flash in the presence of $5 \cdot 10^{-5}$ M DCMU. No quinone treatment. (A) Difference of spectra (4 ms after flash, no dicyclohexyl-18-crown-6)−(4 ms after flash, $+10^{-3}$ M dicyclohexyl-18-crown-6). (B) (100 μs or 4 ms after flash, no dicyclohexyl-18-crown-6)−(100 ms after flash, no dicyclohexyl-18-crown-6). In this experiment, the field decay was almost completed at 100 ms.

in the dark-adapted state, it should be possible to observe this reduction in the presence of an inhibitor blocking electron transfer from PS II. The experiments of Fig. 3 show that such is the case. They were run in the presence of $5 \cdot 10^{-5}$ M DCMU, i.e., a concentration sufficient to block totally the electron transfer from PS II, including the pathway described by Joliot and Joliot [14] which requires higher inhibitory concentrations. Under these conditions, it is not possible to obtain such a high membrane potential as in the experiments of Figs. 1 and 2. About half of the PS II centers are inactivated due to the dark reaction $QB^- \xrightarrow{DCMU} Q^-B$ occurring on those centers with a reduced secondary acceptor B in the dark-adapted state [15,16]. Thus, only half of the the centers will promote a membrane potential upon a saturating flash. As the reopening of the centers in the presence of DCMU proceeds through a recombination process, no higher potential can be built up by further illumination. In order to ascertain a field-induced reduction of cytochrome *b*-6 under conditions of comparatively low membrane potential, we plotted the difference of spectra obtained in the presence and absence of field. This procedure is useful as most of the light-induced change in the blue region is due to the long-lived Q^- (with DCMU present) [17]. It was done in two different ways. In Fig. 3A, the reference spectrum used for subtraction (no field) is measured in the presence of dicyclohexyl-18-crown-6. In Fig. 3B, the reference spectrum is obtained in the absence of dicyclohexyl-18-crown-6, but at a time when the field decay is almost completed. In this particular experiment, the field relaxation was comparatively fast so that the above condition was met as soon as 100 ms after the flash. Only little reoxidation of Q^- has occurred during this time, so that the substraction method should mostly reveal field-dependent phenomena. As can be seen, both methods yield essentially the same result, i.e., confirmation of the field-dependent cytochrome *b*-6 reduction. An interesting point is that this reduction is more than 75% complete in the spectrum obtained 100 μs after the flash: this indicates that the field-dependent equilibrium leading to the reduction of cytochrome *b*-6 is a kinetically fast process.

The experiment of Fig. 3 was repeated with quinone-treated algae (not shown). No significant reduction of cytochrome *b*-6 is then observed. This confirms that in this case, the reductant G is absent, and that the phenomena observed in experiments such as that of Fig. 1 require illumination not only for promoting a light-induced potential, but also for injecting electrons from PS II into the G-cytochrome *b*-6 system.

The relation between the membrane potential and cytochrome *b*-6 reduction is quantitatively investigated in Fig. 4. The difference, 434 nm minus 425 nm, which is mostly an indicator of reduced vs. oxidized cytochrome *b*-6 is plotted against the difference, 511 nm minus 480 nm, mostly an indicator of the electrochromic absorption change. Untreated algae were used, and the points were taken at various times after the last of a series of saturating flashes fired 50 ms apart. As can be seen, the points corresponding to different numbers of flashes lie close to a common straight line. A significant deviation (points situated below the line) was observed with data obtained during the first 50 ms after the last flash (not shown). This deviation is probably due to perturbation by other absorption change(s) rather than to a slow reduction of cytochrome *b*-6. Another (long-lived)

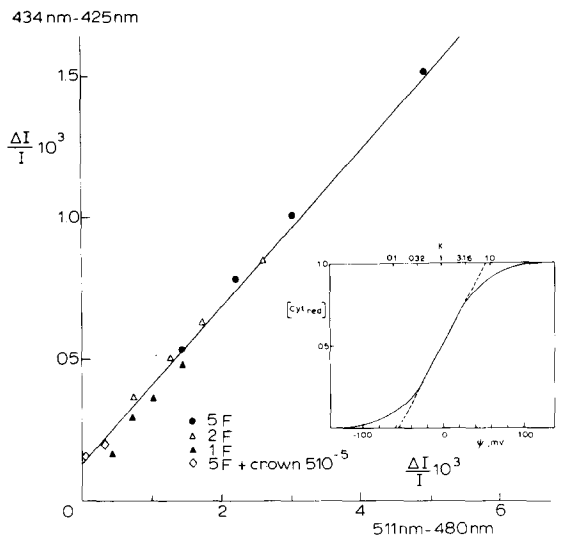

Fig. 4. A plot of the differences of absorption changes 434–425 nm (cytochrome b-6_{red}) vs. 511–480 nm (field-indicating change) measured at various times after the last of a series of saturating flashes (spaced 50 ms apart). Symbols for the number of flashes (F) indicated in the figure. For each experiment, four points were plotted, corresponding to 100 ms, 600 ms 1.1 s and 1.6 s after the last flash. They succeed one another towards decreasing field (or cytochrome b-6_{red}). The symbol ◇ was used for the two first points of an experiment using five flashes in the presence of $5 \cdot 10^{-5}$ M dicyclohexyl-18-crown-6. Inset: theoretical plot (according to Eqn. 1) of the amount of reduced cytochrome b-6 ([Cyt_{red}]) vs. the electric potential difference ψ between G and cytochrome b-6 (bottom scale) or equilibrium constant K (top scale). The origin of ψ is arbitrarily taken at $K = 1$. For other values of K, the ψ scale should be shifted (so that $\psi = 0$ matches the value of K). The broken straight line indicates the linear part of the curve. As can be seen, the maximal linear range is obtained for $K \simeq 0.3$. The maximal potential change in this range is then about 60 mV, corresponding to about 50% of the total cytochrome b-6 being reduced.

absorption change may be responsible for the fact that the line drawn in Fig. 4 has a slightly positive intercept: the wavelength differences we have chosen can hardly be expected to be pure indicators of cytochrome b-6 or membrane potential. The linear dependence shown in Fig. 4 holds over a large range of the light-induced potential, and does not depend on the number of preillumination flashes, i.e., on the number of electrons injected by PS II. It is not modified either when the field relaxation is accelerated by adding a low concentration of dicyclohexyl-18-crown-6 (see Fig. 4).

Discussion

It appears clearly, from the results reported above, that it is possible to reduce cytochrome b-6 in a system which lacks PS I. We have described two different situations. In the case of untreated algae, cytochrome b-6 reduction occurs by shifting an equilibrium constant which is sensitive to the light-induced membrane potential. The concept of potential-dependent redox equilibrium will be discussed later. Strong evidence in favour of this hypothesis may be found in the experiment of Fig. 3, where cytochrome b-6 reduction was observed after one flash in the presence of a saturating concentration of DCMU, which excludes the possibility that the electron could originate from PS II. Therefore, one has to admit that the reductant is already present, and that the photoreaction is required only for generating a delocalized membrane potential. The role of the potential in shifting the equilibrium, towards cytochrome b-6 reduction is evidenced by the kinetic correlations and the effect of dicyclohexyl-18-crown-6. With quinone-treated algae, no field-induced reduction of cytochrome b-6 is observed in the presence of DCMU, which suggests that the injection of reductant from PS II is now required. This is confirmed by the family of spectra of Fig. 1, where the reoxidation of cytochrome b-6 after an illumination is correlated with the appearance of the 420 nm band (and with the field decay), which we interpreted as an indication of the reduction of an unknown carrier G. The field-dependent spectra obtained with untreated algae are consistent with this hypothesis, since the reduction of cytochrome b-6 was accompanied by an absorbance decrease in the 420 nm region. The quinone treatment thus seems to cause an oxidation of G in the dark-adapted state, similarly to its effect on the secondary acceptor B of PS II [16].

The notion of membrane potential-dependent redox equilibrium can be viewed as resulting from a transmembrane location of both carriers, with G closer to the external surface of the thylakoid than cytochrome b-6. If, in the absence of field, the equilibrium constant is (assuming that both species interact within a one-to-one complex) $[G_{ox}cyt_{red}]/[G_{red}cyt_{ox}] = K$, then an electric potential difference ψ (in mV) between G and cytochrome (the

latter being at the more positive potential) will change the equilibrium constant to $K' = K \times 10^{\psi/60}$ (taking $2.3RT/F \simeq 60$ mV at room temperature). A membrane potential-dependent inhibition of the electrogenic transfer responsible for the slow ($t_{1/2} \simeq 5$ ms) phase b of the light-induced potential has already been documented [8]. We are dealing here with an inverse effect – a field-induced electron transfer – which is based on similar physical grounds, and may be connected to the former. From the above expression for K', one obtains:

$$[\text{cytochrome}_{\text{red}}] = (1 + K^{-1} \times 10^{-\psi/60})^{-1} \quad (1)$$

where the total amount of cytochrome *b*-6 (and G) has been taken as equal to one. This sigmoidal relation was plotted in the inset of Fig. 4, as a function of ψ (lower scale, taking $K = 1$) or K (upper scale, taking $\psi = 0$). It is readily seen that the linear relation that was obtained experimentally (Fig. 4) may fit the theoretical curve provided that K is not too different from 1 and that the potential change is not too large. The optimal conditions are found over $K \simeq 0.32$, where the potential change in the linear region may be as high as about 60 mV. The typical membrane potential induced in thylakoids by a single-turnover flash (with only PS II operative) has been estimated to about 20–30 mV [18]. Since we found a linear dependence of [cytochrome $_{\text{red}}$] vs. field in a range as large as 4-times the single-turnover potential, it is likely that the electron transfer between G and cytochrome *b*-6 spans only a fraction of the membrane thickness.

From a comparison of the C-550 signal measured in mutant S44 and spinach chloroplasts, we estimated the concentration of PS II centers in mutant S44. Then, by measuring [cytochrome b-6_{red}] at 563 nm (with a corrected baseline as shown in Fig. 1), using $\epsilon_{563} = 15\ \text{mM}^{-1}$ [19], we obtain a typical figure of 0.17 cytochrome *b*-6 per PS II center being reduced from the potential induced by a single-turnover flash. With the maximum potential that could be built up, about 4-times this value can be obtained, i.e., 0.7 cytochrome *b*-6 per PS II center. Since the maximal linear range in the theoretical curve amounts to 50% of the total reducible cytochrome, this suggests that the 100% value may be greater than one cytochrome *b*-6 per PS II center.

The electron transfer from G to cytochrome *b*-6 is a rapid process, almost completed at 100 μs after a flash (see Fig. 3). This fact and the requirement of a definite transmembrane location with respect to cytochrome *b*-6 suggest that G is bound to the cytochrome *b-f* complex. The reduced vs. oxidized spectrum of G with a peak around 420 nm might suggest an identification of G as cytochrome *f*. However, spectra of the changes occurring in the 540–570 nm region in quinone-treated algae (not shown) did not reveal any appearance of the 554 nm band of cytochrome *f* during the decay of the cytochrome *b*-6 bands and concomitant rise of the 420 nm peak. It thus turns out that G is not cytochrome *f*, which is consistent with the redox potential which may be expected for the G_{ox}/G_{red} couple. As discussed above, a minimum estimate for K is about 0.3, so that the midpoint potential of G is expected to be at most 30 mV more positive than that of cytochrome *b*-6. Values of -100 mV [3] or $+5$ mV [20] for the midpoint potential of cytochrome *b*-6 have been reported in the literature whereas that of cytochrome *f* lies around + 350 mV [21]. The fact that benzoquinone ($E_m \simeq +320$ mV) oxidizes G efficiently is also consistent with a low-potential value for this couple. Another guess as to the identity of G could be a plastoquinone molecule. However, attempts at correlating the changes of G to a semiquinone absorbance peak in the 320 nm region proved unsuccessful (not shown). The Rieske type Fe-S protein does not seem a good candidate either, as its difference spectrum [24] does not match that of G, and due to its too high midpoint potential as well. Accordingly, we are not yet able to propose an identification of G with some known electron-transfer component.

We wish now to discuss the possible role of G in the electron-transfer chain. In our experiments, using a mutant lacking PS I centers, G appears as a dead-end for electron transport. In untreated algae, G seems to be completely or almost completely reduced in the dark-adapted state. In quinone-treated algae, G is initially oxidized but can be reduced by PS II (this will be discussed later). Then again, it remains stably reduced on the seconds time scale. Our point here is that G_{red}

is not spontaneously reoxidized by a higher potential carrier located close to the external surface of the membrane, as might have been expected if G were an intermediate involved in the electrogenic transfer leading to phase b of the light-induced membrane potential. G may be involved in such a process, but one has to admit that the cooperation of PS I is needed somehow. Velthuys [4] suggested that two parallel chains cooperate to yield the two electrons needed for the reduction of a plastoquinone molecule at the external surface of the membrane (with the uptake of two protons) thus completing the Q-cycle. The cytochrome *b*-6–G pathway we have been investigating here may be a segment in one of these chains.

Preliminary experiments with a strain provided with both photosystems show that the field-induced G_{red} → cytochrome *b*-6 transfer is present in such a system, with G being mostly reduced in the dark-adapted state of untreated material, as in the case of the mutant we have studied. After one flash, most of the cytochrome *b*-6 reduction is rapid and correlated with phase b of the light-induced field (this phase is not observed in our PS I-deficient mutant). However, when a higher membrane potential was built up by a series of flashes or continous illumination, no phase b was observed and a slow reoxidation of cytochrome *b*-6 accompanying the field decay took place. This transient reduction-reoxidation was abolished in the presence of dicyclohexyl-18-crown-6, so that we may state that the phenomena described in this paper are not strictly specific to the particular mutant strain we have used.

The last point we wish to discuss is the possibility of injecting one electron into the G-cytochrome *b*-6 system in quinone-treated algae. This reductive pathway is unorthodox, since it does not seem to be correlated to a reduction of cytochrome *f*. We could not detect any absorption change indicating such a reduction in the S44 mutant, and it seems likely that even after quinone treatment, cytochrome *f* remains reduced in the dark-adapted state. The usual concerted reaction: PQH_2 + cyt *b*-6_{ox} + cyt f_{ox} → PQ + $2H^+$ + cyt *b*-6_{red} + cyt f_{red} is thus not allowed to take place. There is no indication either that the reduction of cytochrome *b*-6 in our experiments with quinone-treated algae is correlated to the formation of a stable semiquinone. Preliminary experiments on this problem suggest that (i) this reduction is a low-efficiency process, requiring many single-turnover flashes, (ii) the efficiency is still lowered by the membrane potential, and (iii) only a partial inhibition is observed at $3 \cdot 10^{-7}$ M DCMU (a concentration which according to Joliot and Joliot [14] should block the 'Q_1' but not the 'Q_2' pathway). It may thus be envisaged that this reduction proceeds through a low-potential PS II acceptor such as 'Q_2' [22], 'Q_L' [23], or the 'non-B type acceptor' that we characterized previously [24].

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References

1 Nelson, N. and Neumann, J. (1972) J. Biol. Chem. 247, 1817–1824
2 Hauska, G., Orlich, G., Samoray, D., Hurt, E. and Sane, P.V. (1981) in Proceedings of the 5th International Congress on Photosynthesis (Akoyunoglou, G., ed.), Vol. 2, pp. 903–914, Balaban International Science Services, Philadelphia
3 Rich, P. and Bendall, D.S. (1980) Biochim. Biophys. Acta 592, 153–161
4 Velthuys, B.R. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 6, 2765–2769
5 Mitchell, P. (1976) J. Theor. Biol. 62, 327–367
6 Selak, M.A. and Whitmarsh, J. (1982) FEBS Lett. 150, 2, 286–292
7 Crowther, D. and Hind, G. (1980) Arch. Biochem. Biophys. 204, 2, 568–577
8 Bouges-Bocquet, B. (1981) Biochim. Biophys. Acta 635, 327–340
9 Hurt, E. and Hauska, G. (1982) Photobiochem. Photobiophys. 4, 9–15
10 Joliot, P. and Delosme, R. (1974) Biochim. Biophys. Acta 357, 276–284
11 Renger, G. and Schmidt, G.H. (1977) Z. Naturforsch. 32c, 963–967
12 Joliot, P., Béal, D. and Frilley, B. (1980) J. Chim. Phys. 77, 209–215
13 Bouges-Bocquet, B. (1980) FEBS Lett. 117, 1, 54–58
14 Joliot, P. and Joliot, A. (1983) in Photosynthetic Water Oxidation and Photosystem II Photochemistry (Ryken Internal Symposium, Tokyo) (Inoue, Y., ed.), Academic Press, New York, in the press
15 Velthuys, B.R. and Amesz, J. (1974) Biochim. Biophys. Acta 333, 85–94

16 Wollman, F.A. (1978) Biochim. Biophys. Acta 503, 263–273
17 Diner, B.A. and Bowes, J.M. (1981) in Proceedings of the 5th International Congress on Photosynthesis (Akoyunoglou, G., ed.), Vol. 3, pp. 875–833, Balaban International Science Services, Philadelphia
18 Junge, W. and Jackson, J.B. (1982) in Photosynthesis: Energy Conversion by Plants and Bacteria, Vol. 1 (Govindjee, ed.), pp. 589–646, Academic Press, New York
19 Stuart, A.L. and Wasserman, A.R. (1973) Biochim. Biophys. Acta 314, 284–297
20 Böhme, H. and Cramer, W.A. (1973) Biochim. Biophys. Acta 325, 275–283
21 Wood, P. (1977) Eur. J. Biochem. 72, 605–612
22 Joliot, P. and Joliot, A. (1980) in Proceedings of the 5 th International Congress on Photosynthesis (Akoyunoglou, G., ed), Vol. 3, pp. 885–899, Balaban International Science Services, Philadelphia
23 Horton, P. and Croze, E. (1979) Biochim. Biophys. Acta 545, 188–196
24 Lavergne, J. (1982) Photobiochem. Photobiophys. 3, 273–285