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ELECTRON TRANSFER BETWEEN THE TWO PHOTOSYSTEMS

I. FLASH EXCITATION UNDER OXIDIZING CONDITIONS

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The redox changes of cytochrome *b*-563 (cytochrome *b*), cytochrome *f*, plastocyanin and P-700 were measured on dark-adapted chloroplasts after illumination by a series of flashes in oxidizing conditions (0.1 mM ferricyanide). In these conditions, the plastoquinone pool is fully oxidized and the only available plastoquinol are those formed by Photosystem (PS) II reaction. According to the two-electron gate mechanism proposed by Bouges-Bocquet (Bouges-Bocquet, B. (1973) Biochim. Biophys. Acta 314, 250–256), plastoquinol is mainly formed after the second and the fourth flashes. After the second flash, the reoxidation of plastoquinol occurs by a concerted reaction which reduces most of the cytochrome *b* present and a fraction of PS I donors. Most of these electrons are stored on P-700, which implies a large equilibrium constant between the secondary PS I donors and P-700. One electron is stored on cytochrome *b* during a time ($t_{1/2} \approx 1$ s) much longer than the dark interval between flashes. After the fourth flash, a new plastoquinol molecule is formed, which induces the reduction of PS I donors with no corresponding further reduction of cytochrome *b*. The number of electrons transferred after the fourth flash is larger than that transferred after the second flash although the rate of transfer is lower. To interpret these data, we assume that the plastoquinol formed after the fourth flash is reoxidized by a second concerted reaction: one electron is directly transferred to PS I donors while the other cooperates with the electron stored on cytochrome *b* to reduce a plastoquinone molecule localized on a site close to the outer face of the membrane. This newly formed plastoquinol crosses the membrane and transfers a second electron to PS I donors. This interpretation resembles a model proposed by Velthuys (Velthuys, B.R. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 2765-2769) and which belongs to the modified Q-cycle class of models.

Introduction

The process of electron transfer between the two photosystems has long been described according to the classic Z scheme in which a pool of plastoquinones (PQ) established a direct link between the PS II acceptor Q and the secondary PS I donor cytochrome *f*. As demonstrated by Junge and Witt [1], the electron donors of both photosystems are localized on the inner face of the membrane while the electron acceptors are localized on the outer face. Plastoquinone would then be able to transfer the electrons – or more precisely hydrogen – from one side of the membrane to the other. This model, which is derived from the chemiosmotic theory of Mitchell [2,3], implied that one proton is pumped through the membrane for

Abbreviations: PS, Photosystem; PQ, plastoquinone; cyt *b* or cytochrome *b*, cytochrome *b*-563; cyt *f*, cytochrome *f*; PC, plastocyanin; HQNO, 2-heptyl-4-hydroxyquinoline *N*-oxide; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea.

each electron transferred from PS II to PS I. Nevertheless, the process by which a two-electron carrier as PQ reacts with a one-electron carrier as cytochrome *f* or FeS-Rieske protein was not specified. Moreover, this model did not take into account several experimental results:

(1) the complex behaviour of cytochrome *b* redox changes upon illumination [4,5] makes it impossible to ascribe a well-defined function to this electron carrier in a linear scheme;

(2) the value of the equilibrium constant between Q and P-700 as measured in weak light on uncoupled chloroplasts by Joliot et al. [6] was smaller than 10 although its value should be close to $1 \cdot 10^6$ if calculated from the redox potentials of these two electron carriers;

(3) flash illumination of Chlorella induced a biphasic increase of the transmembrane potential as measured by the 515 nm absorption change [7,8]. The slower phase (phase *b*, $t_{1/2} \approx 6$ ms) was only PS I-dependent and its amplitude was equal to the PS I-induced fast phase. Bouges-Bocquet [9] showed that phase *b* was correlated to cytochrome *f* reduction. The existence of phase *b* proved that an additional electrogenic loop, not included in the classic scheme, is operating. It was first thought that this loop belonged only to a cyclic pathway around PS I. Velthuys [10] and Bouges-Bocquet [11] observed more recently that the amplitude of phase *b* oscillated with a period of 2, which proved that the electrogenic loop could participate in the electron transfer between the two photosystems. Fowler experiments [12] first suggested that an additional site for proton translocation might be localized between the two photosystems: in the presence of ferricyanide, a translocation of two protons per electron was observed associated with the intersystems electron transfer. Saphon and Crofts [13] objected that the buffering properties of methylamine used as an uncoupler by Fowler [12] might perturbe the measurements; however, Velthuys [14] confirmed the stoichiometry found by Fowler and interpreted it as a proof of two sites for proton translocation between the two photosystems.

An essential feature of the Q cycle mechanism proposed by Mitchell [15] is the concept of a concerted reduction of cytochrome b_6 and another one-electron carrier by plastoquinol. In the case of chloroplasts, Velthuys [16] confirmed the validity of this concept and of earlier experiments of Rumberg [4], but proposed a somewhat different interpretation from Mitchell's Q cycle for the cytochrome b_6 reoxidation: a second concerted reaction by another plastoquinol molecule led to the reduction of a second cytochrome *b* molecule; the two reduced cytochrome *b* cooperate to reduce a plastoquinone molecule on a site localized on the outer face of the membrane. Similar hypotheses (modified Q cycles [17]) were proposed to interpret the mechanism of the cyclic electron transfer in photosynthetic bacteria. Whatever the mechanism of cytochrome *b* reoxidation, this process seems to be directly associated with the electrogenic phase as demonstrated by Jackson and Crofts [18] in the case of photosynthetic bacteria, and by Selak and Whitmarsh [19] on chloroplasts.

During the past three years, it has become apparent that these same types of processes were involved in various energy-converting apparatus such as mitochondria, photosynthetic bacteria or chloroplasts. A feature common to these processes is a protein complex which includes the FeS Rieske protein, a cytochrome *f* or *c* and two cytochrome *b* [20–23]. In any case, the process known as oxidant-induced reduction of cytochrome *b* is observed on the complex integrated in the membrane [24] as well as on the isolated complex [25,26].

In this paper, we reinvestigate the process of electron transfer between the two photosystems in oxidizing conditions and under flash illumination. In these experiments, redox changes of cytochrome *b* and of primary and secondary PS I donors were spectrophotometrically measured, in order to establish if the modified Q cycle loop was an obligatory pathway for the transfer of electrons from PS II to PS I.

Material and Methods

Chloroplasts were isolated from market spinach and stored at −70°C as described in Ref. 27. Prior to use, chloroplasts were suspended in 0.05 M phosphate buffer (pH 6.5) with 0.1 M sucrose, 0.1 mM methylviologen, 1 μM gramicidin D and 0.05 M KCl. Experiments were performed at room temperature.

Spectrophotometric measurements were per-

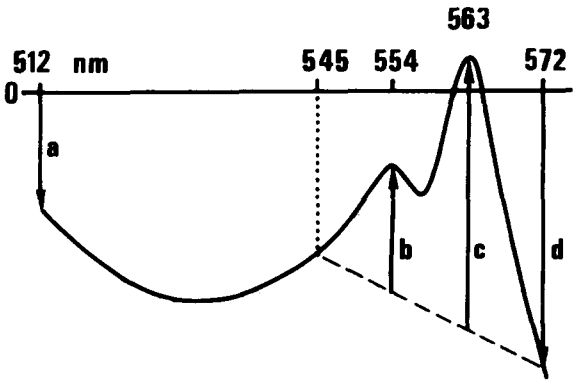


Fig. 1. Schematic representation of the absorption changes associated with the photoreduction of P-700, cytochrome *f*, cytochrome *b* and plastocyanin. The direction of the arrows ***a***, ***b***, ***c***, ***d*** indicates a reduction for each of these components.

formed with an apparatus similar to that described in Ref. 28. The absorption level is sampled using 2 μs monochromatic detecting flashes given at various intervals after the actinic flashes. The following modifications were made to improve the signal-to-noise ratio which is now:

$$1.5 \cdot 10^{-5} < \Delta I/I < 2.5 \cdot 10^{-5}$$

depending upon the available energy of the detecting flash: (a) the monochromator is a Jobin and Yvon HL with a 15 cm diameter concave holographic grating; (b) the detecting beam delivered by the monochromator is split in two, using a Y-shaped fiber optic guide (Schott). In the common window, the fibers of each branch are randomly distributed. One of the arms is used to illuminate the reference cuvette and the other to illuminate the measuring cuvette (optical pathway, 1.6 cm).

Deconvolution of the spectra. The absorption changes due to P-700, plastocyanin, cytochrome *f* and cytochrome *b* were measured in the green region of the spectrum. Five characteristic wavelengths were used in this deconvolution (Fig. 1): as a first approximation, absorption changes ***a***, ***b***, ***c***, ***d***, are characteristic for P-700, cytochrome *f*, cytochrome *b* and plastocyanin, respectively. The following second-order corrections for the overlap of the spectra of these different species were made: it was assumed that the absorption of change ***c*** was a measure of cytochrome *b* redox changes. Contributions of plastocyanin and P-700 are eliminated if one assumes that the spectra of these two species are linear in the 545–572 nm range. ***b*** includes a minor contribution of cytochrome *b*; on the basis of the spectra in Refs. 29–30, we applied the following correction:

$$\Delta I/I(\text{cyt}\,f) = \boldsymbol{b} - 0.17\boldsymbol{c}$$

d includes a minor contribution of cytochrome *f*; we have considered as negligible the contributions of cytochrome *b* and P-700, and we have computed:

$$\Delta I/I(PC) = \boldsymbol{d} - 0.26\Delta I/I(\text{cyt}\,f)$$

a includes a minor contribution of plastocyanin, and we have considered as negligible the contribution of cytochrome *f* and cytochrome *b*. On the basis of the spectrum in Ref. 31, we have computed:

$$\Delta I/I(P\text{-}700) = \boldsymbol{a} - 0.24\Delta I/I(PC)$$

The absorption change due to the reduction of the primary PS II acceptor (C550) significantly perturbes cytochrome-*f* measurements for the shortest time of observation 1 ms after the actinic flash (see Fig. 2A). In Fig. 3A–D, the corresponding values for cytochrome *f* were obtained by extrapolation at time zero of the kinetics obtained for longer detecting times.

Results

Concentrations of primary and secondary donors (Table I)

The maximum absorption changes due to P-700, plastocyanin and cytochrome *f* were measured using chloroplasts in the presence of sodium ascorbate, hydroxylamine and DCMU illuminated by strong d.c.-light (this experiment is detailed in Ref. 33). The maximum absorption change due to cytochrome-*b* reduction was measured in the presence of 0.1 mM ferricyanide either in strong d.c.-light or after four saturating flashes (see Fig. 3D).

The values of ϵ which we used in our deconvolution procedure were computed from Ref. 32 for cytochromes *b* and *f* and from Ref. 31 for plastocyanin. We estimated the concentration of

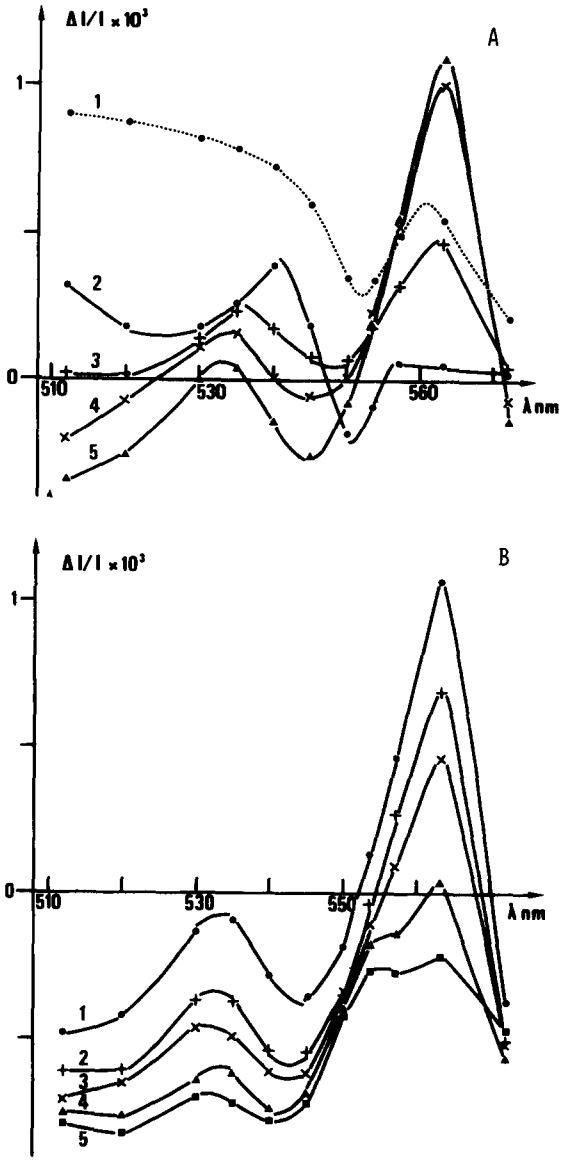


Fig. 2. Absorption changes measured for various dark times following two saturating xenon flashes 100 ms apart. 30 μg/ml chlorophyll. 0.1 mM ferricyanide. (A) curve 1 (dashed line): absorption changes measured 50 ms after the first flash. Curves 2–5: the base line is the absorption level 50 ms after the first flash (curve 1). Time of detection after the second flash 2 (●), 1 ms; 3(+), 5 ms; 4(×), 20 ms; 5(▲), 50 ms. (B) 1 (●), 100 ms; 2(+), 300 ms; 3(×), 500 ms; 4(▲), 1 s, 5(■), 1.5 s.

P-700 by comparing *f* absorption change (421–439 nm) with P-700 absorption change (429 nm). On the basis of the spectra shown in Fig. 1 of Ref. 33 and of the spectra and ε values for cytochrome *f* [29,32] and P-700 [34], we computed a ratio equal to 0.66 for the concentration of cytochrome *f* vs. the concentration of P-700, i.e., one P-700 for 575 chlorophylls. As we did not take into account variations of the flattening factor in the 421–439 nm range, this concentration of P-700 could be 5–10% underestimated. In any case, as large variations in the values are reported in the literature, Table I gives only a rough estimate of the concentration of PS I donors.

Number of electrons stored on primary and secondary PS I donors

Chloroplasts were submitted to a series of short saturating flashes 50 ms apart, in the presence of 3 mM hydroxylamine, 50 μM DCMU and 3 mM sodium ascorbate (data not shown). Under these conditions, we did not observe any cytochrome-*b* reduction, which shows that no dihydroplastoquinone was present prior to the illumination. Assuming an efficiency of 1 for the charge separation, the number of electrons transferred by each flash is proportional to the concentration of reduced P-700 present before each flash, measured at 429 nm in order to minimize the contribution of cytochrome *f*. The sum of the number of electrons transferred along a series of ten flashes is 3.5.

From Table I, we obtained a value of 2.78 for the sum of cytochrome *f*, plastocyanin and P-700 concentrations. One can thus conclude to the presence of a fourth pool of electron donor, the concentration of which is comparable to that of cyto-

TABLE I

R, chlorophyll concentration vs. concentration of each electron carrier. *C*, concentration of each electron carrier vs. P-700 concentration. Chlorophyll concentration, 30 μg/ml. The value of *R* for P-700 was computed by comparison with the concentration of cytochrome *f* (see text).

	$\Delta I/I$ ($\times 10^3$) green region of the spectrum	ε ($\times 10^{-3}$ $M^{-1} \cdot cm^{-1}$)	*R*	*C*	
Cyt *b*	2	14	850	0.67	
Cyt f	2.6	18.5	870	0.66	
PC	1.88	7.8	510	1.12	= *sum* 2.78
P-700	1.41	?	575	1	

chrome *f*. This pool very likely corresponds to the FeS protein.

Effect of a series of flashes given under oxidizing conditions

In the experiments shown in Fig. 2A and B, the chloroplasts were given two saturating xenon flashes 100 ms apart. 0.1 mM ferricyanide was added in order to partially oxidize the secondary PS I donors. 50 ms after the first actinic flash (spectrum 1, Fig. 2A), we observe a large oxidation of P-700 as shown by the absorption increase at 512 nm and minor absorption changes due to plastocyanin and cytochrome-*f* oxidation and to cytochrome-*b* reduction. Within the experimental errors, this spectrum remains rather stable for times as long as 1 · 2 s after the flash (see Fig. 3A). During the first 100 ms following the second flash, a large reduction of cytochrome *b* (which peaks at 563 and 535 nm) associated with a reduction of P-700 and plastocyanin are observed. A significant contribution of cytochrome *f* reduction is seen only when the detecting time is longer than 500 ms. A detailed spectrum in the 563 nm region has

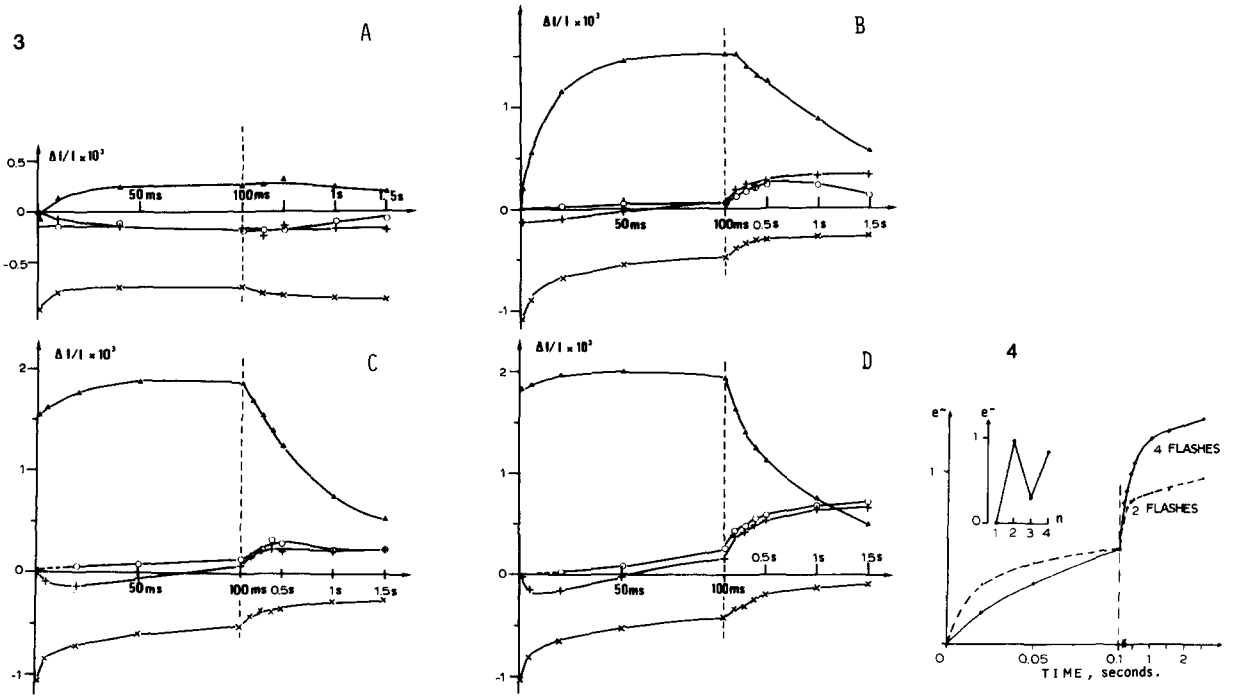


Fig. 3. Kinetics of the redox changes measured after a series of 1–4 flashes. 30 μg/ml chlorophyll. 0.1 mM ferricyanide. P-700 (×); plastocyanin (+); cytochrome *f* (○); cytochrome *b* (▲). Dark interval between actinic flashes: 50 ms. The first detecting flash is given 1 ms after the last actinic flash of the series. For each component, the absorption change is plotted so that increasing values correspond to increasingly reduced state. (A), one flash; (B), series of two flashes; (C), series of three flashes; (D), series of four flashes.

Fig. 4. Number of electrons transferred to PS I donors after a group of two or four flashes (computed from experiments, Fig. 3). For each component, P-700, plastocyanin and cytochrome *f*, the difference between the absorption change at time *t* and the absorption change 1 ms after the last flash of the series was measured. The number of electrons transferred to P-700, plastocyanin and cytochrome *f* has been computed from Fig. 3B and D, taking into account the relative concentration of each carrier as given in Table I. These numbers are thus expressed as a ratio to P-700 concentration. Then the total number of electrons transferred to PS I donors was computed in summing the number of electrons corresponding to each component. This computation does not take into account the electrons stored in the FeS protein that we are not able to detect spectrophotometrically. (●), two flashes; (+), four flashes. Inset: number of electrons transferred to PS I donors after flash *n* minus number of electrons transferred from 50 ms to 2.5 s after flash $(n-1)$, computed from Fig. 3A–D. This difference gives the additional number of electrons transferred to PS I donors after each flash of the series.

been measured 90 ms apart (data not shown). The four spectra peak at the same wavelength (563.5 nm).

In the experiments shown on Fig. 3A–D, chloroplasts were given a series of 1–4 saturating actinic flashes 50 ms apart. The kinetics of the redox changes of P-700, plastocyanin, cytochrome *f* and cytochrome *b* measured during 1.5 s after the last flash of the series are plotted after deconvolutions as described in Material and Methods. On the basis of the experimental results shown on Fig. 3A–D, we computed (Fig. 4) the number of electrons transferred from PS II to PS I donors (i.e. P-700, plastocyanin and cytochrome *f*) from 1 ms to 2.5 s after a series of two or four actinic flashes (see legend Fig. 4).

(1) First flash (Fig. 3A). Illumination by a single flash given to dark-adapted chloroplasts induced in less than 1 ms an oxidation of P-700 with which a minor oxidation of secondary PS I donors plastocyanin and cytochrome *f* is associated. The amount of photooxidized P-700 is probably overestimated, due to the contribution of a long-lived PS II induced signal (approx. 3.5 s), not eliminated by our deconvolution procedure.

During the subsequent dark period, only minor changes occur which shows that very few electrons are transferred from PS II to PS I donors. As already shown by Velthuys [16], only a small fraction of cytochrome *b* is reduced (about 15% of the maximum reducible cytochrome *b*). In the oxidizing conditions we used, the PQ pool including the acceptor B is fully oxidized. Thus, illumination by a single flash should not induce the formation of plastoquinol, the source of electrons required to reduce cytochrome *b* and PS I donors. Preliminary experiments using flashes of different energies suggest that the small amount of reduced cytochrome *b* is due to double-hit processes occuring in PS II centers.

(2) Second flash (Fig. 3B). Unlike a single flash, the second flash induced a large wave of reduction of both cytochrome *b* [16] and PS I donors. The reduction of PS I donors is clearly biphasic (half-times are approx. 10 and 150 ms) while the reduction of cytochrome *b* is monophasic ($t_{1/2} \approx 10$ ms). At the end of the fast phase, i.e., 50 ms after the second flash, 0.47 electron has been transferred to PS I donors and 0.48 electron has been transferred to cytochrome *b*. These results agree with the hypothesis of a concerted oxidation of plastoquinol by the cytochrome *b-f* complex. During the slow phase of PS I donors reduction, no further reduction of cytochrome *b* is observed. Therefore, at the end of the slow phase fewer electrons are stored on cytochrome *b* than on PS I donors.

The reoxidation of cytochrome *b* ($t_{1/2} \approx 1.2$ s) is considerably slower than the reduction of PS I donors. We can then conclude that one electron is stored on cytochrome *b* during a time much longer than the time interval between flashes. The process through which cytochrome *b* is slowly reoxidized remains unknown; the substrate for this reoxidation is not ferricyanide, as an increase of a factor 10 in its concentration does not significantly accelerate this process.

During the first 100 ms after the second flash, most of the electrons transferred to PS I donors are stored in P-700. As there is practically no electron retained in cytochrome *f*, we conclude that in the conditions of this experiment, the equilibrium constant between cytochrome *f* and P-700 is high.

(3) Third flash (Fig. 3C). The number of electrons generated by the third flash and available for the reduction of PS I donors is low if one subtracts the large fraction of the electrons generated by the second flash which has not been yet transferred to PS I donors (see inset, Fig. 4). This behaviour is expected since 50 ms after the second flash, most of the secondary PS II acceptor B is oxidized.

(4) Fourth flash (Fig. 3d). As shown in Figs. 3D and 4, curve 2, the fourth flash induces a large and slow reduction of all PS I donors ($t_{1/2} \approx 100$–200 ms) without a correspondingly significant reduction of cytochrome *b*. As an appreciable fraction of cytochrome *f* is reduced in these conditions, one expects that the FeS carrier is also partially reduced. Therefore, the number of electrons transferred to PS I donors shown in the inset of Fig. 4 is underestimated.

Discussion

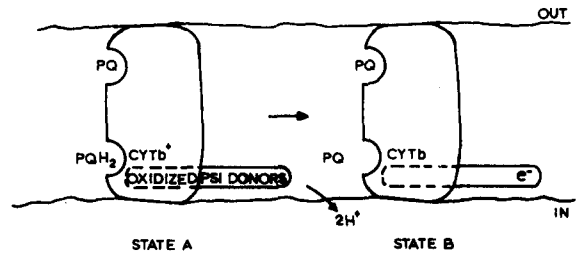
In the conditions of our experiments, the plastoquinone pool is fully oxidized prior to the illumination. In order to simplify the discussion, we will consider that, because of the two-electron

gate mechanism, plastoquinol molecules are mainly formed by PS II after the second and the fourth flashes.

(1) After the second flash (Scheme I). During the 50 ms following the flash, the parallel reduction of cytochrome *b* and PS I donors agrees with a model of a concerted oxidation of plastoquinol as depicted in Scheme I, transition A–B. The absence of a well-defined lag in the reduction of cytochrome *b* shows that state A, in which a plastoquinol molecule is associated with the cytochrome *b-f* complex, is reached in less than 2 ms after the second flash. A possible interpretation, already proposed in Ref. 35, is that a fraction of the cytochrome *b-f* complex is directly associated with PS II centers. In any case, the relative location of cytochrome *b-f* complexes and of PS II is not a critical point in the following discussion.

On the basis of the measurement of the field indicating 515 nm absorption change, we estimate that the concentrations of PS I and II are approximately equal. Thus, after two flashes, about one plastoquinol molecule (i.e., two electrons) per PS I center has been formed, an amount which exceeds the amount of cytochrome *b-f* complexes. At 50 ms after the second flash, only half of these plastoquinol molecules has already been oxidized, as the number of electrons stored on cytochrome *b* and PS I donors does not exceed $0.47 + 0.48 = 0.95$. The mechanism by which the remaining fraction of plastoquinol is slowly oxidized will be discussed below.

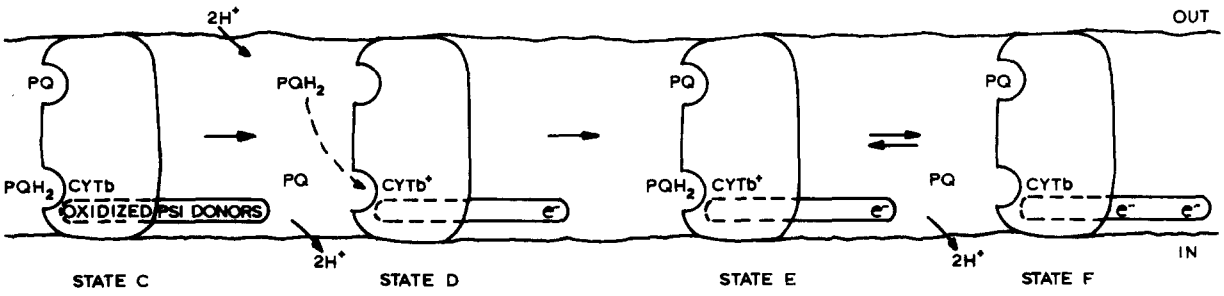
(2) After the fourth flash (Scheme II). Within about the 2 ms following the fourth flash (state C, scheme II), new plastoquinol molecules are available to the cytochrome *b-f* complexes while the PS I donors are back in their oxidized form. Two types



Scheme I. The 'PS I donors box' includes first the FeS carrier and cytochrome *f* which both belong to the cytochrome *b-f* complex, and second, plastocyanin and P-700. This model does not imply a permanent association between the cytochrome *b-f* complex, plastocyanin and P-700.

of hypotheses can be proposed to explain the slow reduction of PS I donors with which is not associated a significant further reduction of cytochrome *b*: (a) plastoquinol transfers its electron to PS I donors by a mechanism in which cytochrome *b* would not be involved; (b) we presently favor a model which resembles the model initially proposed by Velthuys [16] (Scheme II, transitions C–F). First, a plastoquinol molecule is oxidized by a new concerted reaction which leads to state D: one electron is transferred on the PS I donors; the second electron cooperates with the one stored on cytochrome *b* to reduce a plastoquinone molecule localized on the outer face of the membrane. This hypothesis explains the transitory acceleration of cytochrome *b* reoxidation in the 100 ms range after the fourth flash.

This model implies an electrogenic step in which two electrons cross the membrane. Even in the absence of ionophores, this electrogenic reaction is too slow to be easily measured because its half-time ($t_{1/2} \approx 100$–200 ms) is comparable to the decay



Scheme II.

time of the transmembrane potential. The process of the two-electron transfer through the membrane might involve the second cytochrome-*b* molecule known to be present in the cytochrome *b-f* complex. Nevertheless, since neither a significant additional signal in the 563 nm region nor a shift in the cytochrome-*b* peak is induced by the fourth flash, we must assume that both cytochromes *b* cannot coexist in their reduced forms. Most probably, the two electrons required to reduce the plastoquinone molecule cross the membrane almost in synchrony and are finally stabilized on the outer face of the membrane by the formation of plastoquinol.

Preliminary experiments showed us that the slower phase in PS I donors reduction ($t_{1/2} \approx 150$ ms) is highly pH-dependent: at pH 7.5, this slow phase is totally suppressed. Then we conclude that in oxidizing conditions a concentration of protons higher than $1 \cdot 10^{-7}$ is required for the formation of plastoquinol on the outer face of the membrane (state C–D transition); such a high proton concentration is certainly not required under reducing conditions.

During the following transition (state D–E) the newly formed plastoquinol molecule becomes available to the FeS and cytochrome-*b* sites localized on the inner face of the membrane. A last step (E–F) could occur, very similar to the A–B transition. Thus, the transfer of two electrons to PS I donors could be associated with the overall transition state C–F, while only one electron was transferred during the A–B transition. This model explains why a somewhat larger number of electrons is transferred to PS I donors after the fourth flash rather than after the second flash despite a smaller number of plastoquinol formed after the fourth flash (damping of the period-2 oscillations). Nevertheless, if the totality of the cytochrome *b-f* complexes was undergoing the E–F step, we would expect that a larger number of electrons would be transferred to PS I donors after the fourth flash than what is actually observed (see inset Fig. 4). Besides, the transitory acceleration for the cytochrome *b* oxidation (C–D step) would be entirely balanced by its reduction (E–F step), which is not observed. We conclude that state E and F are in equilibrium and that an appreciable fraction of the electrons is stored in the plastoquinone pool. We must stress that the kinetics of cytochrome *b* beyond 100 ms after the fourth flash reflect the superimposition of at least three processes: the oxidation linked to transition C–D, the reduction linked to transition E–F and a slow oxidation of unknown origin already observed after the second flash. As the number of plastoquinone molecules formed after a group of two flashes is larger than the number of cytochrome *b-f* complexes, the transitions C–D or C–F should also occur after the second flash for a fraction (approx. 30%) of the cytochrome *b-f* complexes. This process could account for the slow phase observed in the reduction of PS I donors after the second flash.

Our results indicate that the electron transfer between PS II and I probably operates via the cytochrome *b-f* loop even in oxidizing conditions. This conclusion is consistent with the measurement of proton exchange which shows that the translocation of more than one proton is associated with the transfer of one electron between the two photosystems even in the presence of ferricyanide [12]. Moreover, the rates of proton exchange measured in this condition on both sides of the membrane are slow, in agreement with the slow electron transfer reaction we report. In the model we propose, the reduction of plastoquinone does not imply any cooperation between different cytochrome *b-f* complexes but requires the storage of one electron on cytochrome *b*.

Our data raise an apparent paradox which is that the rate of state C–E transition is considerably slower than the generally admitted limiting step (10–15 ms) for electron transfer between the two photosystems.

Lavergne [36] recently identified in the cytochrome *b-f* complex, an electron carrier, G, located close to the outer face of the membrane which is likely involved in the process of cytochrome *b* oxidation: Lavergne observed that in reducing conditions the equilibrium constant for the electron transfer from cytochrome *b* to G is larger than 1; on the contrary, in oxidizing conditions, electrons cannot be transferred to G and are stored on cytochrome *b*. These problems will be discussed in more details in the following article [33].

Acknowledgments

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