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ELECTRON TRANSPORT BETWEEN PLASTOQUINONE AND CHLOROPHYLL a_I IN CHLOROPLASTS

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SUMMARY

After preillumination with System I light spinach chloroplasts were excited by one flash or a group of saturating flashes. During the following dark period the time courses of the oxidation of plastohydroquinone and of the simultaneous reduction of oxidized cytochrome *f* and chlorophyll a_I (P700) have been measured.

1. From a correlation of these kinetics it can be concluded that at least 85% of the electrons from plastohydroquinone are transferred to chlorophyll a_I.

2. After one flash 93% of the oxidized chlorophyll a_I is reduced. This suggests a high equilibrium constant between chlorophyll a_I and its donor as well as an equilibration between different chlorophyll a_I molecules.

3. Cytochrome *f* is also reduced by plastohydroquinone. A ratio of active cytochrome *f* to chlorophyll a_I of 0.4:1 is observed. The half-life time of the reduction of cytochrome *f* is 17 ms. The time course indicates that in the dark cytochrome *f* does not transfer electrons to chlorophyll a_I and that no more than 15% of the electron transport passes cytochrome *f*. Therefore cytochrome *f* should be situated in a side path of the linear electron transport.

4. The electrons which are released from plastohydroquinone and are not accepted by oxidized cytochrome *f* and chlorophyll a_I have been calculated. From this difference properties of an electron carrier, as yet not identified, between plastoquinone and chlorophyll a_I are predicted.

INTRODUCTION

The two photochemical light reactions of chlorophyll a_I (P700) and chlorophyll a_{II} (P680) transfer electrons from water to the terminal electron acceptor $NADP^+$ through a chain of electron carriers (for a review *cf.* ref. 1). A link between both chlorophylls is the pool of about 7 plastoquinone molecules. The rate-limiting step of the electron transport in strong light is the oxidation of plastohydroquinone[2]. After one flash the oxidation of plastohydroquinone shows the rate-limiting time of approx. 20 ms and corresponds to the simultaneous reduction of chlorophyll a_I[3].

Abbreviation: Tricine, *N*-tris(hydroxymethyl)methylglycine.

Plastoquinone is reduced by light reaction II with a half-life of 0.6 ms[3,4]. In bright light or groups of saturating flashes plastoquinone accumulates in the reduced form, while the electron carriers on the side of light reaction I behind the rate-limiting step accumulate in the oxidized form. Upon darkening the kinetics of the reoxidation of plastohydroquinone indicate electron carriers between plastoquinone and chlorophyll a_{I} with an electron capacity of about two per electron transport chain[3]. Between at least ten chains an electron exchange is possible within a common strand of plastoquinone[5]. Although these and other investigations have contributed detailed information on the reactions in the electron transport chain, there are still several uncertainities on the electron transfer between the pool of plastoquinone and chlorophyll a_{I}.

Cytochrome *f* is one component between the pool and light reaction I[6–9]. A sequential electron transport from plastoquinone *via* cytochrome *f* to chlorophyll a_{I} is mostly assumed. But the reduction time of cytochrome *f* is uncertain. The following values have been reported: 10 ms, 86 ms[10], from 1 to 10 ms and 180 ms[11], and about 20 ms[12]. The oxidation time of cytochrome *f* shows values between 1 ms[13] and 0.3 ms[14] in green plants. But the nature of the electron acceptor is uncertain. After flash excitation the kinetics of the oxidation of cytochrome *f* and the reduction of chlorophyll a_{I} did not prove an electron transfer between these two electron carriers[15]. Also the different equilibrium constants K derived from the redox states of cytochrome *f* and chlorophyll a_{I} in the dark ($K \approx 100$) and during light-limited excitation ($K = 5$–10) are not consistent with a simple in-series arrangement of the two components[16,8]. Similar conclusions have been drawn from the relaxation times of cytochrome *f* and chlorophyll a_{I} in algae[12].

Plastocyanin is another compound supposed to be located between plastoquinone and chlorophyll a_{I}. But the site of plastocyanin has been concluded only indirectly from experiments with added plastocyanin[17,18] or with a mutant of *Chlamydomonas* lacking plastocyanin[19].

Previous investigations dealing with the electron transfer from plastoquinone to chlorophyll a_{I} have been carried out in light-limited conditions[8,9]. In these conditions the time course of the absorption changes is dependent on the intensity of the exciting light, and an equilibrium between the components has to be assumed. More information about the electron transfer mechanisms can be deduced from the kinetics of the reactions.

Recently we have investigated the reduction of chlorophyll a_{I} by electron carriers between plastoquinone and chlorophyll a_{I}. Two fast reaction times have been resolved: 20 μs and 200 μs[20,21]. In this work this fast electron transfer to chlorophyll a_{I} is avoided by preillumination with far-red light. The slower electron transfer from plastoquinone *via* the rate-limiting step to chlorophyll a_{I}, cytochrome *f* and to a non-identified electron carrier is examined in uncoupled conditions. The mechanism of these reactions is discussed in detail.

MATERIALS AND METHODS

Spinach chloroplasts (Class II) were isolated as described in ref. 22 but with 10^{-2} M ascorbate in the grinding solution. In order to prepare broken chloroplasts these chloroplasts have been osmotically shocked in 1 mM 2-(*N*-morpholino)-

ethanesulfonic acid buffer at pH 6.4 for 30 min. The chloroplasts were resuspended in 0.4 M sucrose (with 5% dimethylsulfoxide), frozen in liquid N_2, and stored until use. The reaction mixture contained 10 μM chlorophyll, 20 mM *N*-tris(hydroxymethyl)methylglycine (Tricine) buffer at pH 7.2, 20 mM KCl, 1 mM $MgCl_2$, 10 μM benzylviologen as artificial electron acceptor and 0.5 μM gramicidin D for uncoupling. The temperature of the sample was 22 °C.

Class I (type A) chloroplasts were isolated rapidly in isotonic media as described in ref. 23. (The preparation was carried out by P. Heathcote.) The reaction mixture contained 0.1 M sorbitol in addition to the composition stated.

Titration of electron carriers between the light reactions by flashes

Titration of the oxidized electron carriers between the light reactions by distinct numbers of electrons has been realized by special flash excitation. This technique takes advantage of the different reaction times in the electron transport chain. Light reaction II produces electrons with a half-life of 0.6 ms[4], but the rate-limiting oxidation of plastohydroquinone occurs with a 30-times greater half-life of 20 ms[3]. Therefore nearly all electrons produced by excitation with a group of saturating flashes at distances of 1.6 ms are accumulated in the pool of plastoquinone. After the last flash of a group one should observe the reoxidation of plastohydroquinone and simultaneously the reduction of its electron acceptors. Using different flash groups every redox state of these electron acceptors can be realized. Fig. 1 illustrates the experimental procedure. Far-red light (Schott interference filter IL 720) which excites mainly light reaction I was switched on for 4.5 s with an intensity of $1.3 \cdot 10^4$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}. This preillumination oxidized all electron carriers between the two light reactions. The far-red light was switched off during the measurement of the absorption changes by an electronically controlled shutter to avoid any disturbing reoxidation. The intensity of the monitoring light was lower than 50 ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}.

For registration an improved repetitive flash photometer with double beams was employed similar to that described in ref. 24. For irradiation with repetitive single flashes or groups of flashes four xenon flash lamps (20 μs flash duration) have been used. The intensity of the flashes was saturating. The flashes were passed through Schott filters BG 23 (6 mm) to obtain blue actinic light for simultaneous measurement of plastoquinone at 265 nm ($\Delta\lambda = 2.5$ nm) and chlorophyll a_I at 705 nm ($\Delta\lambda = 3$ nm). Red actinic light for the measurement of cytochrome *f* as difference

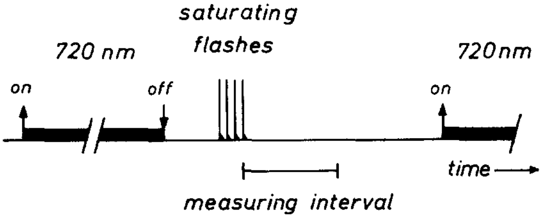

Fig. 1. Experimental conditions. Chloroplasts were periodically excited for 4.5 s with far-red light of 720 nm ($1.3 \cdot 10^4$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}) and during a following dark period of 0.5 s with a flash group. The time between the saturating flashes of the group was 1.6 ms. The flash-induced signals were stored in the averager during every measuring interval.

of the absorption changes at 554 nm *minus* 540 nm ($\Delta\lambda = 2$ nm) was obtained by Schott filters RG 610 (3 mm) and KG 2 (2 mm). Between 32 and 256 signals were averaged with a frequency of 0.2 Hz. The content of the sample cuvette was changed after every 32 signals. The optical path length was 20 mm. The electrical bandwidth ranged from 0.1 Hz to 5 kHz and was limited by the selected distance of the addresses of the signal averager (Fabri-Tek 1072 with plug-in SD-72/2A and SW-70).

RESULTS AND DISCUSSION

The extent of the absorption changes in relation to an increasing number of flashes is shown in Fig. 2. The half-life times of the absorption changes are presented in Fig. 3.

Oxidation of plastohydroquinone

During excitation plastoquinone is reduced. In a following dark period the reoxidation of plastohydroquinone is visible as a positive absorption change at 265 nm[25]. The fast reoxidation after the last flash of a flash group is depicted in Figs 2A and 2D. The amplitude after one saturating flash corresponds to a transfer of one electron per electron transport chain[3]. With increasing flash numbers the

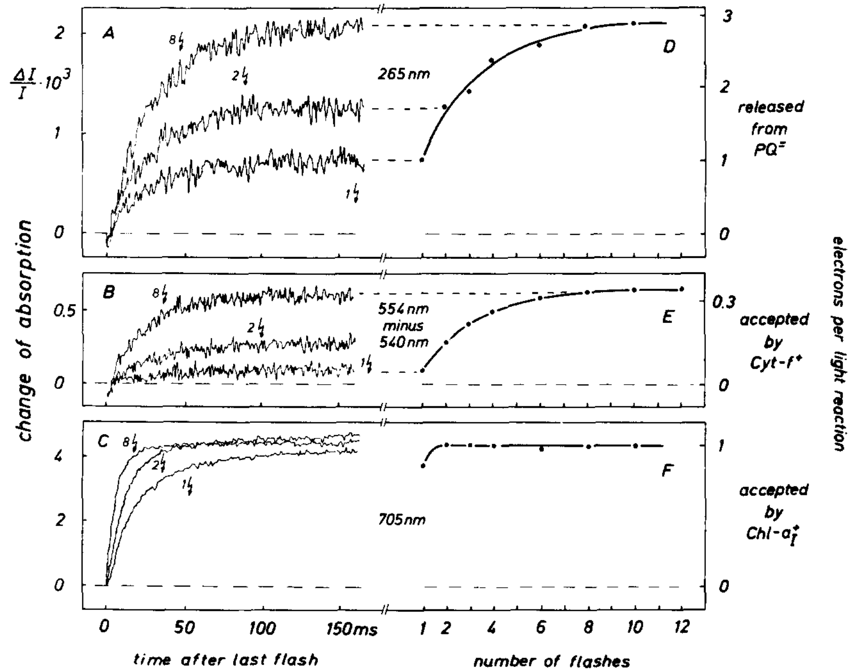

Fig. 2. Absorption changes after the last flash of a flash group as a function of the number of saturating flashes. A and D, changes of absorption at 265 nm (plastoquinone). B and E, differences of absorption changes at 554 nm *minus* 540 nm (cytochrome *f*). C and F, changes of absorption at 705 nm (chlorophyll a_I). Left (A, B and C), time courses after one, two, and eight flashes. Right (D, E and F), extent of the absorption changes. Every trace is the average of 64 signals. The assumptions for the scales on the right-hand side are defined in the text. $PQ^=$, plastohydroquinone.

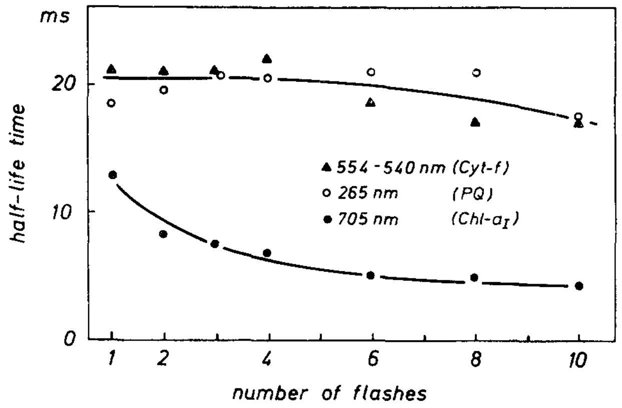

Fig. 3. The half-life time of absorption changes at 265 nm and 705 nm and of the difference of absorption changes at 554 nm *minus* 540 nm after the last flash of a flash group as function of the number of saturating flashes. Conditions as in Fig. 2. PQ, plastoquinone.

amplitude of the fast reoxidation converges to three times the value after a single flash, although the amount of reduced plastoquinone corresponds to about 4.5 electron equivalents after 10 flashes (not shown in Fig. 2A). This demonstrates that the electron acceptors of plastoquinone have an electron capacity of three per electron transport chain. This confirms the previous measurements in ref. 3. The transients fit first-order kinetics and show an approximate constant half-life of about 20 ms as depicted in Fig. 3.

Extinction coefficient of chlorophyll a_I and interaction between different chlorophyll a_I molecules

Chlorophyll a_I is oxidized completely by saturating flashes. The following reduction is presented in Figs 2C and 2F. The amplitude of chlorophyll a_I after one flash indicates a reduction to a very high extent. This result was tested in many preparations: broken chloroplasts yield an average value of 91% (8 different preparations) and Class I and II chloroplasts of 93% of reduced chlorophyll a_I after one saturating flash (7 different preparations). The deviation of the individual results was not greater than ±3%. After two or more flashes chlorophyll a_I is reduced completely.

From the amplitude after one flash it is possible to calculate a value of the differential extinction coefficient of chlorophyll a_I, if the number of electrons produced by one flash is known. This number has been determined by the oxygen yield per flash[2]: The same preparation used in Fig. 2 had a ratio of 530 chlorophyll molecules per light reaction II. (This experiment was carried out by Dr G. Renger.) If one assumes that all electrons produced by one flash are transferred to the oxidized chlorophyll a_I the calculated value would represent the lower limit of the differential extinction coefficient. In case of any distribution of the electrons to other electron carriers, the amplitude of the absorption change is due to a smaller number of reduced chlorophylls a_I and the extinction coefficient must be greater. As can be seen from Fig. 2B a small amount of cytochrome *f* is reduced after one flash. The amplitude indicates that about 7% of the electrons produced by light reaction II are transferred to cytochrome *f*. Taking this into account the differential extinction coefficient of chlorophyll a_I in the intact *in vivo* system has been evaluated: $\Delta\varepsilon(705\ \mathrm{nm}) \geqslant 5.4 \cdot 10^4\ \mathrm{cm}^{-1} \cdot \mathrm{M}^{-1}$. It cannot be excluded that other electron carriers have received electrons. Recently a value of $\Delta\varepsilon(701\ \mathrm{nm}) = 6.4 \cdot 10^4\ \mathrm{cm}^{-1} \cdot \mathrm{M}^{-1}$ was determined with *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine as artificial electron donor in System I particles[26]. The difference between the values is small enough to state a ratio of chlorophyll a_I to chlorophyll a_{II} near one to one. Chlorophyll a_I receives nearly all of the electrons released from water by one flash.

This result enables one to discuss the interaction of the electron carriers. Joliot *et al.*[27] proposed a low equilibrium constant K ($K = 3$–10) of the overall electron transfer between the two light reactions. Since chlorophyll a_I receives most of the electrons, the equilibrium constant, even of the last electron transfer step to chlorophyll a_I, must be greater.

Another, but contrary, model of the electron transport chain was formulated mathematically by Malkin[28]. It consists of independent chains of components with large equilibrium constants for every electron transfer step (*cf.* also ref. 8). This model has been ruled out since the coupling of at least ten light reactions I and II *via* the pool of plastoquinone has been shown[5]. But it can be applied to the electron carriers on the side of light reaction I of plastoquinone. According to their electron capacity, independent chains of three electron carriers are assumed behind the pool of plastoquinone. Every electron transfer step may have a large equilibrium constant and chlorophyll a_I should be at the end of these chains. After one saturating flash the number of electrons produced equals the number of chlorophylls a_I. So after one flash *e.g.* 10 electrons may be transferred to the pool of plastoquinone and then distributed statistically to 10 of these chains. A calculation on this problem shows that oxidized chlorophyll a_I should be reduced to an extent of 66%. A coupling of more than 10 light reactions would decrease this value slightly. Since 93% are found the assumption of independent chains is ruled out and an equilibration between different chlorophyll a_I molecules has to be postulated, possibly *via* a preceding electron carrier.

The electron transfer from plastoquinone to chlorophyll a_I

If the electron carriers between the two light reactions are in an oxidized state chlorophyll a_I is reduced after a flash not before the electrons have passed these redox carriers. Therefore the reduction of chlorophyll a_I after the first flash should start with a lag. A lag of about 2 ms has been resolved. Details will be reported in a subsequent paper. Apart from the short lag in the beginning the reduction of chlorophyll a_I has a similar time course as the reoxidation of plastohydroquinone after one flash (see Figs 2A and 2C) in accordance with the previous results[3]. This is consistent with the conclusion from the differential extinction coefficient that chlorophyll a_I receives most of the electrons from light reaction II. At increasing number of flashes the first half-life time of the chlorophyll a_I reduction decreases down to 4.3 ms (see Fig. 3). To elucidate the electron transfer from plastoquinone to chlorophyll a_I the initial rates of the oxidation of plastohydroquinone and of the reduction of chlorophyll a_I are considered. The initial rate of a first-order reaction is

$$\left(\frac{\mathrm{d}c}{\mathrm{d}t}\right)_{t=0} = -\ln 2 \frac{c_0}{t_{1/2}}$$

c_0 is the initial concentration of the substance and $t_{1/2}$ the half-life time of the reaction. The amplitudes of the absorption changes are proportional to the concentrations. Thus the initial rates can be easily estimated from the data presented in Figs 2 and 3. With increasing flash number the extent of reduced chlorophyll $a_{\mathrm{I}}{}^+$ remains constant and the half-life is diminished. However, the extent of oxidized plastohydroquinone is increased and the half-life remains constant. The relative initial rates calculated from these data are depicted in Fig. 4. The initial rate of the oxidation of plastohydroquinone increases simultaneously with the rate of the reduction of chlorophyll a_{I} up to three times. This increase of the electron transfer rate is the reason for the acceleration of the chlorophyll a_{I} reduction from 13 ms down to 4 ms (Fig. 3). The reduction of chlorophyll a_{I} shows only approximately first-order kinetics and the absolute values of the initial rates are not exactly equal. But with regard to the result that after one flash most of the electrons from water are transferred *via* plastoquinone to chlorophyll a_{I}, the following can be stated as a first approximation also for longer excitation times: During and immediately after excitation nearly all electrons from plastohydroquinone are transferred to oxidized chlorophyll a_{I}.

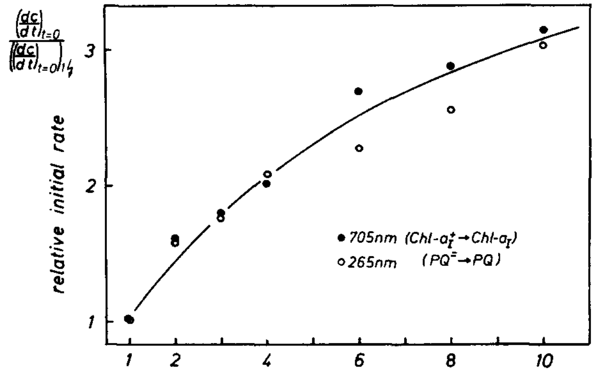

Fig. 4. Initial rates of the absorption changes at 705 nm and 265 nm as a function of the number of preceding flashes of a flash group related to the initial rate after one flash. The values are calculated from the results shown in Figs 2 and 3.

This supports the sequence mostly assumed for linear electron transport that electrons from water must pass through chlorophyll a_{I} on their way to NADP$^+$. But it contradicts the conclusion from experiments on the influence of Mg^{2+}, that linear electron transport to NADP$^+$ does not occur *via* chlorophyll a_{I}[29]. The reported rate of NADP$^+$ reduction greatly exceeds that of chlorophyll a_{I} at the same concentration (*cf.* Materials and Methods) of 1 mM Mg^{2+}. It is likely that a fast electron transfer to chlorophyll a_{I} from its primary donors escaped detection because of the modulation frequency of only 39 Hz. This would result in a lower amplitude and reduction rate, respectively, of chlorophyll a_{I}, *cf.* ref. 20.

Plastoquinone and chlorophyll a_{I} may be either in direct contact or interact *via* other components. This can be decided from the kinetics after excitation with a larger flash group (see Figs 2A and 2C, eight flashes). In the case of a direct contact the probability of the oxidation of plastohydroquinone is decreased after the fast reduction of chlorophyll a_I. The time course is expected to be biphasic. Since the oxidation of plastohydroquinone shows homogeneous first-order kinetics an electron carrier must transfer the electrons from plastoquinone to chlorophyll a_I.

Reduction of cytochrome f

Cytochrome *f* is oxidized completely by far-red light. It remains oxidized during excitation with saturating flashes. By the experimental technique described, it was possible to resolve the time course of the reduction of cytochrome *f* in the following dark period (Fig. 2B). The half-life is $t_{1/2} = 17$ ms [six different preparations of broken, Class II and Class I (Type A) chloroplasts]. This reaction time is little changed by increasing flash numbers (see Fig. 3). The electron transfer from plastoquinone to cytochrome *f* is demonstrated by the equal half-life time of the reduction of cytochrome *f* and the oxidation of plastohydroquinone, within the accuracy of the measurements at all excitation conditions, see Fig. 3.

After one flash not more than 15% of cytochrome *f* is reduced. The maximal amount of cytochrome *f* is not reduced before the amplitude of the plastohydroquinone oxidation also shows its maximum value, Figs 2D and 2E. The spectrum of the absorption changes in the α-band region of the cytochromes is presented in Fig. 5. The *in vitro* oxidized *minus* reduced difference spectrum of cytochrome *f*[30,31] shows a good agreement. Only minor contributions originating from cytochrome *b*-563 and no detectable contributions from cytochrome *b*-559 may be superimposed.

The ratio of total chlorophyll to maximal reduced cytochrome *f* ranged from 1200 to 1500 [six different preparations of broken, Class II and Class I (Type A) chloroplasts]. This has been determined from the maximal difference of absorption changes at 554 nm *minus* 540 nm using the difference molar extinction coefficient of $2.2 \cdot 10^4$ cm$^{-1} \cdot$M^{-1} (ref. 32). The high ratio indicates that only 0.4 cytochrome *f* molecule per one light reaction are engaged in the linear electron transport. Chemical deter-

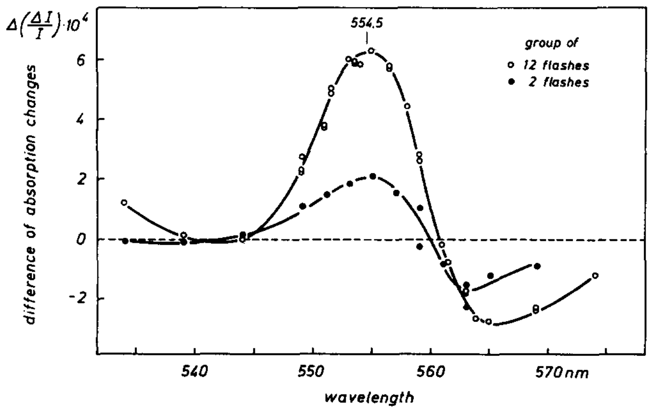

Fig. 5. Difference of absorption changes as a function of the wavelength *minus* the absorption change at 540 nm after a group of two and twelve flashes. Conditions as in Fig. 2.

minations gave a ratio of total chlorophyll to cytochrome *f* of about 500[33] and recently 920[34] for spinach chloroplasts. The latter ratio corresponds to only 0.55 cytochrome *f* molecule per one light reaction.

It should be remembered that during the experiments, alternating excitation with System I light (far-red) and with saturating flashes of System II and System I light, has been used. Therefore only absorption changes are visible of components which are reduced by light reaction II after oxidation by light reaction I. In contrast to grana lamellae, for stroma lamellae a predominant content of Photosystem I has been found[35]. Cytochrome *f* is closely bound to Photosystem I[33]. These results suggest that cytochrome *f* in the stroma lamellae may not be reduced by light reaction II. This may explain the lower amount of cytochrome *f* reduced compared to the chemical determined one.

Errors of cytochrome measurements may result from the field-indicating absorption changes. This has been avoided by the addition of a high concentration of gramicidin D (0.5 μM) which accelerated the field-indicating absorption change to a non-resolved time course[36]. The difference of the field-indicating absorption changes at 554 *minus* 540 nm after one flash[37] has approximately the same amplitude as the maximal absorption change of cytochrome *f*. This superposition has to be taken into consideration if the amount or the redox level of cytochrome *f* is calculated.

Interaction between chlorophyll a_{I} and cytochrome f

The dark electron transfer after excitation leads to an equilibration of the electrons within the transport chain. It seems reasonable to assume that the amplitudes of chlorophyll a_{I} and cytochrome *f* in Figs 2E and 2F correspond to the concentrations of these components at the equilibrium. From the amounts of reduced chlorophyll a_{I} and cytochrome *f* after one and two flashes an equilibrium constant of $K=50$–100 can be decuced. An equal equilibrium constant has been found in the dark after oxidation with System I light[16,8].

In contrast to this large equilibrium constant in the dark during light-limited oxidation an equilibrium constant of only $K=5$–10 has been evaluated[16,8,9]. This is close to $K=13$ which results from the midpoint potentials of chlorophyll a_{I}, $E_0'=0.43$ V[38,39], and cytochrome *f*, $E_0'=0.365$ V[30]. One explanation of the discrepancy of the equilibrium constants would be the change of cytochrome *f* to a low-potential form in the dark. The existence of three forms of different midpoint potentials has been found for cytochrome *b*-559[40].

If there is indeed an equilibrium between a low-potential form of cytochrome *f* and chlorophyll a_{I} this should also be observable from the time course of their reduction. Prerequisite is a faster equilibration of the electrons between cytochrome *f* and chlorophyll a_{I} than the transport from plastoquinone. Cytochrome *f* is oxidized in strong white light. This is only possible if it is oxidized faster by light reaction I than reduced *via* plastoquinone in about 17 ms. The oxidation time of cytochrome *f* of about 0.3 ms[13,14] supports this assumption. Additionally the reduction of chlorophyll a_{I} by its primary electron donors is appreciably faster than the slow reduction *via* plastoquinone[20]. Therefore the equilibrium between chlorophyll a_{I} and cytochrome *f* should be maintained also during the slow dark reduction. In consequence of the high equilibrium constant of $K=50$–100 the reduction of cytochrome *f* should not start until a great amount of chlorophyll a_I is reduced. A lag in the reduction kinetics of cytochrome *f* is expected.

Fig. 6 presents the time course of the reduction of cytochrome *f* and chlorophyll a_I after excitation with a group of ten flashes. No lag of the cytochrome *f* kinetics is found. The time course closely fits first-order kinetics starting at the moment of the last flash. The small negative spikes after the flashes reflect the residual part of the accelerated field-indicating absorption change. Chlorophyll a_I is reduced with a half-life of 4 ms. Related to this time course the expected reduction kinetics of cytochrome *f* in the case of an equilibrium of $K=50$ are shown. The consequence of the difference to the measured time course is that there is no equilibrium between cytochrome *f* and chlorophyll a_I. The change of cytochrome *f* to a low-potential form is ruled out by this result. The dark electron transfer from plastohydroquinone to chlorophyll a_I must run in parallel to the electron transfer to cytochrome *f*. A lag in the reduction kinetics of cytochrome *f* is expected whenever an electron transfer *via* cytochrome *f* to another electron carrier occurs. Consequently even an electron path *via* cytochrome *f* to another light reaction I than chlorophyll a_I[16] is not possible in the dark.

On the other hand, the oxidation time of cytochrome *f* (about 0.3 ms) is much greater than the flash duration[13,14], *cf.* also ref. 10. Therefore cytochrome *f* must be oxidized by another component which is previously oxidized in the flash. Nevertheless, the time course of the subsequent reduction of cytochrome *f* in the dark

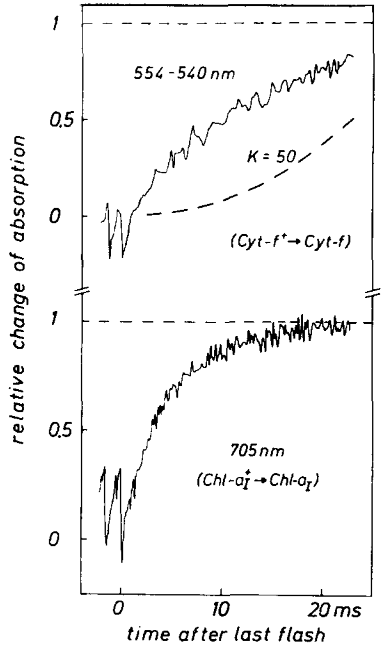

Fig. 6. Absorption changes as a function of time after excitation with a group of ten flashes. Top: difference of absorption changes at 554 nm *minus* 540 nm. 256 signals have been averaged. Bottom: absorption change at 705 nm. 64 signals have been averaged. The depicted time courses start after the eighth flash. The assumptions for the calculation of the dashed line are given in the text.

reflects no electron transfer to an electron acceptor. This inconsistent behaviour could be understood if the electron transfer from cytochrome *f* to the acceptor after excitation of light reaction I is possible only for a short time (about 1 ms or less according to the time resolution in Fig. 6). The mechanism should resemble a light-induced gate. On the molecular basis this would be performed by a light-induced conformation change during which the electron transfer from cytochrome *f* to its acceptor occurs. After excitation a fast oxidation of reduced cytochrome *f* would be possible but during the slow reduction the interaction with the acceptor would be blocked and therefore the expected lag of the kinetics prevented. In this case it is not necessary to assume another light reaction I than chlorophyll a_{I} as electron acceptor of cytochrome *f*. The apparently contradictory data to an electron transfer from cytochrome *f* to chlorophyll a_{I} in refs 8,12,16 can also be explained by this mechanism.

The rates of the electron transfer to cytochrome *f* and chlorophyll a_{I} can be estimated from the initial slope of the reduction kinetics as discussed above. After a group of ten or twelve flashes, the initial rates have been compared: No more than 15% of the electrons from plastoquinone are transferred to cytochrome *f* and at least 85% to chlorophyll a_{I}. This is found in broken as well as in Class I chloroplasts. The result substantiates the first estimation that nearly all electrons are transferred to chlorophyll a_{I}. Because the relative rates of the plastohydroquinone oxidation and the chlorophyll a_{I} reduction increase simultaneously with the flash number (see Fig. 4), a constant portion of the electrons of not more than 15% should pass cytochrome *f*. The parallel electron path *via* cytochrome *f* appears to be much less significant for the electron transport between the two light reactions. This agrees with results found during the greening of a mutant of *Chlamydomonas reinhardi*. Within the first h of illumination the presence of photosynthetic oxygen evolution and Photosystem I activity has been detected but no photooxidation of cytochrome *f*[41].

Properties of an unknown electron carrier between plastoquinone and chlorophyll a_{I}

The electrons donated by plastohydroquinone to its acceptors and the electrons accepted by two of them, cytochrome *f* and chlorophyll a_{I}, have been measured. To balance the donated and accepted electrons one has to state the assumptions: (1) Plastoquinone accepts all electrons from light reaction II. Therefore the amplitude after one saturating flash is proportional to the number of active chlorophyll a_{II}[3]. (2) Chlorophyll a_{I} is present at about the same concentration as chlorophyll a_{II}. (3) Cytochrome *f* has the same extinction coefficient *in vivo* as *in vitro*.

Errors may arise from the signal noise or incorrect assumptions. The amplitudes in Fig. 2 have been converted into electron equivalents related to one light reaction based on these assumptions. They are indicated on the right side of Fig. 2. The difference between the electron equivalents released from plastohydroquinone *minus* the electron equivalents accepted by oxidized cytochrome *f* and chlorophyll a_{I} is presented in Fig. 7. This calculated difference should correspond to the electrons accepted by an electron carrier between plastoquinone and chlorophyll a_{I}. The following properties of this unknown electron carrier may be derived.

The difference of electrons per light reaction as function of the flash number is shown in Fig. 7, right. After one flash the electrons accepted by the electron carrier should amount to about zero and converge to the maximum value at increasing flash numbers. The electron capacity of the electron carrier should be about 1.6 per light reaction. The calculated time course after two and eight flashes is shown in Fig. 7, left. Characteristic for the reduction kinetics of the electron carrier should be a lag in the beginning followed by a rise with a half-life of about 20 ms.

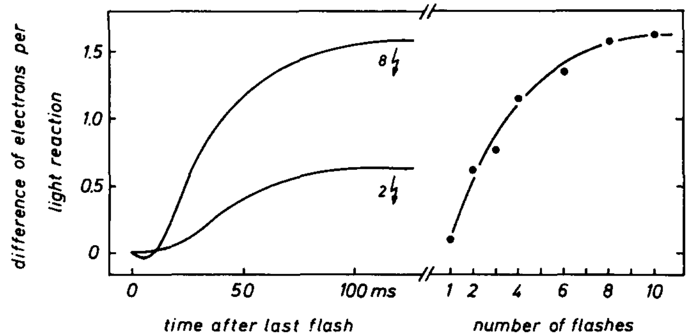

Fig. 7. Difference between the number of electrons released from plastohydroquinone *minus* the sum of electrons accepted by oxidized cytochrome f and chlorophyll a_{I} related to one light reaction. The calculation is based on the results in Fig. 2. Left: time course after two and eight flashes. Right: number of electrons as a function of the number of flashes per flash group. The assumptions are defined in the text.

The dependence of the reduced amount as well as the time course are consistent with the behaviour expected for an electron carrier between plastoquinone and chlorophyll a_{I}. From the oxidation kinetics of plastohydroquinone the existence of such an electron carrier can be deduced, too (see above). The equilibrium constant of the electron transfer from the unknown electron carrier to chlorophyll a_{I} can be calculated as for cytochrome f and chlorophyll a_{I} and gives about $K \approx 50$. A not identified component different from cytochrome f has also been postulated to be in true equilibrium with chlorophyll a_{I} from the area bounded by the transient of chlorophyll a_{I} during oxidation with System I light. But a lower equilibrium constant of $K = 20$ has been determined[8]. Since this unknown electron carrier must be a primary electron donor of chlorophyll a_{I} its oxidation time should correspond at least to one of the fast reduction times of chlorophyll a_{I} of 20 μs and 200 μs[20,21].

The unidentified electron carrier may be plastocyanin. The amplitude at 600 nm due to the reduction of plastocyanin should be as large as the amplitude of cytochrome f after eight flashes according to the extinction coefficient of plastocyanin[42]. However, first measurements at 600 nm did not show absorption changes with the expected kinetics.

Conclusions

The comparison of the oxygen production per flash and the amount of reduced chlorophyll a_{I} resulted in an about equal number of light reactions II and chlorophylls a_{I}. Different chlorophyll a_{I} molecules are in equilibrium with each other during the reduction. The electron equivalents released from the reduced pool of plastoquinone and those accepted by oxidized cytochrome f and chlorophyll a_{I} have been compared. The difference between these electron equivalents shows the property which has to be postulated for an electron carrier between plastoquinone and chlorophyll a_{I}.

The equilibrium of the electron transfer from this non-identified electron carrier to chlorophyll a_I should be far on the side of chlorophyll a_I. The ratio of the electron carrier to chlorophyll a_I is about 1.6:1. At least 85% of the electrons pass this main path of the linear electron transport.

Cytochrome *f* must be situated in a parallel side path. The ratio of cytochrome *f*, engaged in the linear electron transport, to chlorophyll a_I is about 0.4:1. Since a wide range in the ratio of total chlorophyll to cytochrome *f* in different plants has been found[34] this value may vary according to the material. Cytochrome *f* is reduced by plastohydroquinone in the rate-limiting time. But in the dark it transfers no electrons to chlorophyll a_I. A light-induced conformation change is proposed as a mechanism of the oxidation of cytochrome *f* during which a fast oxidation of cytochrome *f* is only possible. A light-induced change in the redox potential[8] is excluded. If the proposed mechanism is valid the electron acceptor of cytochrome *f* may be chlorophyll a_I and not another light reaction I[16].

The quantitative transfer of the electrons from plastohydroquinone to cytochrome *f* and chlorophyll a_I is in accordance with the coupling of two light reactions. It is in contradiction to the concept of three light reactions, which postulates a cyclic electron transport involving chlorophyll a_I and cytochrome *f* and a completely independent linear electron transport *via* plastoquinone with two light reactions II[43].

The method of flash titration makes it possible to measure every redox state of the electron carriers between the two light reactions. Thus it may be possible to identify the electron carrier between plastoquinone and chlorophyll a_I from absorption changes which show the calculated kinetics and to study the fast electron transfer to chlorophyll a_I in detail.

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