Biochimica et Biophysica Acta, 682 (1982) 482–490
Elsevier Biomedical Press

BBA 41216

TRANSIENT KINETICS OF THE ELECTRON TRANSFER BETWEEN P-700, PLASTOCYANIN AND CYTOCHROME *f* IN CHLOROPLASTS SUSPENDED IN FLUID MEDIA AT SUB-ZERO TEMPERATURES

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(Received July 5th, 1982)

Key words: P-700; Plastocyanin; Cytochrome f; Electron transfer; (Spinach chloroplast, Kinetics)

The kinetics of redox changes of P-700, plastocyanin and cytochrome *f* in chloroplasts suspended in a fluid medium at sub-zero temperatures have been studied following excitation of the chloroplasts with either a single-turnover flash, a series of flashes or continuous light. The results show that: (1) The kinetics of reduction of P-700$^+$ and those of oxidation of plastocyanin are consistent with a bimolecular reaction between these two components as previously suggested (Olsen, L.F., Cox, R.P. and Barber, J. (1980) FEBS Lett. 122, 13–16). (2) Cytochrome *f* shows heterogeneity with respect to its kinetics of oxidation by Photosystem I. (3) In contrast to the situation when plastoquinol is the electron donor, reduction of cytochrome *f* by electrons derived from diaminodurene occurs with sigmoidal kinetics that shows a good fit to an apparent equilibrium constant of 12 between the cytochrome and P-700. (4) The rate of electron transfer from plastoquinol to Photosystem I depends on the redox state of the plastoquinone pool. (5) In relation to current ideas about the lateral heterogeneity of Photosystem I and Photosystem II in the thylakoid membrane, the results are consistent with the function of plastocyanin as a mobile carrier of electrons in the intrathylakoid space.

Introduction

The electron-transport chain connecting the two photosystems in chloroplasts has been the subject of intensive research in the past 10–15 years. Nevertheless, there are still several unsolved questions about the sequential arrangement of some of the electron-transport components and the mechanisms of electron transfer. On the oxidising side of plastoquinone the electron-transport chain is known to involve P-700, cytochrome *f* and plastocyanin. It now seems likely that plastocyanin is a more primary electron donor for P-700 than cytochrome *f* [1] but Bouges-Bocquet and Delosme [2] have made a claim for an as yet uncharacterized electron donor situated between plastocyanin and P-700. Haehnel et al. [3] have suggested that this unknown electron donor is complexed plastocyanin whereas Olsen et al. [4] found that the complex kinetics of P-700 reduction following a single-turnover flash [5,6], which is part of the reason for the controversy about electron donation to P-700, could be interpreted in terms of a bimolecular reaction between plastocyanin and P-700 in agreement with previous suggestions by Wood and Bendall [7] and Delosme et al. [8].

Cytochrome *f*, which is almost isopotential with plastocyanin, is now regarded as a secondary donor to P-700. However, previous studies have shown that the kinetics of redox changes of cytochrome *f*

Abbreviations: PS, photosystem; P-700, reaction centre chlorophyll of Photosystem I; DCMU, 3-(3',4'-dichlorophenyl)-1,1-dimethylurea.

0005-2728/82/0000–0000/$02.75 © 1982 Elsevier Biomedical Press

and the effects of inhibitors on these reactions [9–14] are not easily reconciled with this cytochrome being an obligatory component in linear electron transport.

The present work is an investigation of the kinetics of redox changes of P-700, plastocyanin and cytochrome *f* following illumination of chloroplasts with one or a series of single-turnover flashes or with continuous light. Some of these experiments were performed in the presence of DCMU and an artificial electron donor (diaminodurene) in order to obtain a simpler system than when electron transfer to the oxidising side of PS I is from plastoquinol. Addition of DCMU and diaminodurene has furthermore the advantage that oxidation of cytochrome *f* can be measured without interference from C-550 [15]. Finally, these experiments allowed a comparison to be made between electron transfer from diaminodurene to the oxidising side of PS I and the corresponding electron transfer from plastoquinol in order to locate the site responsible for the anomalous kinetics of electron transfer to cytochrome *f* [10–13]. The chloroplasts are suspended in a medium containing ethanediol (50%, v/v) and water at sub-zero temperatures. This experimental procedure results in a more than 100-fold reduction in rate compared to that at room temperature and therefore allows the use of slowly responding but very sensitive equipment to study the electron-transfer reactions. At the same time, the chloroplasts remain in a fluid environment of only moderate viscosity and otherwise with similar physico-chemical properties to an aqueous solution at +20°C [16]. It has previously been shown that these experimental conditions have very little or no effect on the qualitative behaviour of chloroplast electron transport and proton pumping [17,18].

Materials and Methods

Chloroplasts from spinach were prepared on a large scale as described previously [17] and stored frozen in liquid nitrogen until required. This resulted in a very homogeneous material and hence highly reproducible results. The chloroplast material consists of intact thylakoids (no chloroplast envelope) with the ability to perform light-induced proton transport and with a well defined electrochromic absorbance change. Several of the experiments described in the following section were also performed with freshly perpared intact thylakoids derived from intact chloroplasts prepared as described in Ref. 19. The results obtained with this material were more variable but essentially identical to those obtained with the frozen material. The flash-induced redox changes of P-700, plastocyanin and cytochrome *f* were measured in a single-beam spectrophotometer constructed by Applied Photophysics, London, and modified in our workshop (Figs. 1–4), or in a Perkin-Elmer 356 dual-wavelength spectrophotometer (Fig. 5). The outputs from the spectrophotometers were connected to a Nova 1200 minicomputer that also controlled the actinic flash, a Braun 410C photographic flash operated at medium energy output (corresponding to a flash width of 100 μs at half height). To test whether the flash produced single turnovers, I measured the amount of electrons transferred to the PS I acceptor 8-hydroxy-11-methyldibenz(b,e)(1,4)oxazepin-2-(11H)- one ('methyl purple') [20]. Using an extinction coefficient (reduced minus oxidised) at 570 − 543 nm of $2.65 \cdot 10^4\ M^{-1} \cdot cm^{-1}$ [17] (two-electron reduction), it was calculated that under the present experimental conditions illumination by a flash results in the transfer of one electron to methyl purple per 660 chlorophylls. This corresponds to the amount of P-700 oxidised in a flash (see the following section) and hence demonstrates that the flash produced single turnovers. Continuous light was provided by a 150 W slide projector. P-700 was measured at 703 nm or at 703 − 720 nm by accumulating 10–20 single sweeps. In order to compensate for fluorescence artefacts the same number of sweeps were subtracted in the absence of the monitoring light. In some cases (indicated in the respective figure legends) this procedure was not sufficient and therefore the data points in the time interval where fluorescence interferes were automatically discarded by the computer. The flash was filtered through a combination of a Wrattten 47B filter and 3 cm of a 2% $CuSO_4$ solution and the photomultiplier was protected by a 3 mm Schott RG 715 filter. Cytochrome *f* was measured at 554 − 543 nm. In the single-beam instrument this was achieved by accumulating 15–40 single sweeps at the measuring wavelength and subtract-

ing the same number of sweeps at the reference wavelength. Plastocyanin was measured at 584 nm [12] by accumulating 40–80 single sweeps. The flash was filtered through a 2 mm Schott RG 645 filter and the photomultiplier was protected by a combination of a 3 mm Schott BG 18 filter and 5 mm saturated $CuSO_4$ or a 3 mm piece of blue glass with the same spectral characteristics as $CuSO_4$. The response time of the single-beam apparatus was limited by the data sampling rate of the computer (0.3 ms per address) whereas the response time of the dual-wavelength instrument was 100 ms. In order to avoid confusion all measurements indicated in figures are presented in absorption units (ΔA). In the case of measurements performed in the single-beam instrument this was done by using the equation

$$\Delta A = \frac{1}{\ln 10} \cdot \frac{\Delta I}{I}$$

The reaction medium consisted of equal volumes of ethanediol and an aqueous medium giving final concentrations of 0.33 M sorbitol, 3 mM $MgCl_2$ and 50 mM potassium phosphate, pH* 8.0 (pH* = effective pH [16]). Methyl viologen (40 μM) was present as electron acceptor and gramicidin D (4–10 μM) was added to ensure uncoupling of the chloroplasts. Other additions are as indicated in the figure legends. Normally, an Oxford Instruments cryostat was used for cooling of the sample. However, in a few experiments cooling was provided by a cooling bath circulating 96% ethanol to the sample holder (a brass block insulated with polystyrene). Chlorophyll was measured as described by Arnon [21].

The following model has been adopted for the analysis of the results in Figs. 1–4 (Pc, plastocyanin; cyt, cytochrome):

$$\text{P-700}^+ + \text{Pc} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \text{P-700} + \text{Pc}^+ \quad (1)$$

$$\text{Pc}^+ + \text{cyt } f \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} \text{Pc} + \text{cyt } f^+ \quad (2)$$

$$\text{diaminodurene}_{\text{red}} + \text{Pc}^+ \overset{k_3}{\rightarrow} \text{diaminodurene}_{\text{ox}} + \text{Pc} \quad (3)$$

The model was based on the following experimental observations: The reaction between P-700$^+$ in sonicated chloroplasts or in subchloroplast particles enriched in PS I and purified plastocyanin occurs with second-order kinetics [7,22,23]. The reaction between purified plastocyanin and purified cytochrome *f* has been shown to follow second-order kinetics [24]. It was observed here, as in a previous communication [4], that the kinetics of plastocyanin reduction following photo-oxidation by a single-turnover flash were pseudo-first order and with a rate constant that was linearly dependent on the concentration of diaminodurene. Reduction of cytochrome *f* following a single-turnover flash occurred in the same time scale as plastocyanin reduction as expected if the two components have similar redox potentials, and if electron donation from reduced diaminodurene is to plastocyanin only. The possibility that cytochrome *f* reacts slowly with diaminodurene could not be excluded. However, in view of the lack of evidence for this the simplest possible model was chosen.

The redox changes of P-700, plastocyanin and cytochrome *f* were simulated by using the experimentally observed stoicheiometries of the three components and the experimentally determined value of k_3. The relative rate constants k_1, k_{-1}, k_2 and k_{-2} were then varied to obtain the best fit to the experimental curves. The absolute values of k_1, k_{-1}, k_2 and k_{-2} depend on the absolute concentrations of P-700, plastocyanin and cytochrome *f* and hence cannot be determined. Their values are therefore given in the text as the ratios k_1/k_{-1} and k_2/k_{-2}.

Reactions 1–3 were simulated on the NOVA computer by numerical integration of the corresponding differential equations.

Results

Illumination of chloroplasts with a single-turnover flash causes photo-oxidation of P-700. P-700$^+$ is then rereduced by its primary electron donor which itself is reduced by secondary electron donors. A study of the transient kinetics of reduction of P-700$^+$ as well as the kinetics of redox changes of the other electron-transport components on the oxidising side of PS I should give information about the mechanisms of electron transfer and about the sequential arangement of these components.

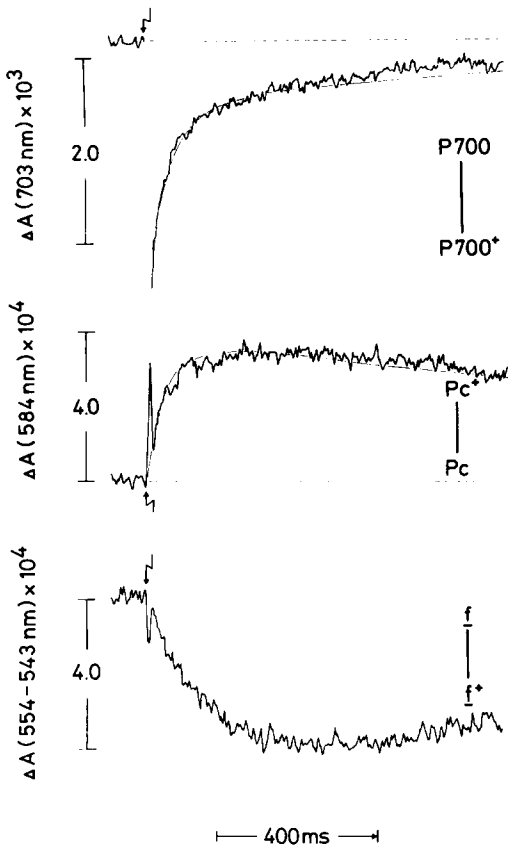

Fig. 1. Absorbance changes due to P-700 (top), plastocyanin (Pc) (middle) and cytochrome *f* (bottom) induced by a single-turnover flash at −25°C. Only 80% of the signal due to P-700 is shown in the figure due to disturbance by a fluorescence artefact lasting for 5 ms. The thin line through the upper two signals represents the expected time course for a reversible bimolecular reaction between P-700 and plastocyanin with an excess of plastocyanin of 1.4-times the amount of P-700 and with $k_1/k_{-1} = 6$ and $k_3 \times$[diaminodurene] = 0.55 s^{-1}. The reaction mixture contained chloroplasts corresponding to 35 μM (top curve) or 70 μM chlorophyll, 10 μM DCMU, 40 μM methyl viologen, 50 μM diaminodurene, 2 mM ascorbate and 10 μM gramicidin. Other conditions as in Materials and Methods.

Fig. 1 shows the redox changes of P-700, plastocyanin and of cytochrome *f* at −25°C induced by a single-turnover flash. The extent of photo-oxidation of P-700 corresponds to about 1 molecule per 660 chlorophylls by using an extinction coefficient at 703 nm of $6.4 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$ [25] whereas the extents of oxidation of plastocyanin and cytochrome *f* correspond to about 1 molecule per 890 and 3600 chlorophylls, respectively, by using extinction coefficients at 584 nm of $4.6 \cdot 10^3$ $M^{-1} \cdot cm^{-1}$ for plastocyanin [26] and at 554 − 543 nm of $2 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$ for cytochrome *f* [27]. The kinetics of reduction of P-700$^+$ ($t_{\frac{1}{2}} = 35$ ms) and oxidation of plastocyanin are in excellent agreement with a reversible bimolecular reaction between P-700 and plastocyanin with $k_1/k_{-1} = 6$ and a stoicheiometry of plastocyanin/P-700 of 1.4 : 1 (see the preceding section for further details). The experiments shown in Fig. 1 were performed in a medium with high ionic strength. Similar experiments using a medium with low ionic strength (data not shown) showed that the rate of reduction of P-700$^+$ was considerably faster ($t_{\frac{1}{2}}$ of the order of 10–15 ms) and the extent of oxidation of plastocyanin was increased. However, the kinetics of reduction of P-700$^+$ and those of oxidation of plastocyanin were still in agreement with a bimolecular reaction between these two components, the only difference being that k_1/k_{-1} was found to be greater than 10. The extent of photo-oxidation of cytochrome *f* is lower than expected if this cytochrome donates electrons to plastocyanin in a reaction with an equilibrium constant of close to 1 and with a stoicheiometry of about 1 : 1. The kinetics of cytochrome *f* oxidation could be simulated by the model described in the previous section when assuming a stoicheiometry of cytochrome *f* to plastocyanin of 0.4 : 1.4 and a value for $k_2/k_{-2} = 2$. The half-time of oxidation of cytochrome *f* did not change when decreasing the ionic strength of the medium, but the extent of oxidation was increased.

Fig. 2 shows the spectrum of the flash-induced redox changes in the cytochrome α-band region. The spectrum is in excellent agreement with the chemical difference spectrum (oxidised minus reduced) of cytochrome *f*.

Illumination of chloroplasts with a series of single-turnover flashes should result in an increase in the extent of oxidation of plastocyanin and cytochrome *f* provided that the time between flashes in the sequence is short compared with the time it takes to reduce these components by electrons originating from diaminodurene. From such experiments we can estimate the stoicheiometric relationship between cytochrome *f*, plastocyanin and P-700 and from the transient kinetics of reduction of the three components following the last

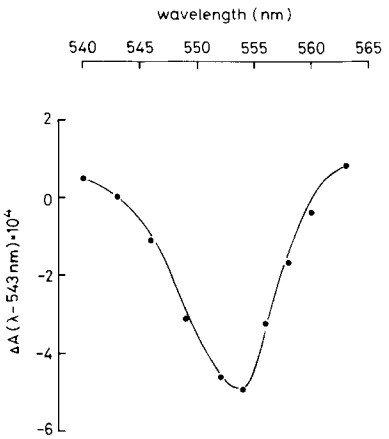

Fig. 2. Spectrum of the flash-induced absorbance changes at 400 ms with 543 nm as the reference wavelength. The curve represents the chemical difference spectrum of cytochrome *f* normalised to the absorbance change at 554 – 543 nm. Experimental conditions as in Fig. 1.

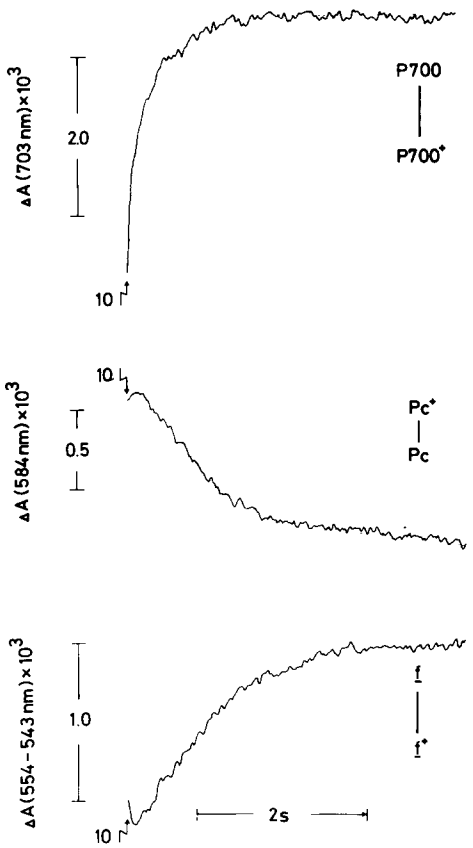

Fig. 3. Absorbance changes due to P-700 (top), plastocyanin (Pc) (middle) and cytochrome *f* (bottom) induced by a series of 10 single-turnover flashes with a flash frequency of 2.5 Hz at – 16°C. Other experimental conditions as in Fig. 1, except that the concentration of diaminodurene was 0.1 mM.

flash in a sequence further information can be obtained about the mechanism of electron transfer between them.

Fig. 3 shows the redox changes of P-700, plastocyanin and cytochrome *f* at – 16°C induced by a series of 10 flashes with a frequency of 2.5 Hz. The extent of photo-oxidation of P-700 is the same as that induced by a single-turnover flash whereas the extent of oxidation of plastocyanin now corresponds to 1 molecule per 490 chlorophylls, equivalent to 1.35 equiv./P-700. For comparison, the extent of oxidation of cytochrome *f* corresponds to only 0.55 equiv./P-700. A plot of the fraction of P-700 reduced vs. the fraction of plastocyanin reduced at different post-illumination times showed a good fit to an equilibrium constant of 5–10 between these two components in agreement with that expected from the simulated time courses shown in Fig. 1. Cytochrome *f* was reduced with a half-time slightly longer than that of plastocyanin reduction, suggesting that the equilibrium constant between cytochrome *f* and plastocyanin is greater than 1 and hence that the midpoint potential of plastocyanin is more positive than the midpoint potential of cytochrome *f*.

Decreasing the time between flashes did not have a significant effect on the extent of oxidation of plastocyanin whereas the extent of oxidation of cytochrome *f* could be increased to about 0.7–0.8 equiv./P-700. Fig. 4 shows the reduction of P-700$^+$ and oxidised cytochrome *f* at – 16°C following a sequence of 10 flashes, with a flash frequency of 4 Hz. As indicated in the figure, the time courses of reduction of the two components show a good fit to the simulated time courses of the model described in Materials and Methods with $k_1/k_{-1} = 6$, $k_2/k_{-2} = 2$ and with stoicheiometries of cytochrome *f*/plastocyanin/P-700 of 0.8 : 1.4 : 1. The ratio of k_2/k_{-2} found here equals that estimated by Bouges-Bocquet [28].

Experiments similar to those described in Fig. 4 were performed in the absence of DCMU and artificial electron donors. Such experiments are different from those in Fig. 4 in that increasing the number of flashes and increasing the flash

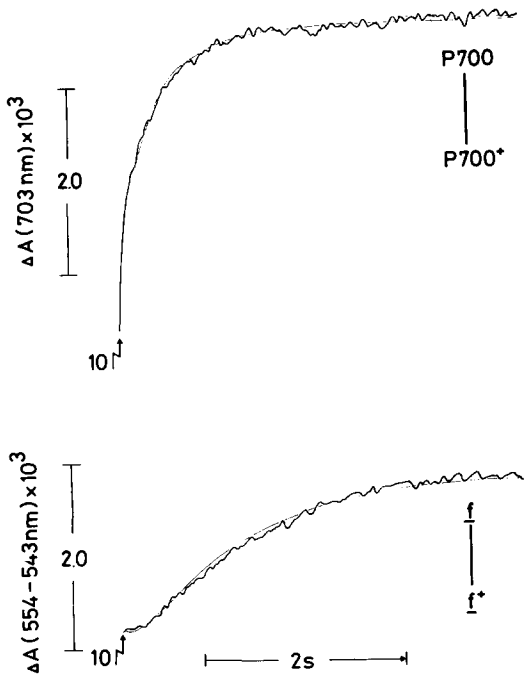

Fig. 4. Absorbance changes due to P-700 and cytochrome *f* induced by a series of 10 flashes with a flash frequency of 4 Hz at −16°C. The thin lines represent the expected time courses of reduction of P-700$^+$ and cytochrome *f* for $k_1/k_{-1} = 6$, $k_2/k_{-2} = 2$ ($k_1/k_2 = 3$), $k_3 \times$[diaminodurene] = 2.5 s^{-1} and relative stoicheiometries of cytochrome *f*/plastocyanin/P-700 of 0.8 : 1.4 : 1. Other experimental conditions as in Fig. 3.

frequency will result not only in increases in the extents of oxidation of plastocyanin and cytochrome *f*, but also in an increase in the extent of reduction of the plastoquinone pool [10]. Fig. 5 shows the reduction by plastoquinol of P-700$^+$ and oxidised cytochrome *f* at −17°C following a series of 10 flashes with two different frequencies, one high compared to the plastoquinol oxidation rate and one allowing significant reoxidation between flashes. Due to the slow response time (100 ms) of the dual-wavelength spectrophotometer used in these experiments, the rapid reduction of P-700$^+$ by plastocyanin is masked by a fluorescence artefact (not shown in the figure). The kinetics of P-700$^+$ reduction were non-exponential at low flash frequencies but approached pseudo-first-order kinetics at high flash frequencies. In contrast to the experiments shown in Figs. 3 and 4, cytochrome *f* is now reduced with pseudo-first-order kinetics. The rates of reduction of cytochrome *f* and P-700 increase as the time between flashes is decreased. The results shown in Fig. 5 were compared with those obtianed when chloroplasts are illuminated with a brief pulse (10–20 s) of continuous light. This comparison revealed that for a sequence of 10 flashes delivered with a frequency of 5 Hz, P-700 was oxidised to the same extent and reduced with nearly the same rate as when illumination is with continuous light. Under the same conditions, cytochrome *f* was oxidised to an extent of only 0.8 equiv./P-700 by a flash sequence whereas the corresponding amount oxidised in continuous light was 1.2 equiv./P-700. The reduction of the cytochrome occurred with nearly the same half-time in both cases.

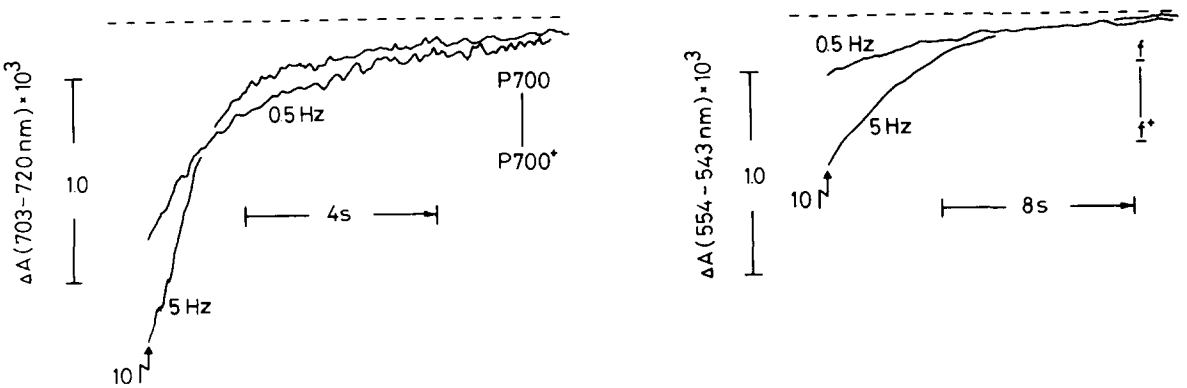

Fig. 5. Reduction of P-700 and cytochrome *f* at −17°C following a series of 10 flashes fired with different frequencies. The traces begin immediately after the last flash in a series. The traces due to P-700 were masked by a fluorescence artefact (not shown) lasting for about 100 ms and hence represent only reduction of P-700$^+$ by electrons from plastoquinol. The reaction mixture contained chloroplasts corresponding to 17 μM (left) or 35 μM (right) chlorophyll, 40 μM methyl viologen and 4 μM gramicidin. Other conditions as given in Materials and Methods.

In order to test whether the failure to observe the expected magnitude of changes due to cytochrome *f* with a flash sequence is due to this component remaining partially oxidised under the experimental conditions used the following experiment was performed: A flash sequence was delivered to the chloroplasts and the extent of oxidation of cytochrome *f* was recorded. Immediately after the cytochrome had become reduced in the dark the chloroplasts were illuminated with continuous light. The experiment showed that during continuous illumination the extent of oxidation of cytochrome *f* was 50% greater than that during flash illumination. The spectra ΔA ($\lambda - 543$ nm) of the changes due to cytochrome *f* were recorded for the steady-state changes during flash illumination and during continuous illumination as well as for the transient changes following illumination. In all cases, these spectra were superimposable on the chemical difference spectrum (oxidised minus reduced) of purified cytochrome *f*, indicating that the measurements of cytochrome *f* in Fig. 5 do not receive significant contributions from C-550. From these experiments we may conclude that cytochrome *f* is fully reduced before the onset of flash illumination.

Discussion

Electron transfer to P-700

The results in Fig. 1 are consistent with a bimolecular reaction between P-700$^+$ and its primary electron donor, plastocyanin, with the latter in slight excess over the former. These results agree with previous findings [4]. However, whereas the measurements by Olsen et al. [4] indicated an essentially irreversible electron transfer from plastocyanin to P-700$^+$, the present results suggest a reversible electron transfer between these two components. This difference was found to be due to the difference in ionic composition of the suspension medium. In the study by Olsen et al. [4] a medium with low ionic strength was used whereas the experiments shown in Fig. 1 were performed in a medium with a relatively high ionic strength. It has previously been shown [7,22] that the second-order rate constant, k_1, decreases when the cation concentration in the suspension medium is increased beyond a certain value. Similarly, we find here that increasing the cation concentration results in a decrease in the rate of P-700$^+$ reduction, and a decrease in the ratio k_1/k_{-1}. This indicates that k_{-1} is less affected by the ionic composition of the medium than k_1.

The value estimated here for the amount of functional plastocyanin is lower than the total amount of plastocyanin in chloroplasts estimated by some workers [29] but agrees well with the value estimated from ESR data for the amount of plastocyanin that can be oxidised by PS I [30].

It is intriguing that estimates of the stoicheiometric relationship between plastocyanin and P-700 in higher plant chloroplasts based on transient kinetic measurements [4,9,10,12] usually give values lower than or equal to 2 plastocyanins per P-700, whereas estimates using other methods give values of 3–4 plastocyanins per P-700 [29,31]. This suggests that some plastocyanin does not participate in electron transport either because it is immobilised at some site or because it is located outside the thylakoid.

Further evidence for a bimolecular reaction between P-700$^+$ and plastocyanin has recently been provided by the finding that the rate of electron transfer from plastocyanin to P-700$^+$ in chloroplasts is linearly dependent on the viscosity [32]. Such behaviour is typcial for diffusion-limited reactions and is incompatible with a scheme where electron transfer to P-700$^+$ is from complexed plastocyanin.

The rate of electron transfer from plastoquinol to P-700$^+$ following a flash sequence (Fig. 5) is low at low flash frequencies and high at high flash frequencies. Since the rate-limiting step of chloroplast electron transfer is plastoquinol oxidation [1], increasing the flash frequency would be expected to result in an increase in the number of plastoquinol molecules following the last flash in a sequence. The results shown in Fig. 5 are therefore consistent with an earlier suggestion that plastoquinol oxidation is a second-order reaction [33,34].

Cytochrome f

The low extent of photo-oxidation of cytochrome *f* and the relatively slow rate of oxidation following a single-turnover flash suggest that this cytochrome is not a direct electron donor to

$P\text{-}700^+$. This is further supported by the fact that the rate of oxidation of cytochrome *f* shows little dependence on the ionic composition of the medium. Neither is the low extent of oxidation of cytochrome *f* easily explained in terms of a scheme where electron donation is from the cytochrome to plastocyanin and with an equilibrium constant of about 1 between these two components as suggested by their similar redox potentials [35]. Furthermore, only 60–70% of the total amount of the cytochrome present in the chloroplasts (corresponding to 0.8 equiv./P-700) can be oxidised in a flash sequence, whereas the corresponding amount of plastocyanin oxidised must be close to 100% as indicated by the fact that when the flash number and the frequency are sufficiently high there is little or no rapid electron donation to $P\text{-}700^+$ (Figs. 3–5). Although the kinetics of reduction of cytochrome *f* by diaminodurene following a flash sequence (Fig. 4) can be explained in terms of reversible electron donation between plastocyanin and cytochrome *f* (with stoicheiometries cytochrome *f*/plastocyanin of 0.8 : 1.4) the low extent of cytochrome *f* oxidation following a single-turnover flash (Fig. 1) calls for an even lower cytochrome *f*/plastocyanin ratio.

The unexpected redox behaviour of cytochrome *f* may have different explanations. One possibility is that cytochrome *f* may exist in different environments. There is good evidence that the stroma region and the unappressed regions of the grana are enriched in PS I whilst the appressed grana regions have a low PS I content [36–38]. Recent results indicate that cytochromes *f* and *b*-563 are evenly distributed in the chloroplast membrane system [39,40]. According to these results, cytochrome *f* should have different degrees of association with P-700 and this could explain why 40% of the cytochrome is apparently not easily oxidised by PS I.

The kinetics of reduction of cytochrome *f* following a flash sequence are clearly different when endogenous plastoquinol or added diaminodurene is the electron donor. In the presence of DCMU, diaminodurene and ascorbate, cytochrome *f* is reduced with sigmoidal kinetics whereas its reduction by plastoquinol appears to be strictly pseudo-first order in agreement with previous studies of the kinetics of its reduction following continuous illumination [11,13]. A simple modification of the model described in Materials and Methods to incorporate electron donation from plastoquinol failed to reproduce the redox behaviour of cytochrome *f* shown in Fig. 5. The possibility that the absorbance changes in the absence of DCMU were masked by the superposition of C-550 [3,14] can be ruled out, since the spectra obtained for the steady-state changes during illumination and for the transient changes following illumination showed absolutely no contribution from this component.

Electron transfer between PS I and PS II

In relation to the current ideas about the lateral heterogeneity of PS I and PS II in chloroplasts stacked by addition of salts [36–38], the experiments described here are consistent with a role for plastocyanin as a mobile redox carrier to 'shuttle' electrons between the two photosystems as previously suggested [41].

Acknowledgements

The author wishes to thank Morten Nielsen and Jørgen Bang Nielsen for excellent technical assistance, Ann Lønholdt for typing the manuscript and Dr. R.P. Cox for stimulating discussions and criticism of the manuscript. This work was supported by grants from the Danish Natural Science Research Council (J. Nos. 11-2107 and 511-15001).

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