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Turn-over of electron donors in Photosystem I: double-flash experiments with pea chloroplasts and Photosystem I particles

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Kinetic properties of the binding of plastocyanin to the Photosystem I (PS I) reaction center have been studied by measuring the reduction kinetics of photo-oxidized P-700 after two consecutive excitations, with flash absorption spectroscopy. In pea chloroplasts and in PS I particles, after two laser flash excitations separated by 50 μs or less, P-700$^+$ is reduced with a $t_{1/2} = 110$ μs. No 12-μs reduction phase, due to 'close' bound plastocyanin electron donation to P-700$^+$, can be detected. By increasing the delay between the flashes, the 12-μs P-700$^+$ reduction phase reappears and its half-time of reappearance is 110 μs. Plastocyanin is shown to be involved in P-700$^+$ reduction after the first and also the second excitations. Results are interpreted in terms of two plastocyanin molecules bound to the reaction center, the 'distant' bound one replacing or re-reducing the 'close' bound one after it has been oxidized.

Introduction

In higher plant chloroplasts, the reduction of photo-oxidized P-700 by plastocyanin proceeds through a complex mechanism which has already been described in some detail [1,2]. After oxidation by a short laser flash, P-700$^+$ returns to the reduced state through a complex decay composed of two major kinetic phases. In chloroplasts, about 50% of the total absorption increase at 820 nm due to P-700 oxidation returns with a $t_{1/2} = 12$ μs. This large kinetic phase was shown to be due to the electron transfer from reduced plastocyanin (PC(I)) to P-700$^+$, plastocyanin being closely bound to the PS I reaction center ('close' bound state: $PC(I)_c \cdot$ P-700). An additional 30% of the total amplitude decays with a $t_{1/2} = 200$ μs. This second kinetic phase was shown to depend upon various parameters such as plastocyanin concentration, medium viscosity and also the addition of mono and divalent cations [1]. This phase of P-700$^+$ reduction is diffusion-controlled and we have proposed that it involves another reaction step associated with a second plastocyanin binding state on the reaction center ('distant' bound state: $PC(I)_d \cdot$ P-700) [2]. In measurements performed at 820 nm, the remaining absorption (20% of the total amplitude) decays slowly ($t_{1/2} > 5$ ms). It is mainly due to an absorption increase originating from the contribution at 820 nm of the near-infrared absorption peak of oxidized plastocyanin (770 nm) [3] and for a small part to a slow phase of P-700$^+$ reduction.

Abbreviations: ΔA, absorption change; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Mes, 4-morpholineethanesulphonic acid; PC (I or II), reduced or oxidized plastocyanin; PS I, Photosystem I; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

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The kinetic properties of the interaction between plastocyanin and the reaction center of PS I were shown to be very similar to those related to the interaction between soluble cytochrome c_2 and the reaction center of the photosynthetic bacterium *Rhodobacter sphaeroides* (formerly called *Rhodopseudomonas sphaeroides*) [4]. Both systems have been proposed to involve a soluble electron donor in equilibrium with two bound states [2,4].

The reaction scheme previously proposed describes the equilibrium state between plastocyanin and the reaction center in the dark and also the electron-transfer reactions occurring just after a single laser flash [2]. No information was obtained on the reactions that return the system to the dark equilibrium state (both 'bound' plastocyanin and P-700 reduced) that exists prior to the flash. Considering the well-established scheme for electron transfer between the cytochrome b_6/f complex and the PS I reaction center, the molecules which can be involved in the reconstitution of the dark equilibrium are either cytochrome *f* or plastocyanin. Cytochrome *f* is known to be the direct electron donor to plastocyanin [5] and its oxidation kinetics have already been investigated [6]. However, it has been proposed that plastocyanin is a mobile electron carrier acting between the two membranous complexes, and it is therefore difficult to draw a direct relationship between $P\text{-}700^+$ reduction and cytochrome *f* oxidation.

One way to investigate the reactions occurring after $P\text{-}700^+$ reduction and leading to the dark equilibrium (reduced state) of the system, is to oxidize P-700 by a second flash following a short delay after the first saturating flash. The reduction kinetics of $P\text{-}700^+$ formed after the second flash should provide information on the reconstitution of the 'close' bound state ($PC(I)_c \cdot$ P-700), on the relations between the two bound states of plastocyanin and on the eventual contribution of cytochrome *f*. In the present study, a new phase of $P\text{-}700^+$ reduction is observed together with the turnover properties of the supramolecular complex formed by the binding of plastocyanin to the PS I reaction center. Analysis of the results provides a more complete scheme describing the electron transfer and the interactions between the two proteins.

Materials and Methods

Intact chloroplasts were isolated from (10–15)-day-old pea plants as described by Nakatani and Barber [7]. The chloroplasts were osmotically shocked in the measuring cuvette as described by Telfer et al. [8]. The final composition of the suspension medium was 0.33 M sorbitol, 20 mM Tricine/NaOH (pH 7.5), 10 mM KCl, 10 mM $MgCl_2$, 1 mM sodium ascorbate, 10 μM DCMU, 40 μM hydroxylamine, and 50 μM methyl viologen, unless otherwise indicated.

Digitonin-solubilized PS I particles from spinach (D-144 particles) were prepared according to Boardman [9]. The particles were suspended in 50 mM Mes-NaOH (pH 6.0), 10 mM $MgCl_2$, 2 mM sodium ascorbate, and 100 μM methyl viologen.

Spinach plastocyanin was purified according to Yocum [10]. The purification was stopped after the second DEAE-cellulose (DE-52, Whatman) column chromatography and a 10 h dialysis against 10 mM potassium phosphate buffer (pH 7.0). Plastocyanin concentration was determined by absorption spectrophotometry using an extinction coefficient of 4900 $M^{-1} \cdot cm^{-1}$ at 597 nm, as described by Davis and San Pietro [11].

Flash-induced absorption changes at 820 nm, 580 nm and 555 nm were measured with an apparatus previously described by Van Best and Mathis [12]. In the case of transient measurements at 580 nm and 555 nm, the water cuvette (thickness, 3 cm) inserted between the tungsten-iodine lamp and the first interference filter was filled with a solution of $CuSO_4$ (50 g/l). A similar $CuSO_4$-containing cuvette was placed between the sample and the detection photodiode, before the second interference filter. Excitation was provided by one or two similar ruby laser flashes (Quantel; $\lambda = 694.3$ nm; $t_{1/2} = 6$ ns; $E_{max} = 40$ mJ). The laser flashes were attenuated with neutral density filters and homogenized by a piece of ground glass. The delay between the two independent lasers was varied from 30 μs to 100 ms.

Absorption changes were recorded with a transient digitizer (Biomation, Model 1010) and transferred to a signal averager (Tracor Northern TN 1710). The kinetics resulting from 4 to 60 excitation flashes were added in the signal averager. The

time between two flashes (or groups of two flashes) was 20 s. For experiments with chloroplasts, a regular 10×10 mm cuvette was used. The measurements with PS I particles were performed using a special lucite cuvette (thickness, 0.7 mm), which was oriented at 45° to the mutually perpendicular measuring and exciting beams.

Other experimental methods and computer analysis of kinetic data were essentially as in Ref. 2.

Results

Chloroplasts

After oxidation by a single saturating laser flash, P-700$^+$ returns to the reduced state via a complex kinetic pattern. Under our experimental conditions, the kinetics were analysed using a sum of three monoexponential components, $t_{1/2} = 12$ μs (50% of the initial ΔA amplitude), $t_{1/2} = 200$ μs (30%), and $t_{1/2} > 5$ ms (20%) (Fig. 1a).

When a second laser flash was fired, at a variable time after the first flash (both flashes being of saturating intensity), the signal induced by the second flash showed the following features (Fig. 1b and c): the amplitude of the second absorption increase was small for short Δt, and reached a maximum for $\Delta t = 80$–100 μs; its amplitude then represented 80% of the signal induced by the first flash. The time course of the amplitude of the second absorption increase followed the kinetics of P-700$^+$ re-reduction, as could be expected if the reactions at the acceptor side were not rate-limiting. The fact that the absolute absorption value was higher on the second flash than on the first one was most likely attributable to an increase of the absorption at 820 nm due to the oxidation of plastocyanin concomitant with the partial reduction of P-700$^+$ after the first flash.

The reduction kinetics of P-700$^+$ induced by the second flash were dependent upon the time interval between flashes. For Δt greater than 400 μs, the decays following the first and the second laser flashes were identical (Fig. 1c). For delays shorter than 50 μs, the size of the 12-μs reduction phase was below the detection level of the apparatus, and at least 70% of P-700$^+$ oxidized by

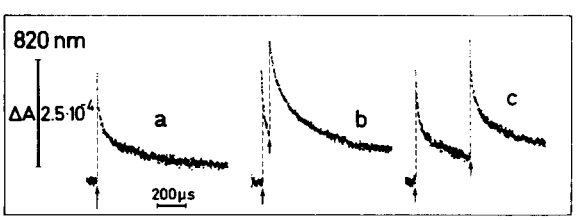

Fig. 1. Kinetics of flash-induced absorption change at 820 nm in pea chloroplasts at a concentration of 25 μg of chlorophyll/ml, at 20 °C. Pea chloroplasts were osmotically shocked in the cuvette in distilled water. The suspension medium was then adjusted to 20 mM Tricine/NaOH (pH 7.5) 0.33 M sorbitol, 10 mM KCl, 10 mM $MgCl_2$, 1 mM sodium ascorbate, 10 μM DCMU, 50 μM methyl viologen and 40 μM hydroxylamine. Cuvette optical path: 10 mm. Average of 16 experiments. (a) Signal induced by a single laser flash. (b) Signal induced by two identical laser flashes separated by 60 μs. (c) Same conditions as (b), but the time between the flashes was 400 μs. The vertical arrows indicate the time of the laser flashes.

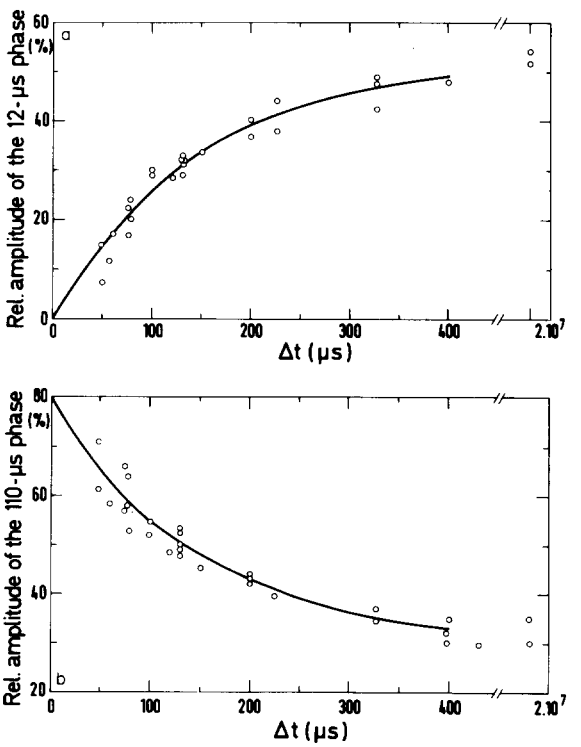

Fig. 2. Effect of the time interval between flashes on the relative amplitudes of the kinetic phases observed after the second laser flash on pea chloroplasts. Same conditions as in Fig. 1. Effect of the delay between the flashes (a) on the relative amplitude of the 12-μs reduction phase of P-700$^+$ and (b) on the relative amplitude of the 110-μs reduction phase of P-700$^+$.

the second flash was reduced monoexponentially with $t_{1/2} = 110 \pm 10$ μs (Fig. 1b).

If the delay between flashes was increased, the 12-μs reduction phase progressivelly reappeared and its half-time of reappearance (after the first flash) was found to be 110 μs (Fig. 2a). The amplitude of the 110-μs reduction phase observed for short delays decreased when the delay was increased, and its half-time of disappearance was also found to be $t_{1/2} = 110$ μs (Fig. 2b).

In single flash experiments with chloroplasts, when Mg^{2+} was absent, the amplitude of the 12-μs reduction phase was largely decreased (from 50% to 22% of the total ΔA) in agreement with previous findings [1]. In double flash experiments, for a delay of 50 μs and in the absence of $MgCl_2$, the amplitude of the 110-μs phase observed after the second flash was decreased in the same proportion as the amplitude of the 12-μs phase measured after the first flash (not shown). However, its half-time was not affected. Therefore, in the presence or in the absence of $MgCl_2$, the ratio of the amplitude of the 12-μs phase observed after the first flash to the amplitude of the 110-μs phase remained constant and close to 1. This supports the proposal that the 110-μs reduction phase is only observed in reaction centers which undergo a 12-μs reduction phase after the first flash.

A possible interpretation of the 110-μs decay observed after the second flash is that this phase originates from a back reaction between $P\text{-}700^+$ and a PS I endogenous electron acceptor. This hypothesis was checked by performing double flash experiments in the presence of various concentrations of methyl viologen (from 0 to 100 μM). The addition of such exogenous PS I electron acceptor had no effect on the kinetics observed after the first and the second flash (not shown). This result is in agreement with the known kinetic properties of the PS I electron acceptors [13–15] which permit to assume that 50 μs after the oxidation of P-700 by a first flash, the first electron has reduced the terminal acceptor P-430 (species likely including iron-sulfur centers A and B). A second electron can then arrive at least at the iron-sulfur center X and may be even at P-430. It is thus unlikely that the 110-μs decay observed after the second flash is due to a charge recombination with $P\text{-}700^+$.

Chloroplasts in the presence of glycerol

In order to check for the possible contribution of diffusion-limited reactions in the kinetics described above, the same double flash experiments were performed in the presence of glycerol (60%, v/v). The overall effect of glycerol addition was the apparent disappearance of the intermediate kinetic phase concomitant with the appearance of very slow phases (Fig. 3a).

In the presence of glycerol and for delay times shorter than 1 ms, the second flash induced an oxidation of $P\text{-}700^+$ which recovered very slowly, without any measurable 110-μs contribution (Fig. 3b). For delays longer than 1 ms, a fast 12-μs phase was detected. Its amplitude increased together with the delay, and for $\Delta t = 60$ ms, signals elicited by the first and the second laser flashes were kinetically similar (Fig. 3c). In the presence of glycerol, the half-time of reappearance of the 12-μs reduction phase was $t_{1/2} = 8.5$ ms (instead of 110 μs without glycerol).

The data show that both the 110-μs reduction phase observed (for short delay) after the second flash, and the half-time of reappearance of the fast phase on the second flash, are sensitive to glycerol addition in the suspension medium. So the mechanism through which $P\text{-}700^+$ is reduced after its second oxidation most likely includes a step which is limited by the diffusion. However, an effect of glycerol on equilibrium constants cannot be ruled out.

Photosystem I particles

Double-flash experiments were also performed using digitonin solubilized PS I particles (D-144

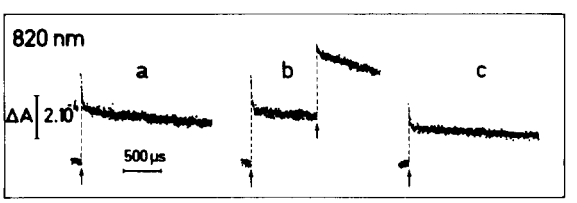

Fig. 3. Time-course of flash-induced ΔA at 820 nm in pea chloroplasts. Same conditions as in Fig. 1, except the addition of 60% (v/v) glycerol and chloroplasts at a concentration of 40 μg of chlorophyll/ml. Average of eight experiments. (a) Signal induced by a single laser flash. (b) Signal induced by two identical laser flashes separated by 1 ms. (c) Same conditions as in (b), but the time between the flashes was 60 ms and the effect of the first flash was not seen on this time scale.

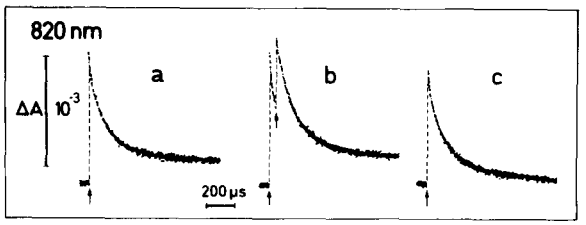

Fig. 4. Time-course of flash-induced ΔA at 820 nm, measured in D-144 particles (460 µg of chlorophyll/ml) at 20 °C. Particles were suspended in 50 mM Mes/NaOH (pH 6.0) 10 mM $MgCl_2$, 2 mM sodium ascorbate, 100 µM methyl viologen and 200 µM spinach plastocyanin. Average of eight experiments. (a) Signal induced by a single laser flash. (b) Signal induced by two laser flashes separated by 50 µs. (c) As (b) but the delay between the flashes was 500 µs and the effect of the first flash was not seen on this time scale. The cuvette (thickness, 0.7 mm) was placed at 45 ° of the measuring beam.

particles) supplemented with purified reduced spinach plastocyanin. This reconstituted system was previously shown to exhibit qualitatively the same P-700$^+$ reduction phases as chloroplasts when the plastocyanin concentration was high enough (above 50 µM) (Fig. 4a) [2].

Under such experimental conditions (with 200 µM plastocyanin), the results of double flash experiments were very similar to those obtained with chloroplasts. For very short delay times, no fast phase was observed and P-700$^+$ was reduced through a 110-µs phase (Fig. 4b), the amplitude of which, extrapolated to $\Delta t = 0$, represented 85% of the total ΔA induced by the second flash. The amplitude of this kinetic phase decreased for longer delays. For $\Delta t = 2$ ms, it represented 65% of the total amplitude. Its disappearance half-time was 110 µs (Fig. 5b). The fast reduction phase, absent for very short delay times, reappeared in parallel (Fig. 4c), with a half-time of reappearance of about 100 µs (Fig. 5).

Results were kinetically identical in the presence of 100 instead of 200 µM plastocyanin, i.e., a 110-µs reduction phase of P-700$^+$ for short delay, and a 110-µs half-time for the reappearance of the 12-µs phase. This indicates that the 110-µs reduction phase and the 110-µs reappearance time for the 12-µs phase do not depend upon plastocyanin concentration, at least at high concentrations. It was not possible to investigate further the effect of plastocyanin concentrations lower than 50 µM

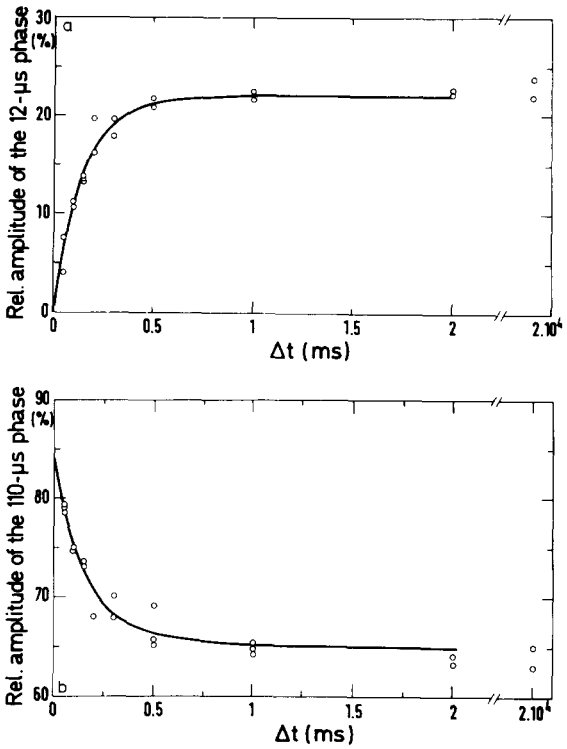

Fig. 5. Effect of the delay between the two flashes, measured in D-144 particles, (a) on the relative amplitude of the fast ($t_{1/2} = 12$ µs) reduction phase of P-700$^+$ and (b) on the relative amplitude of the 110-µs reduction phase of P-700$^+$. Same conditions as in Fig. 4.

mainly because of the small amplitude of the 12-µs phase observed after one flash.

The possible involvement of cytochrome *f* or plastocyanin in the reduction of P-700$^+$ was tested as follows. In D-144 particles, the cytochrome *f* content was measured by difference spectrophotometry according to Boardman and Anderson [16]. We obtained a chlorophyll/cytochrome *f* ratio of 1500, in fair agreement with the value of 1220 already published [16]. Photoinduced P-700$^+$ measured at 820 nm in the same particles gave a chlorophyll/P-700 ratio of 240 in agreement with Ref. 17. In D-144 particles, the amount of cytochrome *f* thus represented 16% of the photoactive P-700. In the same material, the amount of P-700$^+$ reduced through the 110-µs phase after a second flash (for $\Delta t = 50$ µs) was found to be 42% of the photoactive P-700. So the amount of cytochrome *f* present in the D-144 particles appears to be too small for this molecule to be responsible for the

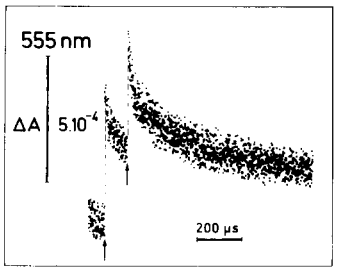

Fig. 6. Time-course of flash-induced ΔA at 555 nm measured in D-144 particles. The delay between the two flashes was 100 μs. Average of 40 experiments. Same conditions as in Fig. 4.

110-μs reduction phase of P-700$^+$.

This was confirmed by measurements of absorption changes at 555 nm in D-144 particles supplemented with plastocyanin. In both single- and double flash experiments the kinetics observed at this wavelength, with a time resolution of 0.5 μs, were very similar to those measured at 820 nm (Fig. 6). According to Bendall et al. [18], the $\Delta\epsilon$ corresponding to cytochrome *f* oxidation at 555 nm is of the order of $-16500\ M^{-1} \cdot cm^{-1}$. Thus, assuming that for a $\Delta t = 100$ μs, at least 50% of the photoactive P-700 is oxidized by the second flash and will be reduced by cytochrome *f*, one can expect to observe a negative signal of amplitude $\Delta A = 1.4 \cdot 10^{-3}$. Despite its large expected amplitude, such a signal was not detected. So, under our experimental conditions, cytochrome *f* is most likely not involved in the 110-μs P-700$^+$ reduction observed after the second flash.

In order to check for the photo-oxidation of plastocyanin after one or two excitation flashes, absorption changes were monitored at 580 nm.

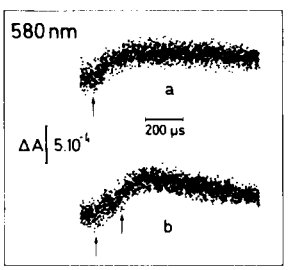

Fig. 7. Time course of flash-induced ΔA at 580 nm, measured in D-144 particles. Average of 60 experiments. Same conditions as in Fig. 4. (a) Signal induced by a single laser flash; (b) signal induced by two laser flashes separated by 150 μs.

This wavelength is near the absorption maximum of oxidized plastocyanin (597 nm) and is, under our experimental conditions, an isobestic point for P-700$^+$ in D-144 particles. Single-flash experiments showed an increase of the absorption with a kinetic pattern compatible with a reaction of $t_{1/2} = 200$ μs (Fig. 7a). The amount of oxidized plastocyanin calculated from the absorption increase at 580 nm ($\epsilon = 4800\ M^{-} \cdot cm^{-1}$) indicated that 0.8–1 plastocyanin was oxidized after one flash when 1 P-700$^+$ was reduced. Double-flash experiments ($\Delta t = 150$ μs) showed an increase of the absorption at 580 nm after the second flash (Fig. 7b). As after the first flash, the amount of plastocyanin oxidized is in good agreement with the amount of P-700$^+$ induced by the second flash. Disregarding the lack of accuracy in the kinetic analysis of the measurements at 580 nm, it clearly appears that both the first and the second flashes induce an increase of the absorption at 580 nm likely due to plastocyanin oxidation by P-700$^+$. We thus conclude that plastocyanin is directly responsible for the fast transfer of two electrons to P-700$^+$, and that cytochrome *f* does not participate in this process.

Discussion

The results of kinetic measurements performed on chloroplasts and PS I particles at 820 nm after two excitation flashes as a function of the delay between the flashes can be summarized as follows. When the second flash was fired within 50 μs after the first one, no 12-μs fast reduction phase was detected. In this case, P-700$^+$ induced by the second flash was reduced through a 110-μs half-time reaction. Upon increasing the delay between the flashes, the fast reduction phase reappeared and its rate of reappearance had $t_{1/2} = 110$ μs. The increase of the fast reduction phase amplitude occurred at the expense of the 110-μs reduction phase.

This new kinetic phase is not related to reactions due to PS II because this photosystem was blocked by addition of DCMU and hydroxylamine in chloroplasts, or almost completely absent in PS I particles.

Considering a simple model for the interaction between plastocyanin and the reaction center of

PS I, in which a bound plastocyanin is in equilibrium with free plastocyanin, the results presented here are rather surprising. The minimum model describing single flash experiments in chloroplasts involves only the 12-μs and 200-μs reduction phases. After two flashes separated by a short delay time, one expects that before P-700$^+$ can be reduced a second time, bound oxidized plastocyanin has to be re-reduced. This can be accomplished through its replacement on the PS I binding site by a diffusing reduced plastocyanin. This reaction should occur with $t_{1/2} = 200$ μs if the site where oxidized plastocyanin was bound has remained functional. The replacement reaction can even be slower if the time for oxidized plastocyanin to leave the binding site is not negligible as compared to 200 μs, and also if the concentration of reduced plastocyanin is lower than before the first flash.

So, within this model, the reduction phase occurring after the second flash and the reappearance of the 12-μs reduction phase cannot be faster than the intermediate phase observed after the first flash (200 μs under our experimental conditions), except if one considers the possibility of some local phenomenon. Such a local property could be a close interaction between the cytochromes b_6/f complex and P-700 via plastocyanin, avoiding any diffusional step in the electron transfer. In this case, 110 μs would be the time for electron transfer from cytochrome f to P-700$^+$, through bound plastocyanin. A half-time of 100 μs for cytochrome f oxidation has already been reported [19] and it could be consistent with the 110-μs phase observed here. This hypothesis, however, seems unlikely, since, under our experimental conditions, in D-144 particles no cytochrome f absorption change was detected after the second excitation flash, although the 110-μs reduction phase was clearly observed. Furthermore, the amount of cytochrome f in the particles is too low for this molecule to be responsible for this reaction.

For short delay times, the amplitude of the 110-μs phase after the second flash was shown to depend directly on the amount of centers that undergo a 12-μs reduction after the first flash. These results can be interpreted as follows. It has been previously proposed that there exists another binding state for plastocyanin on the reaction center [2]. The existence of a 'distant' bound state for plastocyanin, different from the 'close' bound one, was proposed because the rate of P-700$^+$ reduction, which can be accelerated by increasing the plastocyanin concentration, reached a maximum value. This indicates the occurrence of a limiting step in the reaction between free diffusing plastocyanin and P-700$^+$. This rate limitation was detected in D-144 PS I particles supplemented with plastocyanin and the estimated $t_{1/2}$ for this step was 110 μs. It was attributed to the involvement of a 'distant' bound complex between plastocyanin and the reaction center ($PC(I)_d \cdot$ P-700) [2]. Up to now, this 'distant' bound plastocyanin has not been shown to be connected to the 'close' bound plastocyanin.

So we suggest that each reaction center which has a 'close' bound plastocyanin (giving the 12-μs phase) also has a second bound plastocyanin ('distant' bound plastocyanin). This second plastocyanin molecule can reduce or replace the oxidized 'close' bound one with $t_{1/2} = 110$ μs. Thus, for short delay (less than 50 μs), the electron reducing the P-700$^+$ is donated by the 'distant' bound plastocyanin ($t_{1/2} = 110$ μs), directly or via the 'close' bound one. On the contrary, if the delay is longer, the time interval between the two flashes is sufficient to allow the 'distant' bound plastocyanin either to reduce or to replace the oxidized 'close' bound one. Then P-700$^+$ will be reduced with a $t_{1/2} = 12$ μs. Thus, we consider that the process which reactivates the 'close' bound plastocyanin (i.e., repopulation of the 'close' binding site by reduced plastocyanin) is also the process which reduces P-700$^+$ when the delay is too short for the 12-μs phase to operate. Furthermore, we suggest that these two plastocyanin molecules can act in a linear sequence, at least in a short time range, the 'distant' bound plastocyanin being also in equilibrium with the pool of free plastocyanin. In this hypothesis, the effect of glycerol can simply be a viscosity effect on the unbinding of oxidized plastocyanin from the 'close' binding site. However, one cannot exclude that glycerol modifies the binding equilibrium between the component responsible for the 110-μs phase and the reaction center, as proposed for the reaction center of purple bacteria [20].

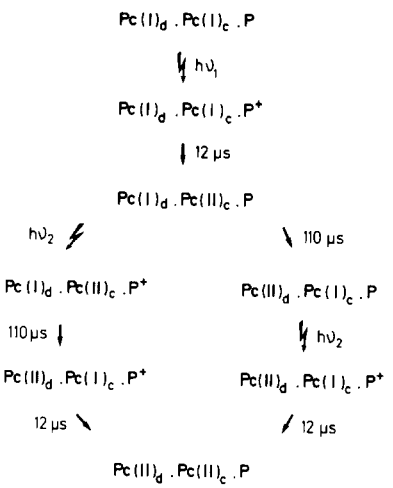

Fig. 8. Scheme of electron transfer between P-700 and the two bound plastocyanin molecules. The left reaction pathway is followed when the second laser flash is fired a short delay after the first one ($\Delta t < 100$ μs). The right reaction pathway is followed for a longer delay between the two flashes. PC(I), PC(II), $PC(\ )_d$, $PC(\ )_c$, P, $h\nu_1$ and $h\nu_2$ represent reduced plastocyanin, oxidized plastocyanin, 'distant' bound plastocyanin, 'close' bound plastocyanin, P-700, the first and the second laser flash, respectively.

The sequence of electron transfer and binding reactions is summarized in Fig. 8. The prerequisite is that each reaction center having a 'close' bound plastocyanin also has a 'distant' bound one. The scheme is incomplete in the sense that binding and unbinding reactions of plastocyanin to the reaction center are not shown explicitly.

The conclusions presented in this work provide a more complete description of the complex reactions of electron transfer and of binding occurring between P-700 and plastocyanin. They also show that electrons can be transferred successively through the PS I at a high rate. However, more data are needed on the biochemical aspects of this problem, namely on the PS I site where plastocyanin interacts, to see how the detailed structures of the proteins correlate with the functions as described by kinetic analysis.

Acknowledgements

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