Biochimica et Biophysica Acta, 396 (1975) 382–391

BBA 46973

ELECTRON TRANSPORT IN PHOTOSYSTEM I IN SPINACH CHLOROPLASTS

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(Received December 6th, 1974)
(Revised manuscript received March 24th, 1975)

SUMMARY

We have studied the recovery of the photochemical activity of Photosystem I after the charge separation induced by a flash under conditions where the secondary donors are in the reduced form.

The rate-limiting steps are on the donor side. The first step is completed within 400 μs. The second step is much slower (half time $\approx$ 1 ms) and corresponds to the transfer of electrons from plastoquinone. Under our conditions, only one intermediate is involved in electron transfer between the centers and the plastoquinone pool.

Electron exchange between the System I centers has been demonstrated.

INTRODUCTION

The primary donor of Photosystem I has been identified as a special chlorophyll molecule (*P*-700) [1]. The electrons are transfered from System II to System I via a pool A of plastoquinones [2, 3]. The relations between the pool A and *P*-700 are not clear (for reviews, see refs 4 and 5) : both cytochrome *f* [6] and plastocyanin [7] seem to be electron transporters between A and *P*-700, but no model accounts for the properties of the transfers in a satisfying way [8–10].

In order to clarify some aspects of these primary electron transfers in Photosystem I, we have studied the recovery of the photochemical activity of Photosystem I after the charge separation induced by a flash.

MATERIALS AND METHODS

Spinach chloroplasts were prepared according to the method of Avron [11] and suspended in 0.05 M Tris-HCl buffer, pH 7.8, containing 0.01 M NaCl/0.1 M KCl/0.4 M sucrose/0.1 mM methylviologen.

Except in experiments shown in Fig. 3, NH_4Cl (10^{-3}M) is added to obtain reproducible conditions.

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The recovery of the photochemical activity of System I following a flash was observed by its ability to reduce methylviologen. A first flash induces a charge separation and the quantity of methylviologen ($MV_2(\Delta t)$) reduced by a second flash given at time Δt after the first one is a measure of the number of System I centers which have recovered their photochemical activity within the time Δt. $MV_2(O)$, the quantity of methylviologen reduced by the second flash when the two flashes are synchronized, is not zero. For the results given here, $MV_2(O)$ was substracted from $MV_2(\Delta t)$ and the result normalized to $MV_2(300\ ms) - MV_2(O)$.

Methylviologen reduction is detected by an amperometric method [12]. This technique is very sensitive but only allows the detection of the System I centers involved in non-cyclic electron transfers.

RESULTS AND DISCUSSION

Recovery of the photochemical activity of system I after a flash

The recovery rate was estimated in chloroplasts by Warden and Bolton [14]. It was measured more precisely by Joliot and Delosme [13] in *Chlorella*. The latter authors observed two phases : a rapid one which is completed within 400 μs and a slow one whose half-time is 10–20 ms. In this paper, we have further extended these studies.

We worked under conditions where the secondary donors are in the reduced form and the secondary acceptors in the oxidized form (following strong white light and a period of darkness, in the presence of methylviologen). We then observed the two phases demonstrated by Joliot and Delosme [13].

Three hypotheses for the cause of this biphasic shape can be proposed:

(1) All centers undergo a biphasic recovery.

(2) There exist two types of centers; some centers undergo a rapid recovery and the others a slow recovery.

(3) For all centers, the first recovery is rapid but the flash is too long and the double hits are numerous. The slow phase represents the recovery after two photochemical reactions.

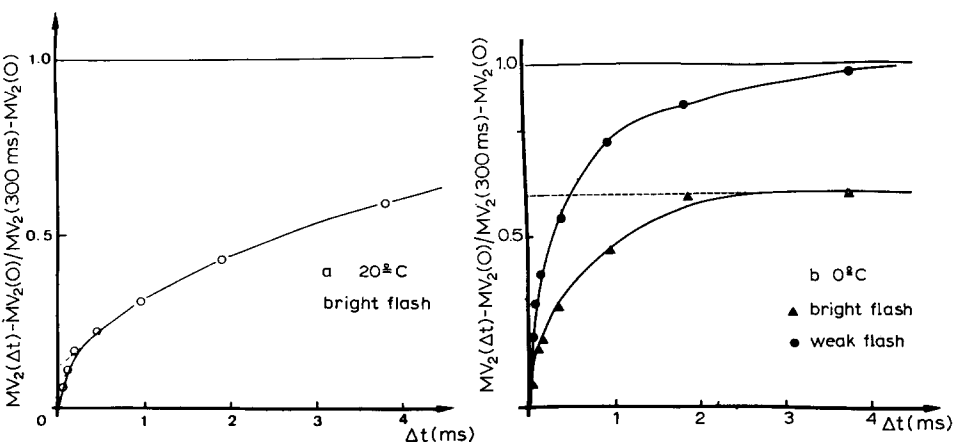

Fig. 1. a and b, see following page for legend.

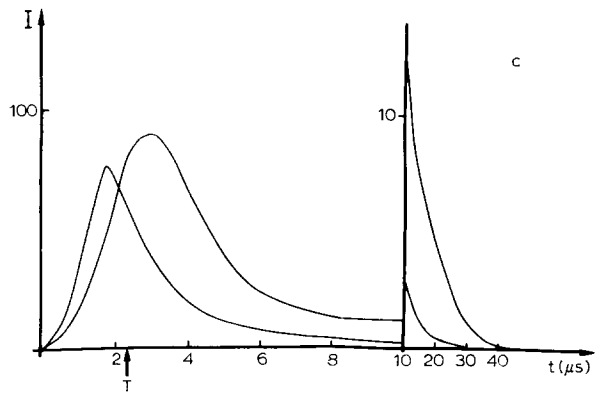

Fig. 1. Recovery kinetics of System I measured in spinach chloroplasts by methylviologen photoreduction (see Materials and Methods). The first flash is given after 10 s of bright white light and 300 ms dark. (a) 20 °C; ○, bright flashes; (b) 0 °C; ▲, bright flashes; ●, weak flashes. (c) relative shape and amplitude of bright and weak flashes.

To choose between these hypotheses, we lowered the temperature to slow down the rapid reaction and then altered the intensity of the flash (Fig. 1). The recovery is still biphasic at 0 °C with the bright flash, but with the weak flash, the slow phase is no longer observed. The possibility of suppressing the slow phase by changing the properties of the flash excludes the first two hypotheses and indicates that the slow phase represents the recovery after two photochemical reactions.

Fig. 2 indicates the theoretical fraction of double hits during a flash as a function of a second order rate constant for the recovery reaction under our flash conditions (see Appendix). It is supposed that the recovery reaction is

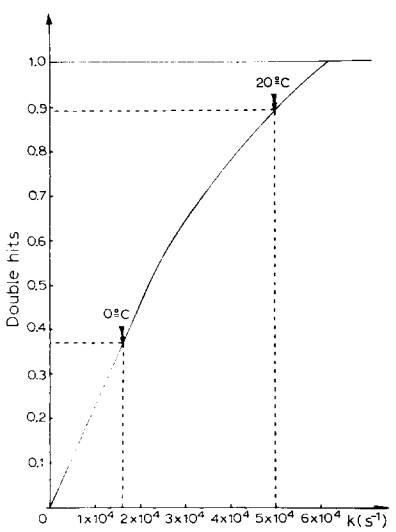

Fig. 2. Computed fraction of double hits as function of the rate constant of a second order limiting step (see Appendix). For the computation, we used the photon repartition of the bright flashes (see Fig. 1, curve c), and saturation coefficient equal to 6 (experimental conditions of Fig. 1, curves a and b).

$$P^+\text{-}700 + \text{PD} \xrightarrow{k} P\text{-}700 \text{ where [1] } \frac{d[P^+\text{-}700}{dt} = -k[P^+\text{-}700][\text{PD}]$$

The rate constant at 0 °C is about $10^4\ s^{-1}$ when computed from the kinetics of the rapid phase (Fig. 1b), and about $1.6 \cdot 10^4\ s^{-1}$ when computed from the fraction of double hits (Fig. 2.).

So we conclude that our results fit with the simple model suggested by Joliot and Delosme [13] : for all the centers, the recovery after one photochemical reaction is rapid, completed in 400 μs; the recovery after two photochemical reactions is slow.

Identification of the two phases

Following strong illumination, the action of an uncoupler shows that the slow phase is dependent upon the coupling conditions (Fig. 3). In the same figure, the lack of change in the amplitude of the rapid phases indicates the same fraction of double hits in the presence and in the absence of uncouplers, and so the same rates for the rapid phase.

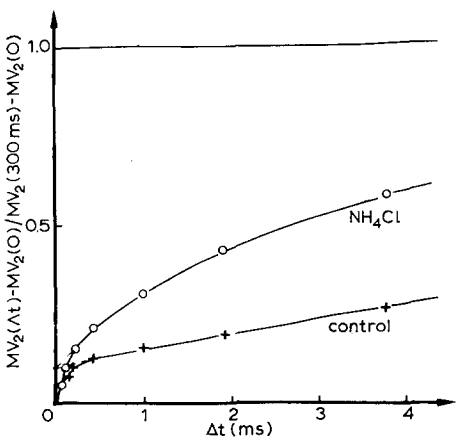

Fig. 3. Recovery kinetics of System I measured on spinach chloroplasts by methylviologen reduction. The first flash is given after 10 s of bright white light and 300 ms dark. (+ — +) control; (○ — ○) NH_4Cl.

The slow phase is exponential. We studied the half-time of this phase as a function of the concentration of pool A^{2-} (Fig. 4). $[A^{2-}]$ was altered either by varying the dark time between the white preillumination and the flashes, or by varying the intensity of the white preillumination. To measure $[A^{2-}]$, far red light is switched on instead of the flashes. The gush of reduced methylviologen observed upon illumination with far red light is a measure of $[A^{2-}]$ at the beginning of the illumination (for more details, see ref. 15).

The half time of the slow phase varies with the inverse of $[A^{2-}]$ and is probably linearly proportional to it.

These two results allow the slow exponential phase to be identified as the transfer of electrons from pool A to the donors of System I studied by Kok et al. [16].

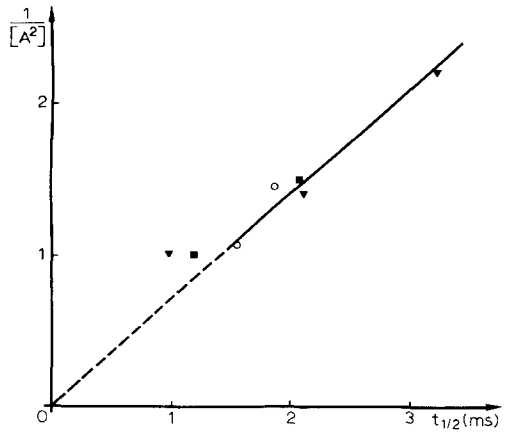

Fig. 4. Half-time of the slow phase of the recovery as a function of $[A^{2-}]$ in spinach chloroplasts, in presence of NH_4Cl 10^{-3}M. ▼ and ■, $[A^{2-}]$ was changed by varying the time between the white light and the first flash. ○ $[A^{2-}]$ was changed by varying the intensity of the white light.

The recovery of a reaction center following one charge separation requires that both transfer reactions, from primary donor to secondary donor and from primary acceptor to secondary acceptor have occurred.

So the rapid phase, which as we showed in the preceeding section corresponds to the first recovery of the reaction center, follows the slower of these two reactions. (In the same way we could say that the slow phase, which corresponds to the second recovery of the reaction centers, follows the slower of the two reactions, from secondary donor to tertiary donor, or from secondary acceptor to tertiary acceptor.)

The kinetics of our rapid phase resemble the kinetics of the rapid phases reported by Haehnel [17] for the reduction of P^+-700.

The absence of any sigmoid character for the kinetics of the rapid phase indicates that the recovery on the acceptor side of System I is several times more rapid than that on the donor side ($t_{\frac{1}{2}} < 20\ \mu s$). A very rapid recovery could be explained either by a very rapid transfer from primary acceptor *P*-430 [18] to the still unknown secondary acceptor, or by the existence of two primary acceptors for one System I center.

The problem of the separation of the chains

Our rapid phase presented as a semi-logarithmic plot has no obvious biphasic character (Fig. 5) and looks like the homogeneous second order kinetics of a reaction:

$$P^+\text{-}700 + \text{PD} \xrightarrow{k} P\text{-}700 + \text{PD}^+$$

where PD is the secondary donor, with a stoichiometry 1 : 1 for *P*-700 and PD. Some recent results of Haehnel [19] confirm this scheme and allow PD to be identified as plastocyanin.

From Figs 1 and 2, we estimated an activation energy of about 9 kcal/mol for this reaction.

In Fig. 5, the normalized kinetics of the rapid phase observed in Fig. 1b are compared. The kinetics are more rapid after the weak flash than after the bright flash. After the bright flash, because of the double hits, some PD^+ is present. The concentra-

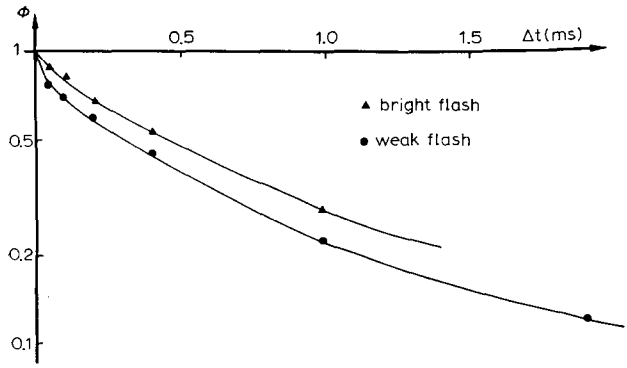

Fig. 5. Kinetics of the rapid recovery phase in spinach chloroplasts at 0 °C. (Semi-log plot). This figure is drawn from Fig. 1b. The slow phase is subtracted, and the amplitude of the rapid phase is normalized to 1.

tion of PD^+ obtained after the flash is equal to the amplitude of the slow phase of the recovery of the centers. Table I (corresponding to Fig. 1b) indicates that $[PD^+]$ is 0.36 immediately after the bright flash and zero after the weak flash. The greater the concentration of PD in the reduced form, the faster the rate of recovery.

The second-order kinetics indicate either transfer from one PD to several *P*-700 molecules, or transfers at the level of *P*-700. In both cases, cooperation between System I chains on the donor side is implied. This explains why the two charges coming from System II on a single plastoquinone molecule may be transferred to two different System I centers [20].

But these chain connections are in contradiction with an assumption of Kok et al. [16] to interpret the observation that, following far red illumination, the time course of the transfer from Q to *P*-700 is first order and the time constant is independent of the intensity of the flash (the number of Q molecules in the sample which are photoreduced).

In fact, they measured the transfer between A^{2-} and the oxidized donors of System I, and the contradiction is between this independence and the connections observed between the different chains through pool A [22–24]. However, it is noteworthy that the independence of the chains is observed under conditions where electrons have just been produced by System II and for low concentrations of A^{2-}.

TABLE I

QUANTITIES OF METHYLVIOLOGEN REDUCED BY FLASH TREATMENT

All values are expressed in arbitrary units. Δt is the time between the first and the second flash. The values correspond to the experiment reported in Fig. 1b.

Light treatment	Bright flashes	Weak flashes
1st flash	1.18	0.465
2nd flash $\Delta t = 0$	0.24	0.293
2nd flash $\Delta t = 1.9$ ms	0.82	0.465
2nd flash $\Delta t = 320$ ms	1.18	0.465

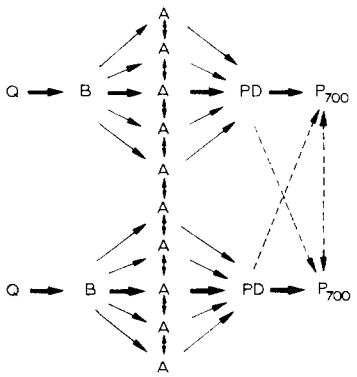

Fig. 6. A possible model for electron transfers between System II and System I.

One possible model to account for these contradictions is presented on Fig. 6. One System I center is associated with each System II center. There are no transfers from one chain to another through Q or B [20, 21]. There are connections between the different chains through pool A [22–24]. But the observation that the transfer rate is independent of $[A^{2-}]$ in the experiment described in ref. 16 implies that there are no transfers at the level of PD, and the transfers from Q to PD are rapid compared to the transfers from one chain to another via pool A. Our experiment indicates either transfer from one PD to several *P*-700 molecules, or transfers at the level of *P*-700. This last point remains to be elucidated.

Cycling and non-cycling centers

In order to detect both cycling and non-cycling System I centers, we also observed the recovery of the photochemical activity through the ability of the centers to generate photochemically the 520-nm absorption change. This technique was developed by Joliot and Delosme [13].

The recovery kinetics of System I exhibit the same pattern as those observed through methylviologen measurements. So there was no evidence for any heterogeneity either between centers involved in the cyclic electron transfer and centers involved in the non-cyclic electron transfer, or between any other types of System I centers.

Our experiments performed in conditions where the donors of System I are in the reduced form, show that only one intermediate is involved in electron transfer between the centers and pool A. If PD is plastocyanin, as seems probable from the experiment of Haehnel [19], a significant transfer of electrons from plastoquinone to the centers through cytochrome f is thus excluded. This is in agreement with the conclusion of Haehnel [10] that cytochrome *f* is situated on a side path of the linear electron transport chain.

APPENDIX

Probability of double hits

When a flash is long compared with the recovery rate of the photochemical centers, several photochemical reactions may occur during the flash. In this appendix,

we shall try to determine the ratio of double hits as a function of the characteristics of the flash and of the recovery reaction.

Let us call:

$J(t)dt$, the probability for a center to have absorbed a first photon during the interval dt located at time t.

$\rho(t',t)$, the probability for a center having absorbed a photon at time t to have recovered photochemical activity at time t'.

$J'(t')dt'$, the probability for a second photon to be absorbed by a center during interval dt' located at time t'.

The probability that the three events happen in the same center is the product of the three probabilities and so the fraction of double hits is

$$\beta = \int_0^\infty J'(t')dt' \int_0^t \rho(t',t)\, J(t)\, dt$$

The probability of absorption is dependent upon the energy transfers between the photosynthetic units. But, this depending induces only second order changes and we shall only study the case where perfect energy transfers occur [25]. Let us call T the time at which all the centers have absorbed one photon, and $I(t)$ the intensity of the flash at time t (normalized so that $\int_0^\infty I(t)dt$ corresponds to the number of photons absorbed per System I center).

If the energy transfers are perfect, we have:

$J(t) = I(t)$ for $0 < t < T$; $J(t) = 0$ for $t < T$;
$J'(t') = 0$ for $0 < t' < T$; $J'(t') = I(t')$ for $t' < T$

To normalize the flash intensity, we performed the following experiment. The flash intensity was decreased by interposing neutral filters of known absorption. When the intensity is low enough, double absorption is negligible and the beginning of the saturation curve can be drawn. The asymptotic value corresponds to the quantity of methylviologen reduced when all system I centers have performed one photochemical reaction. This can be estimated by taking half the amount of methylviologen reduced when the rapid phase is fully developed. Under these conditions, we suppose that 2

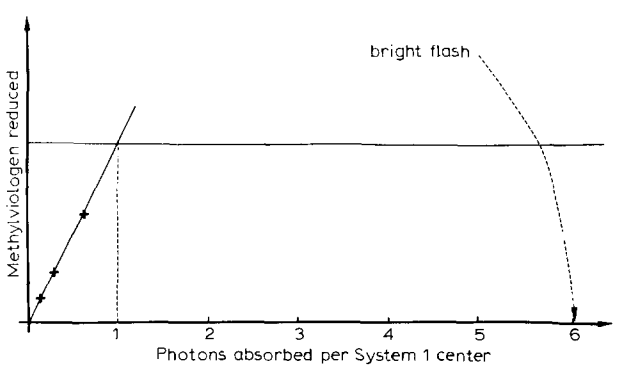

Fig. 7. Methylviologen reduced by a flash after 10 s of bright white light and 300 ms dark, as a function of the intensity of the flash.

photochemical reactions have occurred in each center, i.e. each center has received a double hit.

The results are given in Fig. 7 and we conclude that during our flashes, the ratio between the number of photons absorbed and the number of System I centers is 6.

$\rho(t',t)$ depends upon the recovery kinetics of the centers. If the recovery kinetics are first order with rate constant k,

$$\rho(t',t) = k\ (t'-t)$$

With a bright flash, 90 % of double hits would correspond to a half-time of 9.5 μs, 37 % to 30 μs.

But it has been shown that the recovery kinetics are second order. In this case, no simple computation can be made. To simplify the computation, we assumed that at time T, all the centers are in state P-700 PD. This simplification will give an estimate of the order of magnitude of k. The exact value is overestimated by this simplification.

According to Eqn 1, we found

$$\rho(t',t) = \frac{k(t'-T)}{k(t'-T)-1}\ , \text{for } t' > T$$

if we neglect the contribution of the double hits to the rate of recovery. This simplification also leads to an overestimation of k.

In Fig, 2, β is drawn as a function of k, using these assumptions. The object of this computation is not to give exact values but estimates of orders of magnitude. An exact computation would require the use of an analog computer.

ACKNOWLEDGEMENTS

The author wishes to thank Dr R. P. Cox for his helpful and critical reading of the manuscript.

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