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Electron transfer between the two photosystems in spinach chloroplasts

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SUMMARY

1. We measured the number U_n of electrons transferred from System II to the pool available for System I, by the *n*th flash of a series. After a dark period, the sequence U_n presents damped oscillations with Period 2.

2. With System II limited chloroplasts, the sequence of the quantities MV_n of methylviologen reduced by the *n*th flash of a series exhibits damped oscillations with Period 2.

3. To interpret these phenomena, a model is proposed: the electrons are transferred two by two from System II to the pool available for System I. Some electrons are stored in the dark in the System II chains, on an acceptor we called B. B is not the primary acceptor for System II.

On the other hand, the two electrons may be transferred one by one from the pool to two different System I centers.

In the photosynthetic system, the two photochemical reactions in series transfer electrons from water, through a chain of electron carriers, to a terminal electron acceptor (for review, see ref. 1). The primary electron acceptor Q, for System II, while not chemically identified, has been characterized by its quenching properties[2]. It has been shown that Q is a one electron acceptor[3]. A pool of electron carriers has been located between the two photosystems[4]. Rumberg *et al.*[5] showed that this pool is composed of plastoquinones which are one or two electron carriers.

Abbreviations: DCMU,3-(3,4-dichlorophenyl)-1,1-dimethylurea; MV, methylviologen; PQ, plastoquinone.

Stiehl and Witt[6] proposed a model to account for the transfer between one electron and two electron carriers: two System II centers are in contact with a pair of PQ–PQ. Firstly, plastoquinone is reduced by chlorophyll a_{II} into two semiquinones PQ^-–PQ^-. Secondly, the semiquinone PQ^-–PQ^- dismutates PQ^{2-} and PQ. Thirdly, the electron transfer within the plastoquinone pool results at Light reaction I in a commutation of PQ^{2-} + PQ into two semiquinones molecules. These are oxidized *via* intermediates by chlorophyll a_I^+.

However, these authors used a repetitive flash technique and could not distinguish between a cooperation of two System II centers, and an accumulation of two electrons on a center by two successive photochemical reactions.

Using dark-adapted chloroplasts, we tried to clarify this problem by measurements of methylviologen reduction.

This work was performed on spinach chloroplasts prepared according to Avron[7].

Methylviologen reduction was detected by an amperometric method[8]. Spinach chloroplasts were illuminated either with short flashes (flash lamp General Radio, strobotron, duration 6 μs at 1/3 of peak intensity, 0.4 joule), or with far red (710 nm) modulated light (one photon absorbed per System I center every 0.5 s).

The flashes are saturating, *i.e.* every active photochemical center (System I and System II) absorbs at least one photon upon flash excitation, and are short enough to allow only one photochemical reaction per center. The far red modulated light excites selectively the System I centers[9]. Thus, the amount of methylviologen reduced by far red light is a measure of the reduced state of all the pools located between the two photosystems present at the beginning of the far red illumination (plastoquinone, P_{700}, cytochrome *f*, *etc.*[9]. This may be summarized in Diagram 1.

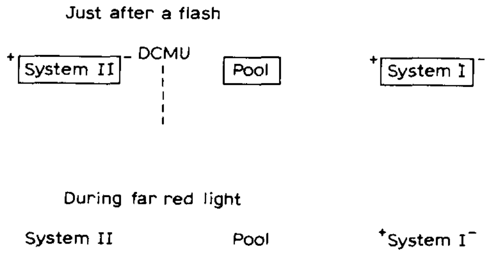

Diagram 1

The amperometric method does not allow absolute measurements. The amount of methylviologen reduced by modulated light is measured *via* an area (see ref. 9) and the flash yield is represented by a number proportional to a tension. Thus, it was necessary to calibrate the apparatus by comparing the quantities of methylviologen reduced by a flash to the quantities reduced by modulated light. We used DCMU-inhibited chloroplasts which only permitted oxidation of the pool by Photosystem I. Under those conditions, the amount of methylviologen reduced by a flash sequence or by modulated light is identical (see Diagram 1).

Electrons transferred from System II

We measured N_n, the quantity of methylviologen reduced by far red light, after n flashes, on dark-adapted chloroplasts (see Diagram 2).

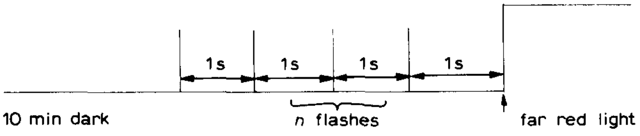

Diagram 2

N_n corresponds to the number of electrons remaining in the pool after n flashes, and available for transfer to System I.

The difference N_n-N_{n-1} equals the number of electrons (U_n) transferred by the nth flash from System II to the pool available to System I, *minus* the number of electrons (MV_n) transferred by the nth flash through System I

$$N_n - N_{n-1} = U_n - MV_n$$

Diagram 3

In fact, a few electrons are transferred by far red light from System II to the pool. But they represent less than 10% of N_n, and are almost equal for N_n and N_{n-1}; so they can be neglected in the difference N_n-N_{n-1}.

As methylviologen is a one electron acceptor, the quantity of methylviologen reduced by the nth flash is a measure of MV_n. From experimental results, N_n and MV_n, we computed the sequence U_n (Fig. 1).

Sequence U_n exhibits damped oscillations with Period 2. We know, from oxygen measurements that the number of photocenters II reacting upon the nth flash is independent of n (ref. 10).

So, we may interpret sequence U_n by making the following assumptions: (a) It is possible to store one *minus* charge on each System II chain. We shall call B the acceptor on which the charge is stored. (b) B^- is stable for, at least, 1 s (time between two flashes). (c) Electron exchange between the pool and the System II electron acceptors occurs two at a time for the main part of the System II chains.

$$B^{2-}+PQ \rightleftarrows B+PQ^{2-} \text{ or } B^{-}+Q^{-}+PQ \rightleftarrows B+Q+PQ^{2-} \quad (1)$$

B could be a special plastoquinone molecule.

In the preceding experiments, we observed oscillations for sequence U_n, but not for sequence MV_n.

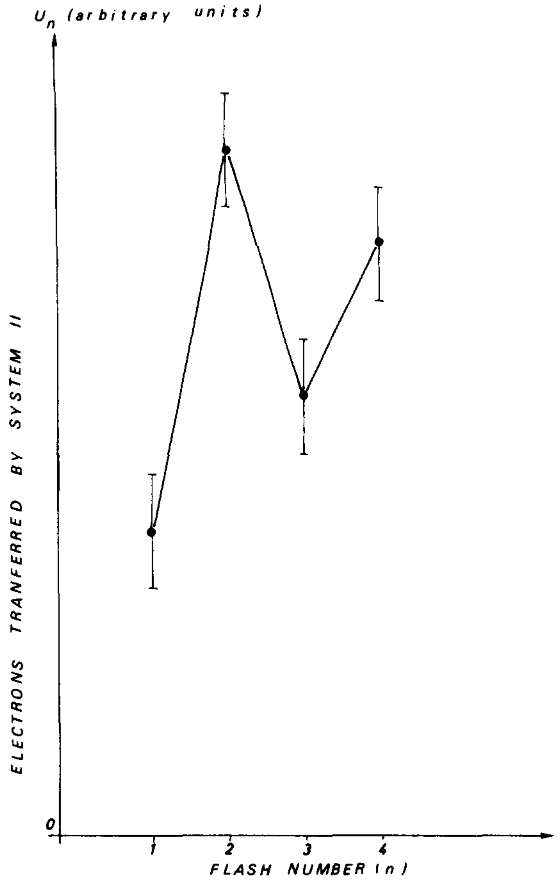

Fig. 1. Numbers U_n of electrons transferred from System II to the pool available for System I, by the *n*th flash of a series, for spinach chloroplasts in presence of 10^{-4} M methylviologen. The time between the flashes was 1 s.

Methylviologen reduction with System II limited chloroplasts

These experiments were performed on chloroplasts partially inhibited by DCMU, *i.e.* chloroplasts where there are fewer System II than System I centers connected to the pool.

The same results could be obtained on aged chloroplasts where there were also fewer System II than System I centers.

After far red preillumination, the suspension is kept in the dark and then illuminated with a series of flashes. We studied the effect of the dark time between the far red preillumination and the flashes, on the flash yields sequence.

The quantities of methylviologen, MV_n, reduced by the *n*th flash, present damped oscillations with Period 2 (Fig. 2). Immediately after far red light, the amplitude of the oscillations is zero. The amplitude grows to maximum in 2 min 30 s,

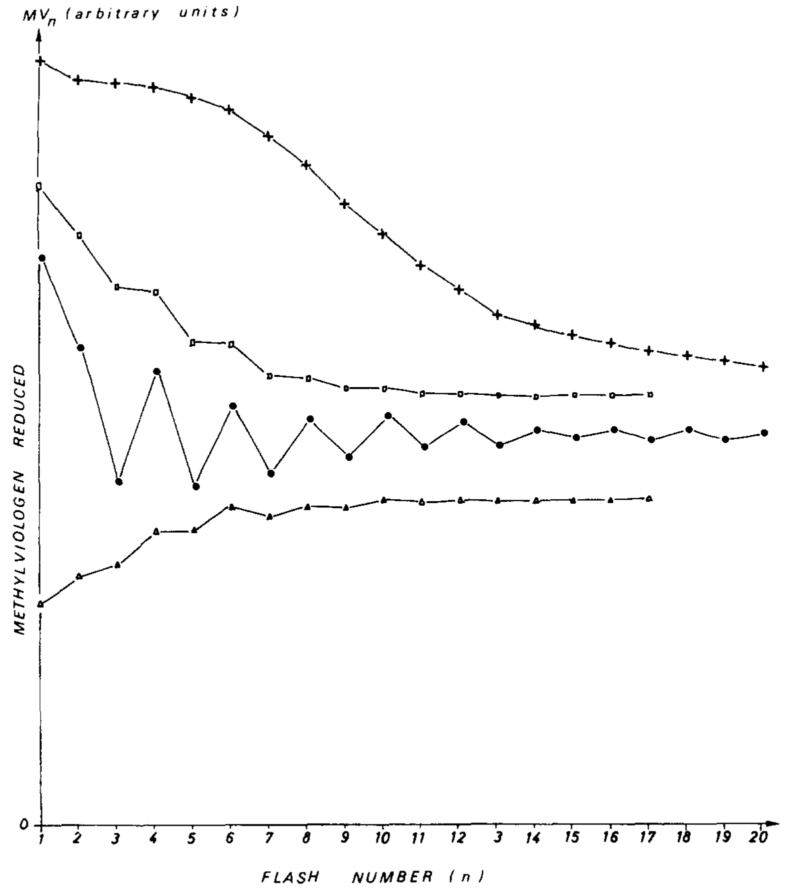

Fig. 2. Quantities MV_n of methylviologen reduced by the nth flash of a series for spinach chloroplasts in presence of $2 \cdot 10^{-7}$ M DCMU (inhibition for oxygen, 50%) and 10^{-4} M methylviologen. The time between two flashes was 0.300 s. The series of flashes were given as follows: △, 4 s dark after 20 s far red light; ○, 2 min 30 s dark after 20 s far red light; □, 10 min dark after 20 s far red light; +,4 s dark after 10 s bright white light.

then diminishes if the dark time after the far red light is increased.

This growth then decrease of the amplitude probably indicates that two reactions affect the oscillations.

2 min 30 s is sufficient time, in this experiment, for deactivation of states S_2 and S_3 in System II[8] (the far red light is strong enough to produce activation of System II centers). The maximum amplitude of the oscillations is probably linked to deactivation. In effect if the same experiment is done on chloroplast batches where deactivation is much slower ($t_{1/2}$ about 6 min), the oscillations are not observed after 2 min 30 s. They appear only at a longer time and with diminished amplitude.

More than 2 min 30 s after far red light, the pool between System I and

System II becomes reduced and the amplitude of the oscillations is diminished. In the same way, when chloroplasts are preilluminated with white light, *i.e.* when the pool is reduced, no oscillations are observed.

As the MV_n oscillations appear only when System II is limiting and when the pool between the two photosystems is largely oxidized, it is reasonable to suppose that these oscillations originate in the electrons coming from System II.

We assumed in the first paragraph that the main part of the System II chain transfers two electrons at a time to the pool available for System I.

Two types of reactions may occur between this pair of electrons and System I donors:

(a) The two electrons are transferred to the same System I center. The System I centers react only once during a short flash; two flashes are necessary for the transfer of the two electrons to the methylviologen. As methylviologen is a one electron acceptor, this type of reaction produces no oscillations for MV_n when System II is limiting (see Diagram 3).

(b) The two electrons may be transferred one by one to two different System I centers; when System II is limiting, and after intermediary pools have been emptied, the number of oxidized System I centers before the *n*th flash equals the number of electrons transferred from System II by the $(n-1)$th flash. According to Diagram 3, we then obtain:

$$MV_n = U_{n-1}$$

which involves oscillations with Period 2 for MVn.

If both reactions a and b took place, the damping for MV_n would be greater that the damping for U_n.

A striking point is that everytime sequence MV_n exhibits oscillations, the damping of these oscillations is 0.12, *i.e.* equal to the damping of oxygen oscillations[13]. To interpret this damping, Kok *et al.*[13] proposed that during each short saturating flash of a sequence, 12% of the System II centers are photochemically inactive. Thus the damping for U_n ought at least to equal 12%, and the equality:

$$\text{Damping for } MV_n = \text{damping for oxygen}$$

involves the equality

$$\text{Damping for } MV_n = \text{damping for } U_n$$

Under those conditions, reactions of type a are unlikely. The electrons are transferred one by one to two different System I centers.

To improve the relation $MV_n = U_{n-1}$, we measured U_1 and U_2 in chloroplasts partially inhibited by DCMU, after far red preillumination and 2 min 30 s dark.

The results obtained were different from those obtained on non-inhibited chloroplasts; the ratio U_1/U_2 was 1.4 instead of 0.5 in Fig. 1. This change may be due to the change of the deactivation mechanisms of the oxygen precursors induced by partially inhibiting concentrations of DCMU[14,15].

Under the same conditions (Fig. 2, black circles), the ratio MV_2/MV_3 was 1.5. Within experimental error, this is in good agreement with the relation, $MV_n = U_{n-1}$.

The electron transfer from System II after the first flash indicates that some B^- is present.

If the quenching properties of B and B^- were different, fluorescence would present, under a series of flashes, oscillation with Period 2. This was not observed[16]. As it is usually assumed that Q, the primary electron acceptor of System II, is a better quencher than Q^-, it seems likely that B differs from Q.

The nature of the primary acceptor (or acceptors) of System II is still to be elucidated as well as details of its exact association with B.

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