Short communication *Bot. Bull. Academia Sinica* 18: 169-177 (1977)

STEP-WISE STABILIZATION OF CHARGE SEPARATION AT PHOTOSYNTHETIC SYSTEM II REACTION CENTER—A HYPOTHESIS[1,2]

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(Received May 10, 1977; Accepted May 31, 1977)

The first steps in photosynthesis are the absorption of light by specific pigment molecules and the transfer of that energy from one pigment molecule to another, until it is eventually conveyed to those few molecules that participate in chemical reactions. Those few molecules together with the associated electron carriers are named reaction centers where the central events of photosynthesis take place. In green plants, a partial reaction of photosynthesis is the photooxidation of water, the pigment system of this partial reaction along with its reaction centers and affiliated enzymes which carry out the photooxidation of water is called Photosynthetic System II (PS II).

The photoenergy conversion at the reaction center (including PS II) is initiated by charge separation. It has only recently been confirmed that the primary charge separation process of PS II involves the photooxidation (Malkin and Bearden, 1973; Ke *et al.*, 1974) of a specilized chlorophyll-a designated as P690 (Döring *et al.*, 1967). This specialized chlorophyll may be a dimer (Fajer *et al.*. 1977; Katz *et al.*, 1977). The corresponding event to photooxidation of P690 is photoreduction of a primary acceptor, the latter is called Q by Duysens and Sweers (1963), or X320 by Stiehl and Witt (1968). The electron transfer between P690 and Q is only one of the many successive electron transfers for the eventual stabilization of light energy; as it will be shown, even this "one step" may involve complicate changes.

The charge separation between P690 and Q occurs in less than or equal to 20 ns according to Wolff *et al.* (1969). $P^{+}690$ has two components (note added in proof) in terms of life-time, namely, a 35 μs component and a 200 μs compo-

(1) This study was supported by the National Science Council, the Republic of China.

(2) Paper No. 197 of the Scientific Journal Series, Institute of Botany, Academia Sinica. Abbreviations: ns=nanosecond=1×10^{-9} second; μs=microsecond=1×10^{-6} second; DCMU=3-(3, 4-dichlorophenyl) -1, 1-dimenthlyurea.

nent (Wolff *et al.*, 1975, disregard a 3 μs component which exists even in chloroplasts either incubated or preincubated at 60°C which incapacitats the reaction center of PS II and both luminescence and variable fluorescence, see latter this report, are abolished). On the other hand, Q^- has a life-time of 600 μs (Forbush and Kok, 1968). During this period, back reaction between P^+690 and Q^- is expected, and indeed it takes place, which is indicated by the luminescence emission.

Luminescence (delayed light or delayed fluorescence) has been considered by Arthur and Strehler (1957) to be the results of the back-reaction (or recombination) of the primary photooxidized and photoreduced products of the light reaction, and more specifically those of PS II (Goedheer, 1962, 1963; for a recent review please refer to Lavorel, 1975a).

From the 35 μs and 200 μs life-time of P^+690 (the half-times of the reduction of P^+690 by secondary and tertiary electron doner respectively, Wolff *et al.*, 1975), it is not surprising to find the 35 μs and the 200 μs components of luminescence lifetime (Zankel, 1971). However, it is not expected to have the one and the five to ten μs components of luminescence (Zankel, 1971; Haug, 1972; Lavorel, 1973; Lavorel, 1975b; van Best and Duysens, 1977). Interestingly and surpringly as well, chloroplast fluorescence is found not to increase until 3 μs after a 10 ns saturating laser flash for the first few flashes (Mauzerall, 1972). That the fluorescence is only increased 3 μs after light absorption being not expected is due to the Q hypothesis of Duysens and Sweers (1963), in which they postulate that a fluorescence yield increase reflects a redox change of Q, from oxidized to reduced form, which completes within 20 ns. In fact, the 20 ns is the rise time of an electric field indicating absorption change at 520 nm (Wolff *et al.*, 1969), the actual electron transferring time may be far shorter than this. (Kaufmann *et al.*, 1975 and Rockley *et al.*, 1975 find in photosynthetic bacterium a rise time for oxidation of the reaction center complex of~150 psec.).

To explain the initial low fluorescence and the 3 μs fluorescence rise, Zankel (1973) proposes that a quencher is formed in the light, this quencher is possibly a carotenoid radical or triplet with a life time of several μs. Duysens (1972) make a similar suggestion to explain the fluorescence lowering phenomenon under strong light. However, this model fails to explain why there are several phases of luminescence decays in the microsecond range shorter than 35 μs, for there is no further electron transfer occurring besides the one of 20 ns life time (note added in proof). The light-induced quencher is expected to quench both fluorescence and luminescence, and as a result one should observe parallel increases of both, but in fact what one observes are antiparallel changes of fluorescence and luminescence in this time range. Furthermore,

recent picosecond flash experiment (Campillo *et al.*, 1976) show that light-induced fluorescence quenching appears in the picosecond range which indicates the existence of singlet-singlet annihilation processes. It is therefore doubtful that the formation of carotenoid triplet can compete with the chlorophyll singlet-singlet annihilation processes during the flash, and that a significant triplet population can be built up in a single flash in the experiment of Mauzerall (1972).

Another way to explain the one μs luminescence decay and the 3 μs fluorescence rise is to assume that there is a reduction of P^+690 at this time range (Duysens *et al.*, 1975), for P^+690 is assumed to be both a precursor of luminescence and a quencher of fluorescence (Butler *et al.*, 1973). However, besides the lack of any direct evidence for the existance of a one μs reduction of P^+690, this explanation encounters a kinetic difficulty. The suggestion of Butler *et al.*, (1973) that P^+690 is a quencher is not supported by their studies of the rise of fluorescence and the kinetics of the oxidation of the electron donor of P^+690 at 77°K, for the fluorescence rise leads in time the kinetics of electron donor oxidation initially and then falls behind the latter (Fig. 3 in Butler *et al.*, 1973). The P^+690 can not therefore be the exclusive quencher, if it is indeed a quencher. P^+690 may be a quencher in the sense that its existence facilitate the regeneration, by means of charge recombination, of a photochemistry active reaction center D.P690.Q which traps and converts the energy chemically.

With both explanations fail in one way or another, we introduce a third one.

Before the introduing of this explanation, we recall an observation made by Bennoun and Li (1973). The experiment includes: (1) to preilluminate hydroxylamine-incubated chloroplast; (2) to put the sample under dark to allow full reoxidation of Q^-; (3) to add 3-(3, 4-dichlorophenyl)-1, 1-dimethylurea (DCMU, a PS II inhibitor) to the chloroplast in the dark; (4) to illuminate the sample with a continuous light, and one inevitably observes a higher initial fluorescence than a control sample with similar treatment except the hydroxylamine addition. They suggest that the above-mentioned treatment may induce a disconnection of part of the Q from the centers, and further speculate that a reversible binding of Q to the centers may be part of the current process of electron transport. Similar dynamic models on the various reactions of photosynthetic electron transport have been postulated by Malkin and Michael (1972), Mauzerall (1972), Bouges-Bocquet (1973) and Lavorel (1976). These dynamic models agree with the membrane dynamism in general (Singer and Nicolson, 1972), and provide a functional explanation for the thylakoid membrane dynamism in particular (Giaquinta *et al.*, 1974).

The model of disconnection of Q^- from P^+690 agrees with both the luminescence decay kinetics at one and at five to ten μs and the fluorescence rise at 3 μs, if one assumes that it is the disconnection following the Q reduction but not the reduction per se which induces a high fluorescence state. The slight difference between fluorescence and luminescence in life time may be real or may be trivial. If it is real, it then shows that the factors which governs the rate of back reaction and the fluorescence yield although closely related are not the same events.

We now summarize all the points we have mentioned above and depict the primary photoevents as follows, and we shall revise it as the discussion progresses. In the absence of any specific evidence which shows the nature of the "disconnection" between Q and P690, we shall call our model as state transformations model instead of disconnection model, the term "disconnection" may have a physical connotation.

$$D \cdot P\,690 \cdot Q$$

$$\uparrow\downarrow\ h\nu \qquad (1)$$

$D \cdot P^{+}690 \cdot Q^-$ (state of low fluorescence and high luminescence)

$\uparrow\downarrow$ State transformations alter the physical relationship between Q^- and P^+690 (2)

$D \cdot P^+\ 690 \cdots Q^-$ (state of high fluorescence and low luminescence)

$\uparrow\downarrow$ (3)

$D^+\ P\ 690 \cdots Q^-$ (high fluorescence state, luminescence is further lowered)

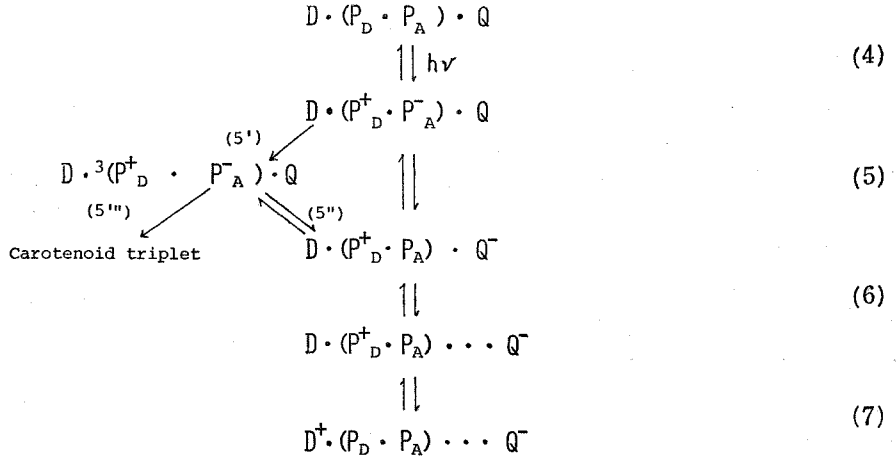
Reaction (1) is the light—induced primary charge separation which results in a complex species $D \cdot P^+\ 690 \cdot Q^-$ which, although Duysens and Sweers (1963) designate it as a state of high fluorescence, represents, in our model, a state of low fluorescence and high luminescence. A state transformation [Reaction (2)] then occurs not later than 1 μs in the reaction center, which gives rise to a state[3], represented by $D \cdot P^+\ 690 \cdots Q^-$, of high fluorescence and low luminescence; there may be a series of state transformations resulting in the one, the five to ten μs luminescence changes and the 3 μs fluorescence rise. Reaction (3) regenerates P 690.

The scheme depicted by the Reactions (1), (2) and (3) may not be a complete description of the primary photoevents. Both the observation of

(3) In the original Q-hypothesis, Q is a hypothetic molecule, it could be a hypothetic state (other than the redox state) as well, the state transformation model is therefore in essence a revised form of the Q-hypothesis of fluorescence.

van Best and Duysens (1977) that at one μs, maximal luminescence is only observed under anaerobic conditions for reaction center in the state P 690 • Q^- before the flash, and the observation of Lavorel (1975b) that the very first phase (5–10 μs under his experimental condition) is even observed in the presence of DCMU under repetitive flashes with flash duration of 50 μs and flash period of 50 ms suggest that Q may not be the primary electron acceptor (this is implied in Lavorel's report, 1975b; and suggested by van Best and Duysens, 1977). The P 690 seems to be composed of P_D and P_A, the primary electron donor and acceptor respectively, or the P 690 is a P_D, and P_A is a pheophytin (Fajer *et al.*, 1975; 1977; van Best and Duysens, 1977 and references quoted therein).

Incorporating this new information into the state transformation model, we have



Light induces a charge separation [Eqn. (4)] in the complex ($P_D \cdot P_A$). During the life time of ($P_D^+ \cdot P_A^-$), an opportunity arises for scrambling of the electron spins in the two components of the biradical, changing its overall spin state from singlet to triplet [Eqn. (5′)]. ($P_D^+ \cdot P_A^-$) can transfer an electron to Q within 20 ns in both singlet and triplet states [Eqns. (5) and (5″)]. The triplet biradical may transfer its energy to a carotenoid [Eqn. (5‴)] when Q is reduced. The crossing of a singlet to a triplet may prolong the life time of ($P_D^+ \cdot P_A^-$) so that the chance of electron transfer from P_A^- to Q is increased. To further stabilize the Q^-, a state transformation then occurs [reaction (6)] which results in a ($P_D^+ \cdot P_A$)$\cdots Q^-$ state, in which the interaction between Q^- and P_A is hindered.

($P_D^+ \cdot P_A$) • Q^- may be a center which still traps an exciton and then crosses into a triplet state and therefore quenches fluorescence. On the other

hand, $(P_D^+ \cdot P_A)\cdots Q^-$ may be a center which does not receive excitons, i.e., it is in a nontrapping state, or which though can be excited but a crossing from excited singlet to triplet is forbidden so that there is a high probability of exciton detrapping and returning it to the bulk chlorophyll where it may fluoresce, in either case, the fluorescence yield is high.

Reaction (6) represents an electron transfer from D to P_D^+, which completes the regeneration process of the $(P_D \cdot P_A)$ complex. For a complete regeneration of the photochemistry active reaction center, $D \cdot (P_D \cdot P_A) \cdot Q$, an electron must transfer to D^+ from a tertiary electron donor and Q^- must give its electron to A, the electron acceptor for Q^-, and finally Q must return to a state which can interact with P_A.

We suggest that the initial (submicro to microsecond) low fluorescence yield under Q^- state may be due to a high probability of triplet formation of the $(P_D^+ \cdot P_A)^* \cdot Q^-$ excited state, and to a less extent to a singlet-triplet annihilation if the center is in $^3(P_D^+ \cdot P_A^-) \cdot Q$ state when a new exciton arrives, therefore the initial low fluorescence is an intrinsic property of the reaction center. While according to Duysens *et al.* (1972) and Zankel (1973), the (carotenoid triplet) quencher formation is only of significant importance if there are excessive light quanta, i.e., more than one hit of the center during the life time of Q^-, and the initial low fluorescence is only observed under excessive light quanta. We believe however that it is important to make distinctions between the fluorescence lowering phenomenon under high light (Duysens *et al.*, 1972) and the initial low fluorescence yield of Q^- state; that they are different is shown by the observation that the fluorescence rise in a later time and the fluorescence lowering phenomenon have different light intensity dependency (Mauzerall, 1976). The fluorescence lowering effect during high light intensity and long duration flash may possibly be due to a combination of the singlet-singlet, the singlet-triplet annihilation processes, singlet-ion quenching (Breton and Geactintov, 1976) and the carotenoid triplet formation, while the initial low fluorescence may be due to the triplet formation of the $(P_D^+ \cdot P_A)^* \cdot Q^-$ excited state.

In summary, in the primary process of photosynthesis a charge pair is light induced, to stabilize this charge pair whose recombination is thermodynamically favorable and it is further facilitated by the fact that the pair are permanent residents of the reaction center (concept of fixity of the centers, Lavorel, 1973) an electron transferring between the primary and secondary acceptor occurs (an auxiliary stabilization mechanism may be a biradical triplet formation), the reduction of the secondary aceeptor triggers state transformations of the center; in the new states the interaction between P_A and Q^- is hindered, and finally an electron transfer on the donor side completes the

stabilization processes at the reaction center and regenerates the active ($P_D \cdot P_A$) complex. Like the electron transfer process in general the stabilization of the primary charge separation may also be accomplished stepwisely.

Note added in proof: For the purpose of interpreting the 3 μs fluorescence rise and the one, the five to ten, the 35, and the 200 μs luminescence decay, it is of interest to find the correspondent components of the half times of P^+690 reduction or Q^- oxidation. Of these, there are direct absorption evidence for the 35 and the 200 μs components of P^+690 reduction. Recently Gläser *et al* (M. Gläser, Ch. Wolff, and G. Renger. 1976. Z. Naturforsch. 31C: 712) suggested that there was a reduction of P^+690 with a half time $\leqslant$ 1 μs. Judging from the kinetic recording they presented (no very well formed initial spike in the curve, in fact the curve for the normal chloroplasts shows an inconspicuous cap in the beginning) and the instrument they employed (allowing a time resolution of 1 μs, G. Renger and Ch. Wolff. 1976. Biochim. Biophys. Acta 423: 610), if this component of P^+690 reduction indeed exists, the half time must be much smaller than one μs.

To explain the 3 μs fluorescence rise in terms of P^+690 reduction, two criteria have to be met: first, P^+690 has to be shown as a major fluorescence quencher which however is not firmly established (P. 171, this report); second, a reduction of P^+690 around 3 μs has to be shown (if measured simultaneously the fluorescence and the reduction kinetics should show exact correspondence), any reduction much earlier than 3 μs, such as the one proposed by Gläser *et al.* can not be considered meeting this criterion. The second criterion applies to the luminescence as well.

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光合作用反應中心電荷分離後之穩定—小步驟法 (一項假設)

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葉綠體在其反應中心將光能轉變爲化學能，其方法爲將電子自初級電子施主轉移至初級電子受子，由是使二個電子載體分別帶有正負電荷。這種以電荷分離來轉變能量形式的方法，普遍存在于植物光合作用的第一，第二系統以及細菌光合作用中，本文僅討論第二光合系統。第二光合系統電荷分離後有再結合之可能，後螢光之存在卽爲一例證。後螢光光譜與螢光同，但以其放射生活期遠較螢光者爲長故名之。各項實驗一再證明後螢光源自第二系統且是該系統初級電荷分離後再結合之表象。所以後螢光是研究再結合現象的有力工具，而對再結合現象的探討可能協助我們對反應中心正常功能的了解。由于有再結合的可能，我們不免要問，反應中心是以何種機構來防止分離後之電荷產生大規模再結合的現象呢？本文主要以文獻中後螢光報告，參證螢光及電子轉移方面的報告，推測反應中心在完成初級電荷分離後係以一系列之狀態變化以及電子轉移來穩定電子的分離狀態，不使其產生大規模之再結合。