Discussion Letter

The emission of chlorophyll in vivo
Antenna fluorescence or ultrafast luminescence from the reaction center pigments

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Low temperature photosystem 2 emission (F_{685}, F_{695}) of chloroplasts may originate from a small pool of pigments. However, the excitons reaching the reaction center have been generated in a much larger pool of chlorophylls constituting the almost non-fluorescent light-harvesting complex. Based upon structural information an interpretation for the molecular origin of the F_{695} emission of variable yield was proposed [FEBS Lett. (1982) 147, 17–20]: in reaction centers having the quinone acceptor reduced, a charge recombination occurring between the primary donor $P680^+$ and Phe^- (the pheophytin primary acceptor) can generate a singlet excited state of Phe which deactivates by emitting F_{695}. Here, an analogous process is discussed for F_{685} with the emission occurring either from P680 directly or from the small pool of core antenna chlorophylls surrounding the reaction center. Furthermore, the presence of F_{695} in the low temperature emission spectra of dark-adapted chloroplasts leads us to propose that charge recombination also takes place in open reaction centers when the quinone acceptor is oxidized. In this case the short lifetime (130 ± 20 ps) observed for the singlet exciton in the intact membrane suggests that the rate-limiting step in conditions of active photosynthesis is more probably determined by the stabilization of the negative charge on the quinone than by either the rate of energy transfer among antenna or the rate of trapping by the reaction center.

Photosynthesis *Primary reaction* *Photosystem 2* *Low temperature fluorescence* *Charge recombination luminescence* *Energy transfer*

1. INTRODUCTION

In photosystem 2 (PS 2) of green plants the reactive center (RC) is described as composed of a primary donor chlorophyll (P680), in close proximity to an intermediary acceptor which is a pheophytin (Phe) molecule, and of a primary quinone (Q) acceptor [1]. In RCs where Q is already in the reduced state (P680–Phe Q^-) a charge separation can still occur leading to the state $P680^+$–Phe^-Q^-. By analogy with observations made on photosynthetic bacteria [2] and also following direct investigations by optical and EPR spectroscopies [1,3], it seems convincingly established that this state relaxes to the ground state with emission of luminescence originating from the recombination of charges according to:

$$P680^+–Phe^-Q^- \longrightarrow P680^*–Phe\ Q^- \longrightarrow P680–Phe\ Q^- + h\nu \quad (1)$$

Furthermore, this scheme provides a mechanism rationalizing the fluorescence of variable yield (F_v), which appears upon light-induced reduction of Q,

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in terms of a fast luminescence [3]. That the recombination of charges at the level of the RC of PS 2 leads to luminescence has been recognized for many years [4] and has provided a wealth of information on the primary processes of photosynthesis [5]. We note however, that it has always been supposed that the excitons created upon charge recombination were reinjected back into the antenna system [5] where they would decay either by fluorescence or by retrapping at a RC. This hypothesis was based on a similar supposition attributing to antenna chlorophyll most of the fluorescence of the excitons created upon direct absorption of the photons in the antenna system. This hypothesis on the antenna origin of the emission of chlorophyll in vivo has been so widely accepted [5–10] that it has become a dogma.

By using polarized light spectroscopy on oriented photosynthetic systems we have observed [11] that in chloroplasts at low temperature the light emitted at 695 nm (F_{695}) stems from an oscillator, the orientation of which could not be correlated with the orientation of the Q_y transitions of any significant pool of antenna chlorophylls [12]. The report that the Q_y transition moment of the Phe intermediary acceptor of PS 2 was oriented [13] with the same geometry as the dipole emitting at 695 nm led us [11] to extend to low temperature the recombination model in [2] and to propose that the F_{695} photons were emitted directly from Phe when the exciton created upon charge recombination becomes localized on this acceptor. The process

$$P680^+-Phe^-Q^- \longrightarrow P680-Phe^* Q^- \longrightarrow$$
$$P680-Phe\ Q^- + h\nu\ F_{695} \qquad (2)$$

analogous to the one described in eq.1 and in which the exciton is not reinjected in the antenna bed, has thus to be considered in the RC of PS 2.

Assuming the validity of this hypothesis we will use some data available in the literature to challenge the currently accepted dogma that the emission stems from a large fraction of the antenna pigments. We will discuss an alternative model in which most of the emission comes from a small pool of pigments and is generated by charge recombination even under conditions of active photosynthesis (state P680–Phe Q). Although we will mainly focus on the low temperature emission of the PS 2 of green plants, we note that, based upon energetic considerations, the importance of charge recombination in the primary processes of bacterial photosynthesis has been stressed in [14].

2. DOES THE ANTENNA CHLOROPHYLL FLUORESCE?

Two main classes of antenna can be distinguished in the photosynthetic membrane of green plants. On the one hand the core antenna is made of chlorophyll *a* molecules surrounding the RCs of PS 1 and PS 2. They can be extracted in their native form together with the RC [15,16]. On the other hand a significant fraction (~50%) of the total chlorophyll *a* and most (or all) of the chlorophyll *b* molecules are associated in a more peripheral antenna system, the light-harvesting complex (LHC). Although it seems commonly accepted that at low temperature most of the fluorescence of chlorophyll in vivo originates from peripheral antenna [5–10], there is strong evidence pointing to a low fluorescence yield of the LHC in vivo. While isolated LHC has its peak emission at about 680 nm [17,18], only a small shoulder at this wavelength can be observed in the fluorescence spectra of normal chloroplasts at 77 K and at lower temperatures. This shoulder, which is absent in organisms deficient in LHC (chlorophyll *b*-less mutant of barley, blue-green algae), has been attributed to the F_{680} emission of LHC [19,20]. The contribution of F_{680} to the total emission spectrum at 77 K thus appears to be very small. On the other hand, normal F_{685} and F_{695} are still present in the low temperature spectra of organisms deficient in LHC, thus indicating that these emissions do not originate from LHC.

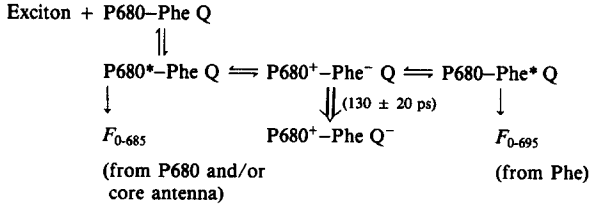
It is important at this point to recognize that the excitons which lead to F_{685} and F_{695} at low temperature have been primarily generated in the LHC bed. This is clearly indicated by the observation that the excitation spectra of F_{685} and F_{695} are almost identical to the LHC absorption spectrum even at temperatures as low as 4 K [21] and is further demonstrated by the extreme similarity between the polarized fluorescence excitation spectra for F_{685} and F_{695} on oriented chloroplasts [22] and the linear dichroism spectra of oriented LHC [23] at low temperature. These observations illustrate the small size of the core of antenna surrounding

the RC of PS 2, for which as few as 40 chlorophyll *a* are involved [16]. Furthermore they indicate that a very efficient energy transfer process with a low probability of radiative decay, even at 4 K, leads the excitons created in the LHC towards a small core of antenna surrounding the RC of PS 2.

3. THE IMPORTANCE OF RECOMBINATION LUMINESCENCE

According to authors in [2,3] the variable fluorescence (F_V) at room temperature is in fact a luminescence originating from charge recombination as described in eq.1. In [11] we presented circumstantial evidence, based upon structural observations, indicating that at low temperature, F_{695} leaves the RC directly from the Phe partner of the pair without returning to the antenna core as described in eq.2. Observing that the absorption of P680 and Phe peak at almost the same wavelength [13], it seems possible to attribute the variable F_{685} ($F_{V\text{-}685}$) to the process described in eq.1 and with the emission originating directly from P680. Since the Q_y transition of P680 has an orientation similar to the one of the PS 2 core antenna (i.e., rather parallel to the membrane plane [12,23–25]), we cannot yet use a structural information to decide whether $F_{V\text{-}685}$ originates from P680, from the small core of chlorophyll surrounding the RC, or from both (excitons oscillating between the core and P680).

Fluorescence induction experiments at 77 K indicate that the variable fluorescence represents a large fraction (~80%) of the total PS 2 emission although the presence of F_{685} and F_{695} is still easily recognized in the F_O spectra [19,20]. At still lower temperatures an even larger contribution to the F_O level is made by F_{685} and F_{695} ($F_{O\text{-}695}/F_{V\text{-}695} \sim 1$ at 4 K [19]). These observations, and more specifically the presence at low temperature of a large F_{695} emission when all the RCs are in the open state (P680–Phe Q), suggest the possibility of a common origin for the F_V emission and for at least a fraction of the F_O emission. Accordingly, we propose as a possible mechanism for the low temperature emission of PS 2, that charge recombination can occur in the state $P680^+$–Phe^- Q. By an ultra-fast luminescence process this leads to $F_{O\text{-}685}$ and $F_{O\text{-}695}$ according to the following scheme:



(3)

In this scheme (which does not consider other possible non-radiative and/or triplet pathways [26]) the main deactivation channel for the radical pair is the transfer of the electron on the primary quinone acceptor. The second competing channel, which even at 77 K represents a minor (<5%) pathway, is an ultrafast luminescence occurring in these RCs where the charge stabilization on Q has not been successful. As already noticed in the case of the closed RC [11], the equilibrium between the two singlet-excited states associated with the radical pair provides a rationale for the large variations in the F_{685}/F_{695} ratio observed as a function of temperature and of a variety of physical or chemical treatments which affect the RC complex.

Upon direct excitation of isolated bacterial RCs it has been observed that the electron transfer step from the bacteriopheophytin intermediary acceptor to the primary quinone takes place with a half-time of 150–250 ps [27–29]. If this observation is extrapolated to the case of the PS 2 RC in the intact membrane and at low temperatures, the rate-limiting step of the overall equilibrium described in eq.3 (and which also includes the migration of the exciton in the antenna system) could then be determined by the stabilization of the negative charge on the primary quinone. A short lifetime (130 ± 20 ps) has been detected in chloroplasts and algae under a variety of conditions [30,31], including low temperature [32]. Furthermore, the strict dependence of the amplitude of this short-lived component on the state of the RCs of PS 2 at room temperature has been described in [33]. Its contribution to the overall emission is rather large at the F_O level but becomes vanishingly small at the F_M level when all the RCs are closed. Accordingly, we propose that the 130 ± 20 ps fluorescence decay might reflect the electron transfer step depicted in eq.3. The observation that: (i) the electron transfer step is rather temperature-independent for bacterial RCs in the temperature range 300–77 K

[29]; and (ii) the 130 ± 20 ps component of chloroplasts is present both at room and low temperature suggests that the mechanism proposed in eq.3 also occurs at ambient temperature.

4. CONCLUSIONS

Here, we have indicated that various fluorescence properties of PS 2 at low temperature could be rationalized in a frame different from the one currently accepted, which assumes that most of the fluorescence originates from the peripheral antenna. This alternate model emphasizes the role of the RC pigments and/or core antenna in the fluorescence process.

We note that this model, which extends the original hypothesis [2,4] to the case of the open RCs and unifies in terms of luminescence the origin of the various PS 2 emissions, might also help to explain some properties of bacterial photosynthesis. In this respect it is of interest to recall that, for chromatophores from various bacteria at the F_O level, fluorescence lifetimes of the order of 100–300 ps have been reported [14,34,35] and that they are comparable to the kinetic of primary charge stabilization in the isolated RCs. This model also suggests that the rate-limiting step for the exciton lifetime is neither at the level of energy transfer within the antenna nor limited by the trapping of the exciton by the RC, but is determined by the stabilization of the negative charge on the quinone acceptor. Assuming the validity of this last point, the transfer time from the antenna (peripheral and core) to the RC of PS 2 could then be estimated from a measurement of the rise-time of $F_{O\text{-}695}$ at low temperature.

Finally, to avoid the replacement of one dogma by another, I stress that the two models which have been discussed here (antenna fluorescence or ultrafast luminescence) are not mutually exclusive. There are indeed several observations that peripheral antenna do fluoresce: an emission from the peripheral antenna of *Rhodopseudomonas sphaeroides* (B800–850) has been detected in the F_O (but not in the F_V) spectrum at room temperature [19,36]. A specific contribution of F_{680}, the emission from LHC, to the F_O spectrum of chloroplasts has been reported at 4 K [19]. Furthermore, the heterogeneity in terms of orientation of the emission dipoles which has been described for the fluorescence band of chloroplasts [37,38], might reflect a contribution of both LHC and PS 2 core antenna to the emission spectrum at room temperature. Further experiments at room and low temperature are clearly needed to discriminate better between the 'dead' fluorescence from unconnected pigments, the fluorescence from pigments which transfer excitons to the RC but do not sense the state of the RC and the 'active' fluorescence from the pool of pigments which senses the state of the RC. In addition, the limiting possibility that for the PS 2 emission F_{685} at low temperature this last pool of pigment is constituted by P680 itself, should also be investigated.

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