RECOMBINATIONAL LIGHT EMISSION FROM PHOTOSYSTEM II REACTION CENTERS

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Abstract. The variable yield of Chl a fluorescence from PS II is either (1) prompt fluorescence originating in competition with photochemical trapping, or (2) recombinational luminescence originating from the reaction between $P680^+$ and $Pheo^-$. Arguments for and against the recombinational hypothesis are presented. Our current data on Chl a fluorescence lifetime distributions in open and closed PS II reaction center preparations show that a significant amount of 2-30 ns lifetime light emission is recombinational luminescence.

1. INTRODUCTION. Chl a fluorescence is a non-destructive and highly sensitive probe of photosynthesis, particularly of PS II (1). PS II serves to oxidize water to O_2 and to reduce plastoquinone to plastoquinol (2,3). Its antenna system includes Chl a/b - containing LHC-II, a 29 kDa polypeptide (CP-29), and Chl a - containing protein complexes of 43 and 47 kDa (CP-43 and CP-47). The reaction center (RC)-II complex is composed of two major polypeptides, D-1 and D-2 of 32-kDa; Cyt b-559, whose function is, as yet, unknown; and a 5-kDa protein whose function is also unknown. Light energy, either directly absorbed by the RC-II complex, or transferred to it by the antenna pigments is converted into chemical energy at the RC-II complex: the primary electron donor P680 is oxidized and the primary electron acceptor pheophytin (Pheo) is reduced in the primary photochemical reaction:

$$^1\text{Chl antenna} + h\nu \longrightarrow {}^1\text{Chl}^*\text{antenna} \quad (1a)$$

$$^1\text{Chl}^*\text{antenna} + {}^1\text{P680} \cdot \text{Pheo} \rightarrow {}^1\text{Chl antenna} + {}^1\text{P680}^* \cdot \text{Pheo} \quad (1b)$$

$$^1\text{P680}^* \cdot \text{Pheo} \longrightarrow {}^1\text{P680}^+ + \text{Pheo}^- \quad (1c)$$

(primary charge separation occurs in 3ps, as shown by Wasielewski et al. [4] in isolated RC-II)

Fluorescence yield (ϕ_f) is: $$\frac{k_f}{k_f + k_h + k_t + k_p [Q_A]} \quad (2)$$

where, k_f = rate constant of fluorescence, k_h = rate constant of heat loss, k_t = rate constant of energy transfer, k_p = rate constant of photochemistry, and $[Q_A]$ is the concentration of Q_A, the primary bound plastoquinone. At F_o, ϕ_f is minimal, when $[Q_A]$ is maximal and taken as 1. This fluorescence is basically in competition with trapping at the RC-II. The variable fluorescence, Fv, is observed either as an increase in fluorescence as the time of illumination increases or as a decrease in fluorescence after a strong brief flash of light, measured by a weak measuring flash. The former has been explained to reflect the reduction Q_A to Q_A^-; when $[Q_A]$ is high, Chl a fluorescence is low and when $[Q_A^-]$ is high, Chl a fluorescence is high (5). The decay of fluorescence is explained to be due to the recovery of Q_A from Q_A^- (see e.g., [6]). Both the Fo and Fv had been considered as "prompt" fluorescence originating in competition with the various constants in eq. (2) until Klimov et al. [7] proposed that Fv is delayed,not prompt,

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fluorescence. It was suggested that when $[Q_A] = 0$, i.e., when $[Q_A^-] = 1$, the following reactions occur (cf. with equation 1):

$$^1Chl\ antenna + h\nu \longrightarrow {}^1Chl^*antenna \quad (3a)$$

$$^1Chl^*antenna + {}^1P680 \cdot Pheo \cdot Q_A^- \longrightarrow {}^1Chl + {}^1P680^* \cdot Pheo \cdot Q_A^- \quad (3b)$$

$$^1P680^* \cdot Pheo \cdot Q_A^- \longrightarrow {}^1P680^+\ Pheo^-\ Q_A^- \quad (3c)$$

$$^1P680^+ \cdot Pheo^-\ Q_A^- \longrightarrow {}^1P680^* \cdot Pheo \cdot Q_A^- \quad (3d)$$

$$^1P680^* \cdot Pheo \cdot Q_A^- + {}^1Chl\ antenna \rightarrow {}^1P680 \cdot Pheo \cdot Q_A + {}^1Chl^*antenna \quad (3e)$$

$$^1Chl^*antenna \longrightarrow {}^1Chl\ antenna + h\nu' \quad (3f)$$

Here, $h\nu'$ is the recombinational luminescence as it originates by charge recombination in equation (3d).

2. RECOMBINATIONAL LUMINESCENCE HYPOTHESIS. In PS II membranes (DT-20), Klimov et al. [7; also see 8] discovered that when Q_A was reduced, light emission with lifetime of 4 ns paralleled the disappearance of $Pheo^-$, as expected from eq. (3). In this hypothesis, an activation energy (Ea) for light emission is expected. It was observed that this emission diminishes with decrease in temperature from 0 to -100°C, and an Ea of 0.06 eV, consistent with the redox potential difference between $P680^*/P680^+$ and $Pheo/Pheo^-$ was found (9). A strong case was made for "recombinational luminescence". A serious challenge came from Mathis [10] who showed that, in their PS II membranes, the Fv increased with decreasing temperature in the 5K-300K range. Also Schlodder and Brettel [11] did not find the expected correlation between the lifetime of light emission and the reoxidation of $Pheo^-$. On the other hand, Mauzerall [12] found a delay of 200 ps in the appearance of Fv and thought that it supported the recombinational hypothesis. Schatz and Holzwarth [13], however, argued that Mauzerall's method of subtraction of kinetics with open reaction centers from those with closed reaction centers produced artifactual components. Moya et al. [14] failed to obtain a lag in Fv measured by a fluorometer that had a ten times better resolution; moreover, such a lag is not even necessary in the recombinational hypothesis [15]. In addition, (1) Mutants of barley [16] and Chlamydomonas [14] that lacked RC-II had the same lifetime of fluorescence (2 ns) as that in the F_{max} state in the wild type; furthermore, in both the PS II-lacking mutant and the wild type cells, similar effect of DNB was observed on both the lifetime and amplitude of the ns component. The ns fluorescence component in the mutant cannot be due to recombinational luminescence since RC-II do not exist there, and, since DNB has similar effects, the wild type ns component was also suggested to be independent of the reaction center. However, see arguments in [15]. (2) Schatz and Holzwarth [13] note that their result on the decrease and the increase of the amplitudes of the fast (200 to 500 ps) and of the slow (1200 to 2000 ps) components, respectively, observed upon closure of the reaction center, cannot be explained by the Klimov hypothesis because the latter requires that the slow fluorescence components add to the fast components, not replace it. Van Gorkom [17] has listed additional arguments against the recombinational hypothesis. Geacintov and Breton [15] have pointed out that the lifetime results of Hodges and Moya [18] cannot be easily reconciled with the Klimov hypothesis, but, Geacintov et al. [19] stressed that the arguments of [13] are not necessarily correct since

in the random walk model, the recombinational mechanism can be viewed as a reduced efficiency of photochemical conversion at the trap (lower kp); thus, the shorter lifetime is converted into a longer lifetime due to reinjection of excitons from the reaction centers to the antenna. Thus, the above do not allow definite conclusions about the recombinational hypothesis.

3. EXPERIMENTS WITH REACTION CENTER COMPLEXES. In the case of isolated reaction centers, the probability of radical pair formation is close to 1 and, thus, significant recombination is expected there [13]. Barber et al. [20] observed that the quantum yield of pheo reduction in isolated RC-II was three orders of magnitude higher than that in PS II membranes. On the basis of these results, the Klimov hypothesis was questioned [20]. We, however, favor the existence of significant amount of recombinational luminescence in isolated reaction center preparations (also see [21]). From measurements on the decay of Chl a fluorescence at 4°C under anaerobic conditions in stabilized spinach PS II reaction center preparations using multifrequency (2-400 MHz) cross-correlation phase fluorometry, we have shown [22] that the Chl fluorescence of the open reaction centers can be analyzed as a lifetime distribution with fast (2-300 ps range) components decay merging into a broad shoulder in the ns range (2-30 ns). When the reaction centers are closed (i.e., when pheophytin is pre-reduced), a dramatic decrease in the contribution of slow components is observed (Fig. 1). These results clearly suggest that the ns distributed lifetime components in

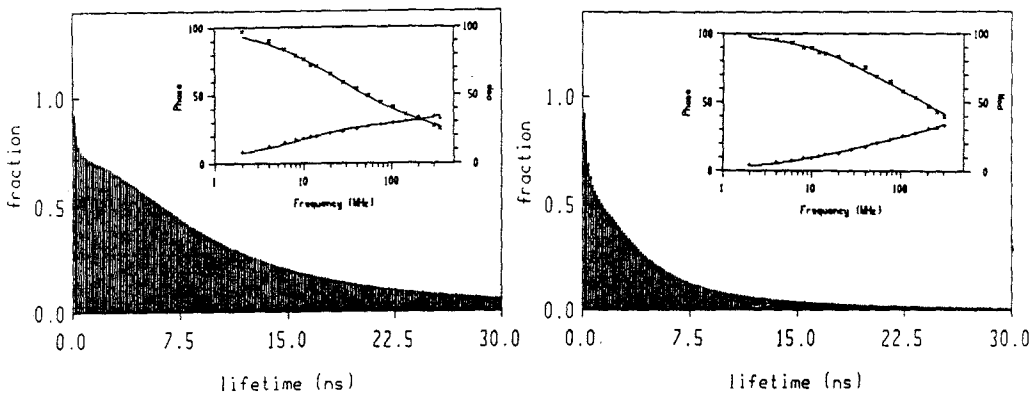

FIGURE 1. Continuous bimodal lifetime distribution function (fractional contribution to the total intensity versus lifetime) for open (left) and closed (right) reaction center of Photosystem II at 4°C under anaerobic conditions. Inserts show the respective phase (▲) and modulation (X) data as a function of frequency (MHz) with the best bimodal distribution fit (-). Samples were excited at 580 nm with a collimated 1 mW laser beam. Emission was observed through a monochromator at 680 nm (FWHM bandpass 16 nm) and a Hoya R-64 cut-off filter to eliminate scattered excitation light.

the isolated reaction centers (Q_A is absent) originate in the back reaction of the charge separated state (see eq. 3). Thus in isolated reaction center preparations, a major fraction of the observed ns light emission is recombinational luminescence.

In conclusion, Klimov's hypothesis of recombinational luminescence is fully supported in our studies on light emission from stabilized open and closed (prereduced pheo) PS II reaction centers at 4°C.

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