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Kinetic Properties of Prompt and Delayed Fluorescence of Chlorophyll a with λ_{max}=685 nm when Electron Transport is Blocked on Acceptor Side of Photosystem II

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Abstract

This work is a theoretical consideration of steady-state kinetics of prompt and delayed fluorescence of chlorophyll a entering into the pigment matrices of photosynthetic units of photosystem II when the electron transport from the primary to secondary acceptor of this system is blocked. It has been shown that in such a system of quantum yields of prompt and delayed fluorescence are complementary. At low intensities of excitation light the quantum yield of delayed fluorescence is several times more than that of prompt fluorescence. With an increase in the light intensity the reverse situation is observed. The literature data given sustain the results obtained. It has also been unambiguously shown what values, when changed, may be responsible for the corresponding changes in prompt and delayed fluorescence yields.

Introduction

Prompt and delayed fluorescence of chlorophyll a with λ_{max} = 685 nm emitted by molecules of the pigment matrix of photosystem II (PhS II) is now widely used when solving the problem of arrangement and functioning of noncyclic electron transport chains and coupling of phosphorylation with electron flow. Thus, for instance, it is possible to explain such questions as the localization of electron carriers, termed cytochrome-b-559, C-550 [1, 2], different states of water decomposition system [3, 4], high energy (phosphorylating) state of chloroplasts [5, 6], etc. However, the relationship between these types of

fluorescence and especially their dependence on the noncyclic electron transport and high energy state of thylakoids are still not clearly understood. A kinetic model of the processes in photosystem II could help in studying these questions, since only a model makes possible the comprehensive consideration of the processes and relations between them.

In [7] an attempt was made to construct a model. It was shown that some of its properties agreed well with experimental data [8–10]. In this paper we would like to consider the properties of steady-state kinetics of prompt and delayed fluorescence of chlorophyll a (λ_{max} = 685 nm) which are observed in the case of blocked electron transport on the acceptor side of PhS II.

Theory

We need the following set of equations:

$$\begin{aligned}
&\dot{c} = I\epsilon - c\{k_f + k_t + kQ(1-\alpha)\} + k_0(p^+a^-) \\
&(\dot{p}^+) = ckQ(1-\alpha) - \Sigma_{H_2O}(p^+) - k_0(p^+a^-) + k_{-H_2O} \\
&(\dot{a}^-) = ckQ(1-\alpha) - k_0(p^+a^-) \\
&(\dot{p^+a^-}) = ckQ(1-\alpha) - (\Sigma_{H_2O} + k_0)(p^+a^-) + k_{-H_2O}(a^-) \\
&\alpha = (p^+) + (a^-) - (p^+a^-)
\end{aligned} \tag{1}$$

It holds true for the multicentral model of the photosynthetic unit (PhSU) and results from system (1) in paper [8] when $k_1 = k_{-1} = 0$. The last condition means that there is no electron transport between the first acceptor of PhS II and plastoquinone pool.

In (1) I is the excitation light intensity expressed as a number of quanta per cm^2 in seconds; ϵ is the effective cross-section for capture of light quanta by a set of light-collecting chlorophyll molecules present in a reactive center; (p^+), (a^-), (p^+a^-), and α are the relative concentrations (fractions) of the oxidized form of the primary donor P^+, reduced primary acceptor A^-, double-charged P^+A^-, and closed $P^+A,^- + P^+A + PA^-$ centers, respectively; c is the concentration of singlet-excited states of pigment matrix of PhSU; Q is the number of centers in a PhSU of multicentral type; k_f, k_t, and k are the rate constants of fluorescence, thermal deactivation, and the capture of excitation by the reactive centers, respectively; k_0 is the effective value of the constant of the reverse electron transfer in double-charged centers; $\Sigma_{H_2O} = k_{H_2O} + k_{-H_2O}$ where k_{H_2O} is the rate constant of P^+ reduction by the water decomposition system and k_{-H_2O} is the constant of the reverse reaction; $(\dot{p}^+)$, etc., are the rates of the change of the corresponding values.

Steady-state solution of system (1) is given below.

$$(p^+) = \frac{k_{-H_2O}}{\Sigma_{H_2O}} \equiv \alpha_1 \tag{2.1}$$

$$(a^-) = \frac{I\epsilon L(1-\alpha_1)}{I\epsilon L(1-\alpha_1) + k_0(1-L)\alpha_1} \tag{2.2}$$

$$(p^+ a^-) = \alpha_1 (a^-) \tag{2.3}$$

$$\alpha = \alpha_1 + (1-\alpha_1)(a^-) \tag{2.4}$$

where α_1 is the equilibrium (dark) value (p^+) and α; $L = kQ/(k_f + k_t + kQ)$ is the quantum efficiency of the use of singlet-excited states of PhSU pigment matrix separation of the charges in a center at $\alpha_1 = 0$.

In a multicentral model of a photosynthetic unit there is the following relationship between the fluorescence quantum yield and α [11]:

$$\rho = \frac{\rho_0}{1-L\alpha} \tag{3}$$

where $\rho_0 = k_f/(k_f + k_t + kQ)$, and the delayed fluorescence yield is given by the expression [8]:

$$\rho_d = \rho \frac{k_o (p^+ a^-)}{I\epsilon} \tag{4}$$

By means of (2)–(4) it is easy to show that

$$\rho + \rho_d = \rho_\infty \tag{5}$$

where $\rho_\infty = k_f/(k_f + k_t)$ is the quantum yield of prompt fluorescence at $\alpha = 1$, i.e. when all reactive centers are in a closed state. From here it follows that ρ and ρ_d are complementary.

Thus, when electron transport on an acceptor side of PhS II is blocked, the steady-state concentration of the oxidized form of the primary donor (2.1) does not depend on the excitation light intensity and is equal to the equilibrium (dark) value. Under these conditions the steady-state value of the quantum yield of fast fluorescence is completely determined by the degree of reduction of the primary acceptor. The rate of its reoxidation depends, in its turn, on the product of three values: the constant of the reverse electron transfer in centers, k_0, the sum of quantum yields of fluorescence and thermal quenching $1\text{-}L = (k_f + k_t)/(k_f + k_t + kQ)$, the degree of oxidation of the primary donor in an equilibrium state $\alpha_1 = (p^+)_{eq} = k_{-H_2O}/\Sigma_{H_2O}$. The value $\tau = 1/(k_0(1\text{-}L)\alpha_1)$ is the mean relaxation lifetime of the reduced form of the primary acceptor. During this period of time the complete oxidation of (a^-) and, hence, a change of ρ and ρ_d take place after switching of the light, whereas before this the steady-state has been attained in light [12].

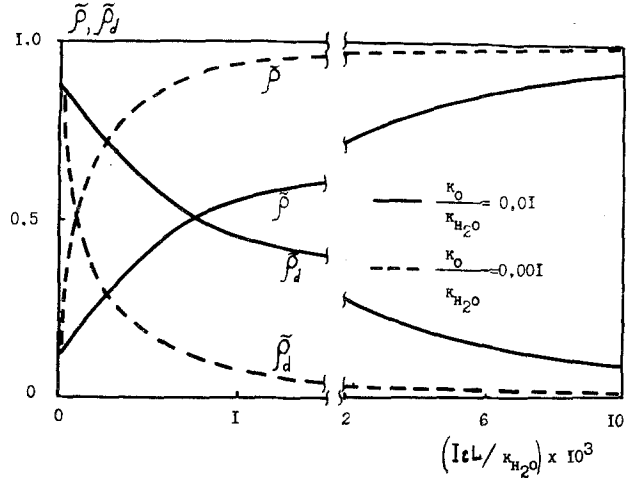

Figure 1. Theoretical dependence of quantum yield of prompt $\tilde{\rho} = \rho/\rho_\infty$ and delayed $\tilde{\rho}_d = \rho_d/\rho_\infty$ chlorophyll fluorescence on excitation light intensity at two values of k_0 differing by one order of magnitude. Calculated from formulas (2)–(5). In the calculations it is taken that $\alpha_1 = 1/11$, $L = 0.9$.

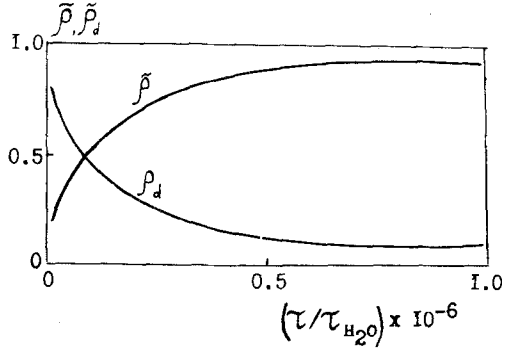

Figure 2. Theoretical curves $\tilde{\rho}$ and $\tilde{\rho}_d$ vs $\tau = 1/k_0(1\text{-}L)\alpha_1)$ at $I\epsilon L/k_{H_2O} = 10^{-4}$. The rest is the same as in Fig. 1; $\tau_{H_2O} = 1/k_{H_2O}$.

Figure 1 shows two types of ρ and ρ_d dependence on the excitation light intensity on the basis of formulas (2)–(4) at two different values of k_0. Figure 2 illustrates the curves of ρ and ρ_d variation with τ.

Comparison with Experiment and Discussion

It is seen from graphs in Fig. 1 and formula (5) that if the electron transport on the acceptor side of PhS II is blocked the quantum yield of

fluorescence induced with continuous light $\rho + \rho_d = \rho_\infty$ does not depend on the excitation light intensity. In weak light the yield of delayed fluorescence is several times more than that of prompt fluorescence. With an increase in light intensity the reverse situation is observed, and at sufficiently large intensities the contribution of delayed fluorescence to the value of ρ_∞ becomes negligibly small.

If the frequency of the modulated excitation light is more than $k_0(1\text{-}L)\ \alpha_1$ the amplitude of modulated fluorescence is proportional to ρ only and hence is dependent on I.

The data of Cramer and Böhme [1] (Fig. 3) and Malkin [13] (Fig. 4) obtained with chloroplasts poisoned by DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea] confirm the above statements. In the first case (Fig. 3) the fluorescence was induced by monochromatic ($\lambda = 652$ nm)

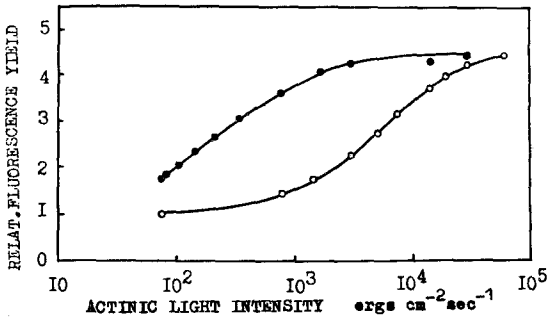

Figure 3. Increase in fluorescence yield as a function of actinic light intensity in the presence (●) and absence (○) of DCMU. Taken from [1], Fig. 11.

light modulated at 300 Hz [14] and intensity 75 ergs · cm^2 · sec_{-1}. As is seen, the yield of modulated fluorescence depends on the actinic light intensity. The data in Fig. 4 are obtained with the help of continuous excitation. In control chloroplasts *Fst* (designations of Malkin) depends on I, but at $I \to 0$ *Fst* tends to the value more than F_0. The difference $(F_{st} - F_0)_{I \to 0}$ is the contribution of the delayed fluorescence observed in the presence of O_2 when the slow electron flow takes place. After depriving the chloroplasts of O_2 or upon addition of DCMU to them, the electron flow stops and the delayed fluorescence yield at $I \to 0$ increases up to its limiting value $(F_{st,\,\mathrm{DCMU}} - F_0)_{I \to 0}$ and the yield of the total fluorescence no longer depends on I.

Thus, the fact that the yield of the fluorescence observed under the conditions of continuous light is independent of the excitation intensity unambiguously indicates that in the presence of DCMU reoxidation of the primary acceptor of PhS II takes place only due to the reverse electron transfer in the centers.

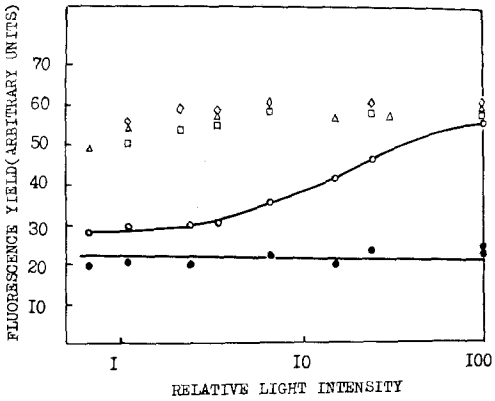

Figure 4. The dependence of the fluorescence yield on the light intensity. (○) Steady-state fluorescence yield (F); (●) initial fluorescence yield at the beginning of the induction period (F_0); (◇) fluorescence yield in presence of sodium hydrosulpfite; (△) fluorescence yield under anaerobic conditions; (□) fluorescence yield in prescence of DCMU. The values for the last three conditions are approximately equal, representing F_m. In this particular experiment there are large variations in F_m. The slight decrease of F_m in anaerobic conditions at low light intensity may be real, since anaerobiosis is not strictly complete. The reaction mixture contained about 20 μM chlorophyll in a 1.5 ml sucrose-Tris-NaCl medium. Sodium hydrosulfite was added as a solid; DCMU was added to a final concentration of about 10 μM. Anaerobic conditions were established by evacuation and addition of 0.04 M glucose and glucose oxidase. The maximum light intensity (100%) was 10^{-8} Einstein/sec and the absorption of the suspension was estimated to be 11%. Taken from [13], Fig. 1.

Then, as follows from formulas (2)–(4), the further variations in ρ and ρ_d in such objects may be caused only by the change of the product $k_0(1\text{-}L)\alpha_1$. The light curves $\rho(I)$ and $\rho_d(I)$ will shift along the axis of intensity depending on the concrete value of $k_0(1\text{-}L)\alpha_1$ (Fig. 1). At some $I \neq 0$ ρ increases (ρ_d decreases) as the product $k_0(1\text{-}L)\alpha_1$ decreases (Fig. 2).

This is experimentally confirmed by the curves ρ vs $\tau = 1/k_0\ (1\text{-}L)\alpha_1$ plotted according to the data of Cramer and Böhme [1] and shown in Fig. 5. The changes in ρ and τ are caused here by the various concentrations of antimicine A, hydroxylamine, and FCCP (carbonylcyanide p-trifluoromethoxyphenylhydrazone).

Similar data are given in a number of other papers [15–19]. For instance, in [19] it is shown that under the action of hydrazobenzene in particles of PhS II (where, probably, the electron transport is blocked on the acceptor side as well) prompt and delayed fluorescence change antibatically, i.e. in accordance with formula (5). Since in the majority of experiments L is constant, the changes of ρ, ρ_d, and τ observed are

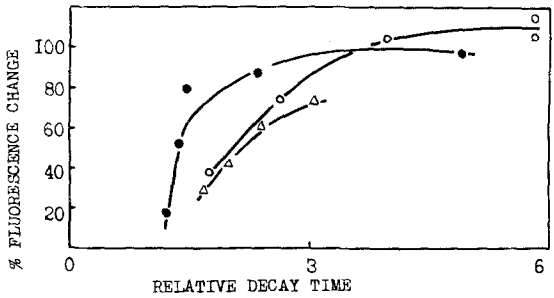

Figure 5. Experimental dependence of ρ on τ in chloroplasts poisoned with DCMU. Variations in ρ and τ are caused by different concentrations of hydroxylamine (●), antimicine A (△), and FCCP (○). Plotted on the basis of data given in [1], Figs. 6–8.

most likely due to the variations in k_0 and α_1. In papers [3, 16–19] a change in ρ, ρ_d, and τ is explained by value of α_1 variation. It should be borne in mind that if the alteration of α_1 takes place it should be accompanied by a change in the initial value of the quantum yield of fast fluorescence $\rho_1 = \rho_0/(1\text{-}L\alpha_1)$ on the induction curve of fluorescence $\rho(t)$ when passing from darkness to light at $t \to 0$. However, this change in ρ_1, at best, may attain several percent. If k_0 changes under the influence of some compounds, as was assumed in [5] and [6], the initial value of ρ_1 must remain unchanged.

In conclusion it should be especially noted that the above consideration of kinetic regularities of prompt and delayed fluorescence is based on the assumption that the same chlorophyll a molecules are responsible for both types of fluorescence and, therefore, there is no spectral difference between them. This is inconsistent with the data of Bonaventura and Kindergan [20] who observed the difference between the spectrum of slow fluorescence in chlorella cells poisoned with DCMU and the spectrum of fast fluorescence.

If the origin of prompt and delayed fluorescence is really different then the condition of complementarity of ρ and ρ_d [formula (5)] must not take place.

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