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THE FLUORESCENCE LIFETIME AND ENERGY MIGRATION MECHANISM IN PHOTOSYSTEM I OF PLANTS

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SUMMARY

1. The active chloroplasts and subchloroplast particles sedimenting at 10000 and $165000 \times g$ were isolated from pea leaves. The ratios of chlorophyll *a* belonging to Photosystem I and II were shown to be equal to 2.7, 0.4 and 5.9, respectively.

2. Separate values of fluorescence lifetimes were obtained for chlorophyll *a* of Photosystem I ($\tau \leqslant 0.03$ ns) and Photosystem II ($1.6 \leqslant \tau_2 \leqslant 1.8$ ns in saturating light conditions).

3. On the basis of the above estimation of the maximal τ_1 value, the minimal chlorophyll *a* molecular interaction energy is 0.02 eV. These moderate interactions correspond to the exciton type of energy migration in Photosystem I.

INTRODUCTION

It is known that light absorption induces singlet electronic excited states of molecules. Different photoprocesses can be caused by either short-lived singlet or considerably more stable triplet excited molecules. In the latter case, a photoprocess is preceded by an intersystem crossing.

It has been well established in a great number of studies, the first being the classical treatise by Emerson and Arnold[1], that a pool of chlorophyll molecules (light harvesting or antenna chlorophyll) serves one reaction center, in photosynthesis. There are approximately 10^2 chlorophyll molecules per reaction center. Duysens[2] was the first to advance detailed and extensive experimental evidence in favour of a resonance type of energy migration from chlorophyll to the reaction center. However, the question about the possible role of triplets remained open. It has been shown[3,4] that in the main fraction of bacteriochlorophyll in three purple bacteria, the fluorescence lifetime (τ) is equal to 0.015–0.03 ns when reaction centres P_{870} are in the active state. If they become inactive (for example, by photobleaching) τ increases up to 10–13 times, according to refs 3 and 4. These facts are an irrevocable indication[5] that energy migrates to P_{870} *via* the lowest singlet excited level of antenna bacteriochlorophyll and produces P_{870} photobleaching with a quantum yield equal to 0.88–0.94 (ref. 6). This means that the maximal possible value of quantum yields of singlet quenching by other processes (fluorescence, interconversion on some wasteful centers and intersystem crossing) are not more than 0.06–0.12 or, in other words,

the contribution of triplets may be neglected in an energy migration process. The new values of excitation lifetimes obtained in refs 3 and 4 strongly suggest that energy migrates from bacteriochlorophyll to P_{870} not by a slow inductive resonance mechanism, but by an excitonic one[3,5] in correspondence with moderate bacteriochlorophyll interaction energies of the order of 0.01–0.02 eV.

It is well known[7] that in plants, fluorescence light is mostly emitted by chlorophyll of Photosystem II. Corresponding τ values are 3–4 times as low as for chlorophyll *a in vitro*[8,9], thus indicating the qualitatively important role of singlet excited states. It was shown that after several hours of greening of etiolated leaves when O_2 evolution starts, τ of chlorophyll drops from 4.0 to 1.5 ns[10]. On the other hand, the increase in exciting light intensity from several units to several thousands erg·cm^{-2}·s^{-1} has been shown[11] to result in a τ rise from 0.4 to 1.8 ns. All these facts appear to prove that a considerable part of excited states are delivered to the reaction centers of Photosystem II *via* the singlet excited level of chlorophyll, although, unlike the case observed with purple bacteria, in none of refs 7–11 were the states of reaction centers controlled. These values leave uncertainty about the mechanism of energy migration in Photosystem II. It may be of a slow inductive resonance type, according to the Förster–Dexter theory[12], which was first experimentally corroborated by Galanin[13,14], if the efficiency of energy trapping by excited reaction centers is supposed to be high ($\rightarrow$100%); or it may be of an exciton type if this efficiency is not more than 20–30%. This problem, originally formulated by Robinson[15], is still to be solved. There are almost no experimental data on the nature of excited states, excitation lifetimes and energy migration mechanisms in Photosystem I. In our first contribution[16], the fluorescence lifetime for chlorophyll of Photosystem I was estimated to be less than 0.07 ns.

Now we are undertaking a more detailed study of this parameter, with a better accuracy and higher sensitivity, in order to throw light on the problem of energy migration and trapping mechanisms in Photosystem I.

METHODS

Chloroplast preparations were obtained from 3–4-week-old pea seedlings as described elsewhere[17]. Subchloroplast heavy particles were prepared by treating chloroplasts with 0.5% digitonin and subsequently sedimenting them at $10000 \times g$, according to Anderson and Vernon[18]. Light particles were sedimented at $165000 \times g$ from the supernatant obtained at $50000 \times g$ (ref. 18). Both chloroplasts and heavy particles exhibit a Hill reaction activity with ferricyanide. The chloroplasts were capable of cyclic photophosphorylation when catalyzed by phenazine methosulfate and flavine mononucleotide. The light-particle preparations were able to carry out cyclic (either without any additions or phenazine methosulfate-stimulated) and non-cyclic (with an exogenous donor–acceptor couple) electron transports.

RESULTS

Let us consider photosynthetic preparations, containing both photochemical

systems, I and II, in different ratios. Then the light energies absorbed by two photosystems ($\mathscr{I}_1, \mathscr{I}_2$) will be the following:

$$\mathscr{I}_1 \cong \frac{c_1 \cdot \int_\lambda \varepsilon_1(\lambda) \cdot \mathscr{I}(\lambda) \mathrm{d}\lambda}{c_1 \cdot \int_\lambda \varepsilon_1(\lambda) \mathrm{d}\lambda + c_2 \cdot \int_\lambda \varepsilon_2(\lambda) \mathrm{d}\lambda} \tag{1a}$$

$$\mathscr{I}_2 \cong \frac{c_2 \cdot \int_\lambda \varepsilon_2(\lambda) \cdot \mathscr{I}(\lambda) \mathrm{d}\lambda}{c_1 \int_\lambda \varepsilon_1(\lambda) \mathrm{d}\lambda + c_2 \cdot \int_\lambda \varepsilon_2(\lambda) \mathrm{d}\lambda} \tag{1b}$$

where c_1, c_2 and $\varepsilon_1(\lambda)$, $\varepsilon_2(\lambda)$ are concentrations and molar extinction coefficients for chlorophyll of Photosystem I and Photosystem II, respectively; $\mathscr{I}(\lambda)$ = spectral distribution of exciting light. By definition:

$$\mathscr{I}_a = \int_\lambda \mathscr{I}(\lambda) \cdot \{1 - 10^{-l \cdot [\varepsilon_1(\lambda) \cdot c_1 + \varepsilon_2(\lambda) \cdot c_2]}\} \mathrm{d}\lambda \tag{2}$$

where $\mathscr{I}_a$ = light absorbed by the whole chlorophyll preparation; l = optical path length.

It is known that, in contrast to red absorption peaks, the Soret bands of different chlorophyll forms approximately coincide, *i.e.* $\varepsilon_1(\lambda) \cong \varepsilon_2(\lambda)$ in this optical region. In our particular case, exciting light in the phase fluorimeter was obtained from the 436-nm mercury line. Therefore, we obtain from Formula 1ab:

$$\mathscr{I}_1 \cong \mathscr{I}_a \frac{c_1}{c_1 + c_2}, \qquad \mathscr{I}_2 \cong \mathscr{I}_a \frac{c_2}{c_1 + c_2} \tag{3ab}$$

It is evident that for low values of fluorescence lifetimes (τ), the fundamental formula of a phase-type fluorimeter is:

$$2\pi\nu \cdot \tau = \mathrm{tg}\psi \cong \psi \tag{4}$$

where ν = frequency of light modulation; ψ = phase shift between the first harmonics of exciting and fluorescent lights.

The accuracy of $tg\psi \cong \psi$ is better than 1% if $\psi \leqslant 10°$. In our instrument (ν = 12.33 mHz), this corresponds to $\tau \leqslant 1.8$ ns. Hence, we obtain for two individual emissions the following experimental value of mean measured fluorescence lifetime (τ_m):

$$\tau_m \cong \frac{F_1 \cdot \tau_1 + F_2 \cdot \tau_2}{F_1 + F_2} \cong \frac{\mathscr{I}_1 \cdot \Phi_1 \cdot \tau_1 + \mathscr{I}_2 \cdot \Phi_2 \cdot \tau_2}{\mathscr{I}_1 \cdot \Phi_1 + \mathscr{I}_2 \cdot \Phi_2} \tag{5}$$

where F_1, F_2, τ_1, τ_2, Φ_1, Φ_2 are fluorescence intensities, lifetimes and quantum yields for the first and second emissions, respectively, coming from chlorophyll of Photosystem I and Photosystem II.

To be more accurate, F_1 and F_2 in Formula 5 must be amplitudes of photomultiplier currents (i_1,i_2); hence:

$$i_1 = \int_\lambda \mathscr{I}_1(\lambda) \cdot \rho(\lambda) \mathrm{d}\lambda, \qquad i_2 = \int_\lambda \mathscr{I}_2(\lambda) \cdot \rho(\lambda) \mathrm{d}\lambda,$$

where $\rho(\lambda)$ = spectral distribution of photocathode quantum yield. But in our instruments $i_1/i_2 \cong F_1/F_2$, as the photomultiplier used (S-1 type) was characterized by an approximately constant sensitivity in the region of 650–750 nm.

It has recently been shown[16] that fluorescence intrinsic lifetimes are equal (within the error of 15%) for the main chlorophyll fractions of Photosystem I and Photosystem II, *i.e.* $\tau_{01} \cong \tau_{02} = \tau_0$. Therefore:

$$\tau_1 \cong \tau_0 \cdot \Phi_1, \qquad \tau_2 \cong \tau_0 \cdot \Phi_2 \tag{6}$$

Taking into account Formulas 6 and 3 we obtain from Formula 5:

$$\tau_m = \frac{c_1 \cdot \tau_0 \cdot \Phi_1^2 + c_2 \cdot \tau_0 \cdot \Phi_2^2}{c_1 \Phi_1 + c_2 \cdot \Phi_2} = \Phi_2 \cdot \tau_0 \frac{1 + \frac{c_1}{c_2} \cdot \left(\frac{\Phi_1}{\Phi_2}\right)^2}{1 + \frac{c_1}{c_2} \cdot \frac{\Phi_1}{\Phi_2}}$$

$$= \tau_2 \frac{1 + \alpha x^2}{1 + \alpha x} \tag{7}$$

where

$$\alpha = c_1/c_2, \qquad x = \Phi_1/\Phi_2 \cong \tau_1/\tau_2$$

Formula 7 shows how the value of the average fluorescence lifetime, τ_m, measured with a phase-type fluorimeter, is governed by the ratios of concentrations (α) and fluorescence yields or lifetimes (x) of the chlorophyll belonging to Photosystem I and Photosystem II. The values of α were determined for pea chloroplasts and both types of subchloroplast particles[16] enriched with Photosystem I and Photosystem II by means of the fluorescence method.

$\alpha = c_1/c_2$ was found to be 2.7 for pea chloroplasts; 0.4 for $10000 \times g$ particles; and 5.9 for $165000 \times g$ particles.

The theoretical dependences of $\tau_m = f(x)$ corresponding to $\alpha = 0.4$ (heavy particles), $\alpha = 5.9$ (light particles) and $\alpha = 2.7$ (chloroplasts) are shown in Fig. 1A. According to these curves, the more the difference in τ_m for different preparations the greater is the ratio of $x = \Phi_1/\Phi_2 \cong \tau_1/\tau_2$. Hence, it is possible to estimate τ_1 and τ_2 separately by measuring τ_m for heavy and light particles, respectively.

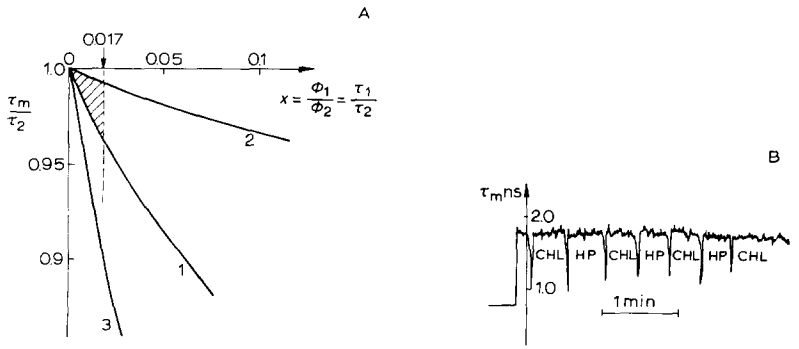

Fig. 1. A. The theoretical dependences of measured fluorescence lifetime (τ_m) on the ratio of fluorescence yields of two photosystems (x): 1, for heavy particles; 2, for pea chloroplasts; 3, for light particles. B. An example of comparison of experimental τ_m values for pea chloroplasts (CHL) and heavy particles (HP). The exciting light intensity is $4 \cdot 10^{-10}$ einstein·cm^{-2}·s^{-1} ($\lambda = 436$ nm).

Absolute values of fluorescence lifetimes

Pea chloroplasts, heavy particles and light particles were isolated eight times. The light dependences of τ_m for two individual chloroplast preparations are shown in Fig. 2. The τ_m values were saturated at light intensities exceeding 10^{-10} einstein·$cm^{-2}\cdot s^{-1}$ for all chloroplast preparations, as well as for heavy particles. Light particles were saturated at 20 times lower light intensities. All of the saturated values of τ_m for eight chloroplast and eight heavy-particle preparations were determined from 6–8 separate experiments with a relative accuracy of ± 0.03 ns. Such a series is given below for one of the chloroplast preparations:

(ns):	1.76 ± 0.06	1.76 ± 0.06
1.83 ± 0.06	1.82 ± 0.06	1.79 ± 0.06
1.86 ± 0.06	1.75 ± 0.06	1.81 ± 0.06

the average value: $\tau_m = 1.80\pm0.03$ ns.

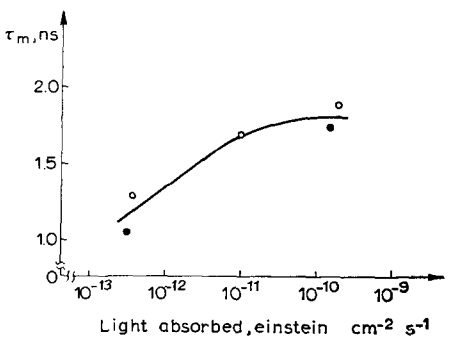

Fig. 2. The light intensity dependence of pea chloroplasts of measured fluorescence lifetime (τ_m) for two individual preparations. The exciting light is at 436 nm; absorbance of samples is equal to 0.3.

All averaged τ_m values ($\lambda=436$ nm, $\mathcal{I}_a=2\cdot10^{10}$ einstein·$cm^{-2}\cdot s^{-1}$) for chloroplast and heavy particles were in the region of 1.7–1.9 ns.

Two systematical errors should be taken into account:

(a) The spectral difference between exciting ($\lambda=436$ nm) and fluorescence ($\lambda\simeq680$ nm) lights may cause a false τ_m increase (up to 0.1–0.15 ns in our instrument) due to the difference in the initial velocities of photoelectrons released by blue and red light, respectively.

(b) The difference in light scattering from measured and reference samples may change the distribution of the corresponding light beams on the photomultiplier cathode ($D=1.2$ cm), thus adding uncertainty (about ±0.04 ns for these particle suspensions in our instrument) to lifetime measurements.

Taking (a) and (b) into account, we had to subtract 0.1 ns from the experimental τ_m values. This means that absolute values for eight chloroplast and eight heavy-particle preparations in saturating light conditions were in the region of 1.6–1.8 ns (that means biological uncertainty), and physical accuracy for each individual absolute result is not better than ±0.15 ns for a series of 4–6 measurements.

These figures are in good agreement with those obtained earlier by Müller *et al.*[11] for *Chlorella* cells in saturated light conditions.

Estimation of separate τ values for Photosystem I and Photosystem II

In order to eliminate the source of greatest systematical error, we performed a series of experiments to directly compare τ_m values for heavy particles and chloroplasts. An example of such a series of experiments is shown in Fig. 1B. It is clear that the respective τ_m values do not differ within the limits of the experimental error. [8–10] individual experiments were made with each of eight chloroplast and eight heavy-particle preparations and thus the relative statistical error was lowered from ± 0.06–± 0.01 ns. In each pair of the particle systems, τ_m values were similar within the experimental error and, consequently, after overall averaging of approximately 70 individual experiments with eight individual preparations, we obtained the following values for light scattering uncertainty:

$$\Delta\tau_m = (\tau_{m(\text{chloroplast})} - \tau_{m(\text{heary particles})}) \leqslant 0.04 + 0.01 = 0.05 \text{ ns}$$

This $\Delta\tau_m$ corresponds to the x values: $0 \leqslant x \leqslant 0.017$ (see Fig. 1A, the dashed region between the Curves 1 and 2). This means:

$$x = \frac{\Phi_1}{\Phi_2} = \frac{\tau_1}{\tau_2} \leqslant 0.017 \tag{8}$$

This dashed triangle corresponds to:

$$1 \geqslant \frac{\tau_m}{\tau_2} \geqslant 0.962$$

Therefore the maximal possible τ_2 value is equal to

$$\tau_2^{max} = \frac{\tau_m^{max}}{0.962} = \frac{1.8 \text{ ns}}{0.962} \cong 1.9 \text{ ns}$$

Or eventually:

Photosystem II: $1.6 \pm 0.1 \leqslant \tau_2^{max} \leqslant 1.8 \pm 0.1$ ns

and from this data and Formula 8 we obtain $\tau_1 \leqslant 0.017 \cdot \tau_2^{max}$ or eventually:

Photosystem I: $\tau_1 \leqslant 0.03$ ns

DISCUSSION

It seems likely that our data may be obscured by the energy migration from Photosystem II to Photosystem I ("spill-over" phenomenon), that would make Formula 3ab incorrect. But our experiments have shown that τ_m values do not differ for heavy particles and chloroplast particles, although $\alpha = c_1/c_2$ varies significantly. It appears unlikely that the efficiency of the "spill-over" depends on the α value. Therefore, we had to conclude that the "spill-over" efficiency is small enough and may be neglected. Moreover, we have even obtained some heavy-particle preparations with τ_m/Φ_m values equal to those for chlorophyll *a* in solution and hence without detectable energy migration to Photosystem I. Only for light particles was the averaged τ_m value 0.06–0.1 ns lower than those for heavy particles and chloroplasts. Accord-

ing to our calculations, this gives $\tau_1 \leqslant 0.04$ ns. But, we believe that this small difference in τ_m is caused by some additional fluorescence quenching on some centers.

Photosystem I

According to our data[20,21], the quantum yield of primary energy trapping in Photosystem I is high enough (0.7–0.8). This means that the averaged rate constant of singlet excitation trapping by active reaction centers is 2.5–3.5 times greater than the sum of those for all other processes. Consequently, the fluorescence lifetime τ_1 (and τ_2 as well) gives a rise time for the primary process of energy trapping that seems to be the electron donation by excited reaction centers P_{700}. According to the random walk method[15,22], the number of excitation jumps (n) providing the trapping of 63% of excitation quanta in active reaction centers is equal to

$$n = 0.72\, N \log N + 0.26\, N^* \tag{9}$$

where N is the ratio of bulk chlorophyll and active reaction center concentrations For $N=220$ we obtain $n=500$. The spectral heterogeneity of the pigment complex of Photosystem I may reduce n by 3–5 times[23], *i.e.* $n_{het} \cong n/4 \cong 125$. Nevertheless, the value of jumptime calculated:

$$\tau_j = \frac{\tau_1}{n_{het}} \leqslant \frac{3 \cdot 10^{-11}}{125} \cong 2.5 \cdot 10^{-13}\,\text{s}$$

corresponds to the exciton type of energy migration with moderate chlorophyll interaction energies $W_i \geqslant 0.02$ eV. On the other hand, the similarity of chlorophyll absorption spectra *in vivo* and *in vitro* proves that the maximal value of W_i is not more than 0.06–0.09 eV. Therefore,

Photosystem I: $0.02 \leqslant W_i \leqslant 0.09$ eV

This means that:

(a) The efficiency of excitation trapping in the reaction center may be $<100\%$ according to Robinson's views[15]. This indicates that the rate constant of primary electron transfer in the reaction center is comparable with that for reversed energy migration from the reaction center to the bulk chlorophyll.

(b) The excitation is delocalized through a number of chlorophyll molecules.

(c) The random walk method is not good enough for such a system.

Photosystem II

According to previous data[11], chlorophyll fluorescence lifetime is equal to 0.4 ns for active photosynthesis in Photosystem II. If one considers $N \simeq 100$, then, according to Formula 9, $n \simeq 170$ and consequently:

$$\tau_j \cong \frac{4 \cdot 10^{-10}\,\text{s}}{170} \cong 2.5 \cdot 10^{-12}\,\text{s}$$

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* Formula 9 is valid for a two-dimensional pigment complex, but that for a three-dimensional complex does not differ very much.

This value corresponds to 100% trapping efficiency in the reaction centers. But, if this efficiency is of the order of 0.2–0.8, the jumptime may be equal to 10^{-12} s. In this case, the energy migration in chlorophyll of Photosystem II is also of the exciton type.

It is interesting to compare the values of $\tau_1 \leqslant 0.03$ ns obtained in this work with those measured by Witt and co-workers[24] for the risetime of membrane potential in thylakoids of spinach ($\tau < 2 \cdot 10^{-8}$ s). The question is whether our value of τ_1 (*i.e.* the rise time of the trapping of electron excitation in reaction centers of Photosystem I) is the same as that required for creating a membrane potential. It seems more realistic that the primary process of electron transfer creates only an electric dipole of $\leqslant 10$ Å, whereas the separation of opposite charges to the two sides of membrane (the distance is approx. 100 Å) should require longer time periods.

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