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# ENERGY TRANSFER IN PHOTOACTIVE COMPLEXES OBTAINED FROM GREEN BACTERIUM *CHLOROBIUM LIMICOLA*

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## SUMMARY

The pigment · protein complexes enriched with bacteriochlorophyll *a* were derived from green bacterium *Chlorobium limicola*. The light dependences of bacteriochlorophyll fluorescence yield and lifetime as well as the portion of photooxidized reaction centers *P*-840 were recorded. These combined data revealed that in addition to the known fluorescence with a lifetime exceeding 2 ns, the short-living emission exists in a picosecond time scale. The latter belongs to the main portion of bacteriochlorophyll *a* and its lifetime and yield are governed by the *P*-840 state.

According to the time-lever method developed for phase fluorimetry, its physiological lifetime amounts to no more than 40–100 ps and the limiting value to 10–25 ps. The fluorescence yield and the portion of molecules belonging to different funds are also determined.

The above data prove the energy migration in bacteriochlorophyll *a* to be of the excitonic type at a moderate rate of molecular interaction. The quantum yield of triplet formation in antenna bacteriochlorophyll is negligible in a state of active photosynthesis, as in purple bacteria and Photosystem I of plants.

## INTRODUCTION

The pioneering study of energy migration in antenna chlorophylls of green bacteria was undertaken by Sybesma and Olson [1]. Recently, the pigment · protein complexes enriched with bacteriochlorophyll *a* and reaction center *P*-840 were isolated from green bacteria *Chlorobium limicola* and *Chlorobium thiosulfatophilum* [2]. In our previous work [3] dealing with similar complexes, the conclusion was made that the background bacteriochlorophyll *a* fluorescence with an approximately constant yield ($\varphi_{bg}$) and lifetime ($\tau_{bg}$) exists together with photosynthetic bacteriochlorophyll *a* emission the quantum yield ($\varphi_{ph}$) and lifetime ($\tau_{ph}$) of which correlate with the redox state of reaction centers *P*-840. This fact accounts for the small increase in the measured value of bacteriochlorophyll *a* fluorescence yield ($\varphi_m$) observed earlier [1–4] in the transition from unsaturated to light-saturated photosynthesis, whereas the yield of photosynthetic bacteriochlorophyll *a* emission increases significantly, thus proving

that energy migration in corresponding molecules occurs essentially via singlet levels [9]. In the present work, we have used pigment · protein complexes isolated from *Chlorobium limicola* in order to study the excitation lifetime as related to the reaction center state and the mechanism of energy migration in antennae chlorophylls.

## MATERIALS AND METHODS

The photochemically active complexes enriched with bacteriochlorophyll *a* and *P*-840 were isolated from *Chlorobium limicola* as described previously [3]. Light-induced changes in the relative fluorescence yield and the absorption changes corresponding to photobleaching of the reaction centres were registered as described elsewhere [5]. Absorption spectra were measured with a Hitachi EPS-3 spectrophotometer. Fluorescence spectra were measured with the instrument described previously [6].

The phase fluorimeter (a modified version of the instrument described in ref. 7) operating at the frequency $\nu = 12.3 \cdot 10^6$ MHz was used for fluorescence lifetime measurements. Time resolution was about $5 \cdot 10^{-11}$ s.

*Measurements in the picosecond time scale (time-lever method)*

We were the first [8, 9] to show that the excitation lifetime of antennae chlorophyll in vivo amounts to several tens of picoseconds under conditions of unsaturated photosynthesis. A special method was developed for phase fluorimetry, based on the measurements of mean lifetime ($\tau_m$) of a heterogeneous emission consisting of two individual ones, with their lifetimes differing greatly (see earlier publications [8, 9]).

The method permits measurements with a time resolution of up to several picoseconds which is considerably better than that of the phase fluorimeter used. It is restricted to spatial cases of two component emissions. The intensity ($I_1$) and the lifetime ($\tau_1$) of the first component to be investigated should be variable. The second component, which is introduced into this method on purpose, should be constant and its lifetime ($\tau_2$) must by far exceed $\tau_1$. The measured $\tau_m$ value of two-component emission evidently approximates (see Appendix):

$$\tau_m \cong \frac{I_1\tau_1 + I_2\tau_2}{I_1 + I_2} = \frac{\alpha\tau_1 + \tau_2}{\alpha + 1} \qquad \alpha = \frac{I_1}{I_2} \tag{1}$$

It can easily be derived from Eqn. 1 that small changes in $\tau_1$ (even in a picosecond time scale) should cause profound, readily measurable changes in $\tau_m$ in a nanosecond time scale.

Let us give an example for nearly optimal conditions: $\tau_2 = 5 \cdot 10^{-9}$ s; $\tau_1 = 5 \cdot 10^{-11}$ s; $\nu = 10^7$ s$^{-1}$; $I_2/I_1 = 2 : 1$. Then, according to Eqn. 1:

$$\tau_m = \frac{5 \cdot 10^{-11} + 2 \cdot 5 \cdot 10^{-9}}{1+2} = 3.35 \cdot 10^{-9} \text{ s}$$

If the investigated emission doubled its lifetime and yield ($\tau'_1 = 2\tau_1 = 10^{-10}$s; $I'_1 = 2I_1 = I_2$), it would result in a considerable decrease in the $\tau_m$ value:

$$\tau'_m = \frac{2 \cdot 10^{-10} + 2 \cdot 5 \cdot 10^{-9}}{2+2} = 2.55 \cdot 10^{-9} \text{ s}$$

Thus, a relatively small increase ($\Delta\tau_1 = 5 \cdot 10^{-11}$ s) produces a much more pronounced change in $\tau_m$:

$$\Delta\tau_m = -0.8 \cdot 10^{-9} \text{ s}$$

For example, in our particular phase fluorimeter [7], $\tau_m$ is measured with an accuracy of $\pm 0.05 \cdot 10^{-9}$ s (time resolution of the instrument). Consequently, $\Delta\tau_m = -800 \pm 50$ ps corresponds in the above example to:

$$\Delta\tau_1 = 50 \pm 3 \text{ ps}$$

Note that the negative change in $\tau_m$ value is induced by a positive deviation in measured $\tau_1$. Thus, for the measured parameters $\tau_m$ and $\varphi_m$, the changes are of opposite signs, as was the case with the object investigated.

The determination of $\tau_1$ values by the time-lever method requires two independent measurements of the lifetimes $\tau_m$ and $\tau_m + \Delta\tau_m$ and solution of the system of two type (1) equations with known $I_1/I_2$ corresponding ratios (for details, see Results and Appendix).

Below, we use the time-lever method for quantitative characterization of fluorescence emitted by chlorophylls of green bacteria *Chlorobium limicola.*

## RESULTS AND DISCUSSION

### *1. Fluorescence yields and lifetimes in the bacteriochlorophyll a antenna complex*

Various photochemically active complexes enriched with the reaction center *P*-840 and containing in various portions the bacteriochlorophyll *a* with absorption peaks around 600 and 810 nm, bound bacterioviridin with an absorption peak about 750 nm and bacterioviridin both in monomeric and pheophytinized forms (absorption about 670 nm) may be obtained in the above mentioned method [3] with variations in the sonication regime. These complexes were used in this work as being most suitable for controlling the redox state of *P*-840 and correlating it with fluorescence parameters of antenna pigments. Besides, they exhibit hardly any light scattering, due to their

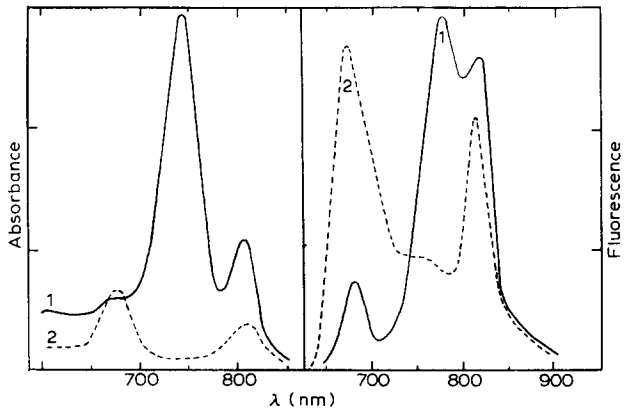


Fig. 1. Absorption and fluorescence spectra of subchromatophore particles isolated from *Chlorobium limicola.* Curves 1 and 2, two types of subchromatophore particles obtained with a change in cell sonication regime. Excitation with mercury lines 365, 404 and 436 nm.

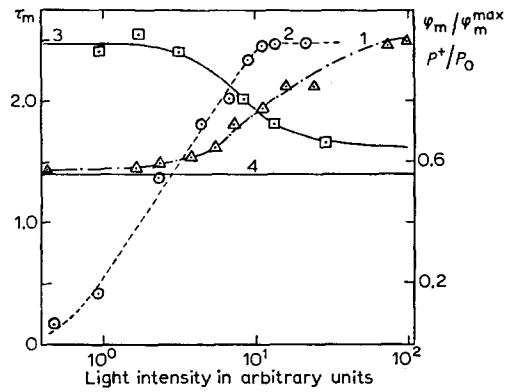


Fig. 2. Relative bacteriochlorophyll *a* fluorescence yield in arbitrary units (1), portion of oxidized reaction centers (2), and bacteriochlorophyll *a* fluorescence lifetime (3) as a function of light intensity. The portion of oxidized reaction centers $P^+/P_0$ was determined as a normalized absorption change at 828 nm (one of the characteristic maxima of longwave *P*-840 photobleaching). Solid line (4) indicates the background bacteriochlorophyll *a* fluorescence level. Curve (1), related to background fluorescence level as zero, represents the relative quantum yield of photosynthetic bacteriochlorophyll *a* fluorescence.

small dimensions. The respective absorption and fluorescence spectra of these complexes are shown in Fig. 1. The dependence of $\varphi_m$ and $\tau_m$ of bacteriochlorophyll *a* fluorescence on the excitation intensity (as well as the portion of photooxidized *P*-840) are shown in Fig. 2. The decrease in $\tau_m$ following the increase in $\varphi_m$ indicates that more than one emission is present. The analysis performed previously for chromatophores of purple bacteria [8, 9] revealed that the only possible conditions responsible for such situation are the same as those needed for the application of the time-lever method. This means that the complexes investigated produce two bacteriochlorophyll *a* emissions. The first emission (designated the photosynthetic emission) has $\varphi_{ph}$ and $\tau_{ph}$ variable respective to the reaction centre state and considerably lower than those for the second constant emission(s) ($\varphi_{bg}$, $\tau_{bg}$) (called the background emission). These emissions correspond to the above mentioned ones (see Methods), i.e., $\tau_{ph} \rightarrow \tau_1$; $\tau_{bg} \rightarrow \tau_2$. In order to determine $\tau_{ph}$ by use of Eqn. 1, it is necessary to measure the ratio of background and photosynthetic intensities ($I_{ph}/I_{bg}$) and $\tau_m$ values for at least two distinct points with different $\tau_{ph}$ and $I_{ph}$ values. These data were obtained following the method of Borisov et al. [3, 8, 9] from the experimental light intensity dependence of $\tau_m$, $\varphi_m$ and normalized portions of photooxidized *P*-840 (shown in Fig. 2).

The solid line represents the level of bacteriochlorophyll *a* background emission(s) determined by the method developed previously in this laboratory [5] and first applied to the object investigated earlier [3]. Thus, for each intensity of exciting light (Fig. 2):

$$I_m = I_{bg} + I_{ph}$$

and these relative values may be used in Eqn. 1 for obtaining $\alpha = I_{ph}/I_{bg}$. To determine $\tau_{ph}$ as dependent on the portion of active *P*-840 and $\tau_{bg}$, two independent measurements of $\tau_m$ were performed at weak (open traps) and saturating (closed traps)

exciting illuminations. Thus two equations were obtained according to Eqn. 1, weak illumination:

$$\tau_m = \frac{\tau_{bg} + \alpha\tau_{ph}^{min}}{1+\alpha} \tag{2a}$$

saturating illumination:

$$\tau'_m = \frac{\tau_{bg} + \alpha'\tau_{ph}^{max}}{1+\alpha'} \tag{2b}$$

where $\alpha$ and $\alpha'$ are the ratios of $I_{ph}$ to $I_{bg}$ for weak and saturating illuminations respectively; $\tau_m$ and $\tau'_m$ are the measured fluorescence lifetimes for the above conditions; $\tau_{ph}^{min}$ and $\tau_{ph}^{max}$ are extreme values of $\tau_{ph}$ for the above illumination conditions.

In fact, the system of two equations (2a and 2b) contains three unknown parameters: $\tau_{bg}$, $\tau_{ph}^{min}$, $\tau_{ph}^{max}$. Suppose that:

(a) relation $\tau = \varphi\tau_0$ ($\tau_0$ is the intrinsic lifetime) is valid for photosynthetic emission also in a picosecond region;

(b) the fluorescence intensities measured ($I_{bg}$, $I_{ph}$) are equal to:

$$I_{bg} = I_0\varepsilon_{bg}C_{bg}\varphi_{bg}$$

$$I_{ph} = I_0\varepsilon_{ph}C_{ph}\varphi_{ph},$$

where $I_0$ is excitation intensity, $\varepsilon$ is the bacteriochlorophyll *a* extinction coefficient, $C_{ph}$ and $C_{bg}$ are the relative concentrations of molecules, emitting photosynthetic and background fluorescence, and $\varphi$ is the fluorescence quantum yield. Then

$$\tau_{ph}^{max} = \frac{\alpha'}{\alpha}\tau_{ph}^{min}$$

Thus, one of three unknown parameters of Eqns. 2a and 2b is easily ruled out, i.e. the system of two equations is reduced to two unknown parameters. The results are summarized in Table I.

To test the validity of the assumptions used in above determinations of the $\tau_{ph} = f(P^+/P_0)$ the whole $\tau_m = f(I_{ex})$ was analytically reconstructed from the values $\alpha$, $\tau_{bg}$ and $\tau_{ph}$ (curve 3, Fig. 2). It can be seen that the experimental results are in a good agreement with this curve.

The data in Table I show that bacteriochlorophyll *a* photosynthetic emission is characterized by a lifetime value as short as $20 \cdot 10^{-12}$–$60 \cdot 10^{-12}$ s in the active state of all reaction centers, and 40–120 ps when 50 % of the reaction centers are in an

TABLE I

THE CHARACTERISTICS OF BACTERIOCHLOROPHYLL *a* EMISSIONS

| $\tau_m^{max}$ (ns) | $\tau_m^{min}$ (ns) | $(J_{ph}/J_{bg})_{min}$ | $(J_{ph}/J_{bg})_{max}$ | $\tau_{ph}^{min}$ (s) | $\tau_{ph}^{max}$ (s) | $\tau^{bg}$ (ns) |
|---|---|---|---|---|---|---|
| 2.5±0.1 | 1.6±0.1 | 0.036 | 0.786 | $4 \cdot 10^{-11} \pm 2 \cdot 10^{-11}$ | $4 \cdot 10^{-10} \pm 2 \cdot 10^{-10}$ | 2.7±0.1 |

TABLE II

THE CHARACTERISTICS OF BACTERIOCHLOROPHYLL *a* EMISSIONS

| $\varphi_{ph}^{min}$ | $\varphi_{ph}^{max}$ | $\varphi_{bg}$ | $C_{bg}$ | $C_{ph}$ |
|---|---|---|---|---|
| $2 \cdot 10^{-3}$ | $2 \cdot 10^{-2}$ | $1.5 \cdot 10^{-1}$ | $0.1 \pm 0.03$ | $0.9 \pm 0.03$ |

active state [16]. The lifetime of bacteriochlorophyll *a* background emission was determined as 2.7 ns.

The absolute quantum yields of both bacteriochlorophyll *a* emissions, given in Table II, are calculated from the relation $\varphi = \tau/\tau_0$ (where the bacteriochlorophyll *a* intrinsic lifetime [10] $\tau_0 = 18$ ns).

The relative concentrations of the bacteriochlorophyll *a* molecules $C_{ph}$ and $C_{bg}$ were determined from the relation

$$\frac{C_{ph}}{C_{bg}} = \frac{I_{ph}^{min}}{I_{bg}} \cdot \frac{\varphi_{bg}}{\varphi_{ph}^{min}} = \alpha \frac{\tau_{bg}}{\tau_{ph}^{min}}$$

which follows from assumptions (a) and (b).

The data obtained are presented in Table II. It might be well to point out that the portion of bacteriochlorophyll *a* background molecules obtained is less than 20 % and yet approx. 92 % of all bacteriochlorophyll *a* emission originates from this small bacteriochlorophyll *a* fraction in conditions of active photosynthesis.

*2. The mechanism of energy migration in bulk bacteriochlorophyll a of Chlorobium limicola*

The prominent increase in bacteriochlorophyll *a* fluorescence yield and lifetime (10-fold at least) in the transition to saturated photosynthesis (Table II) indicates that the overall rate constant of excitation trapping considerably exceeds the sum of rate constants for wasteful processes when all the *P*-840 are photoactive. In this case, the limiting value of fluorescence lifetime of antenna bacteriochlorophyll obtained in this work, $\tau_{fl}^{min} = 20$–$60$ ps, is evidently the mean time interval between creation of singlet excitation due to absorption of quantum or excitation migration from accessory pigments and its subsequent trapping by the active reaction center. The rate of the latter process, which approximates the inverse value of $\tau_{fl}^{min}$, is governed by the trapping constant of the reaction centers, by the ratio of reaction centers in open and closed states and by the rate of energy migration in antenna bacteriochlorophyll. The latter rate constant for resonance intermolecular transfer of singlet excitations is equal to the inverse value of the mean period between two successive excitation jumps (jump-time $\tau_j$) and plays a decisive role in the determination of the particular mechanism of resonance energy migration.

It was shown by Robinson [11] that in a spectrally homogeneous pigment domain containing one trap per $N$ molecules, $n_j$ excitation jumps ($n_j = 0.72\ N \log N + 0.26\ N$) are needed for ($1-e^{-1}$) portion to be trapped. For the complexes investigated, each reaction center is served by $N \cong 90$ molecules of bacteriochlorophyll *a*, thus providing $n_j \cong 150$. Consequently, for bacteriochlorophyll *a* antenna molecules, the mean value of $\tau_j$ equals:

$$\tau_j = \tau_{fl}^{min}/n_j = \frac{20 \cdot 10^{-12}\text{–}60 \cdot 10^{-12}}{150} S \cong 1.5 \cdot 10^{-13}\text{–}4 \cdot 10^{-13} \text{ s}$$

Thus for objects investigated, the value $\tau_j$ obtained falls into the range of molecular oscillations which are just in the gap between strong excitonic molecular interactions ($\tau_j \ll 10^{-12}$–$10^{-13}$ s) and resonance transfer of the Förster type ($\tau_j \gg 10^{-12}$–$10^{-13}$ s, weak molecular interactions). This region usually comprises excitons with a moderate interaction energy, which means excitation localized in a limited number of molecules.

Two different theories exist now for moderate dipole-dipole molecular interactions. The first one was developed by Simpson and Peterson [14] and the other was recently advanced by Kenkre and Knox [15]. According to the Förster theory for weak molecular interactions [17], $\tau_p$ may be expressed via the mean intermolecular distance $R_m$: $\tau_p \cong \tau_{fl} \cdot (R_m/R_0)^6$, where $\tau_p$ is the pairwise molecular jumptime.

For strong molecular interaction, $R_m/R_0$ is in the third power. This transition, i.e. $(R_m/R_0)^6 \to (R_m/R_0)^3$ is half accomplished at $\tau_p$ equal to $10^{-11}$ and $5 \cdot 10^{-13}$ s for corresponding theories [14, 15]. For $\tau_{fl} = 5 \cdot 10^{-9}$ s, the critical distance of energy migration is estimated for bacteriochlorophyll *a* to be 70–75 Å.

For a realistic case, each chlorophyll molecule having 2–3 neighbours, as was proved for this green bacterium [13] we obtain $\tau_p = (2\text{–}3)\ \tau_j \cong (0.3 \cdot 10^{-12}\text{–}1.0 \cdot 10^{-12}$ s. It gives the following $R_m$ values: (1) according to Simpson and Peterson [14]: $R_m \cong$ 7–13 Å; (2) according to Kenkre and Knox [15]: $R_m \cong$ 12–20 Å.

The last figures seem to fit best the direct experimental data [13] obtained by Fenna and Mathews ($R_m = 12$ Å inside the bacteriochlorophyll-protein complex and $R_m \cong 24$ Å between separate complexes. It thus appears likely that the exciton is spread over 7 bacteriochlorophylls of the individual pigment-protein block [13].

Small values of singlet excitation lifetimes obtained in this work prove that in the physiologically active state of photosynthesis (the lifetime of singlet excitations $\gtrsim$ 100 ps), the quantum yield of triplet formation in antenna bacteriochlorophyll *a* of green bacterium is less than 1 %.

It was originally established that the minimal values of fluorescence lifetime for antenna pigments of purple bacteria [8, 9] and Photosystem I of pea [12] do not exceed 30–50 ps, and their $n_j$ values are of the order of 100–300, which led to the same conclusions about energy migration mechanisms and the negligible role of triplets in energy delivery to reaction centers [8, 9, 12].

## APPENDIX

The basic features of the time-lever method will be grounded and formulated here. By using this method, the authors succeeded in measuring picosecond excitation lifetimes in active chlorophyll complexes of purple and green bacteria, and in Photosystem I of plants. These lifetimes were obtained with an accuracy of the order of 10 ps, whereas the time resolution of phase fluorimeter employed was 50 ps.

In phase fluorimetry, the fluorescence of the samples is excited with modulated light beam

$$S_{ex}(t) = S_0[1 + m \cos 2\pi\nu t]$$

where $S_0$ is the amplitude of light beam, $m$, the modulation coefficient, $\nu$, the modulation frequency and $t$ the time.

In this case, the intensity of fluorescence emission is modulated with the same frequency $\nu$ and is represented by the following formula [18]

$$F(t) = F_0[1+m_{fl}\cos(2\pi\nu t+\psi)]$$

For a simple case of exponential decay of homogeneous emission:

$$F(t) = F_0 e^{-t/\tau}$$

it was well established [18] that:

$$\tau = \frac{tg\,\psi}{2\pi\nu}; \qquad m_{fl} = \frac{m}{\sqrt{1+(2\pi\nu\tau)^2}} \tag{I, a,b}$$

It can be seen from Eqn. Ia that the determination of $\tau$ may be obtained via measurements of phase shifts between photocurrents induced by exciting and fluorescent light beams. Evidently, for small phase shifts Eqn. Ia is simplified:

$$\tau \cong \frac{\psi}{2\pi\nu} \tag{II}$$

The accuracy is better than 3 % for $\psi \leqslant \frac{1}{3}$ radian or $\tau \leqslant (6\pi\nu)^{-1}$.

For a two-component emission, i.e.

$$F_1(t) = F_1 e^{-t/\tau_1}; \qquad F_2(t) = F_2 e^{-t/\tau_2}$$

the phase fluorimeter produces a phase shift $\psi_m$ which may be related to the "measured" value of the lifetime $\tau_m$ representing the weight center of the area restricted by the complex decay curve:

$$F_m(t) = F_1 e^{-t/\tau_1} + F_2 e^{-t/\tau_2}$$

This $\tau_m$ may be expressed via $\tau_1$ and $\tau_2$ in the following way:

$$\tau_m = \frac{1}{2\pi\nu} \cdot \frac{I_1 \sin\psi_1 + I_2 \sin\psi_2}{I_1\cos\psi_1 + I_2\cos\psi_2} \tag{III}$$

Again when $\tau_1$ and $\tau_2 \leqslant (6\pi\nu)^{-1}$, Eqn. III may be simplified to the following:

$$\tau_m \cong \frac{1}{2\pi\nu} \cdot \frac{I_1\psi_1 + I_2\psi_2}{I_1+I_2} = \frac{\alpha\tau_1+\tau_2}{1+\alpha} \tag{IV}$$

where $\alpha = I_1/I_2$ and $\psi_1$ and $\psi_2$ were substituted according to Eqn. II.

Equation IV is essentially used in the time-lever method. Let us consider the case of two emissions (as was mentioned in the text), one of which has its fluorescent parameters (lifetime $\tau_1$ and quantum yield $\varphi_1$) variable. The second emission ($\tau_2$, $\varphi_2$) is constant. If $\tau_1$ undergoes a small change, $\tau'_1 \to \tau_1 + \Delta\tau_1$, then it can be easily derived from Eqn. IV:

$$\frac{\Delta\tau_m}{\Delta\tau_1} = \frac{\alpha(1+2\alpha)}{(1+\alpha)^2} - \frac{\alpha}{(1+\alpha)^2} \cdot \frac{\tau_2}{\tau_1} \tag{Va}$$

In order to obtain the maximal value of $\Delta\tau_m/\Delta\tau_1$ (i.e. negligible $\Delta\tau_1$ results in profound, readily measurable change in $\Delta\tau_m$), the following requirements should be satisfied:

(a) The ratio $\tau_2/\tau_1$ should be as great as possible ($\tau_2 \gg \tau_1$), hence the contribution of the first term in Eqn. Va could be neglected:

$$\frac{\Delta\tau_m}{\Delta\tau_1} \approxeq -\frac{\alpha}{(1+\alpha)^2}\cdot\frac{\tau_2}{\tau_1} \tag{Vb}$$

(b) The ratio $\alpha = I_1/I_2$ should be close to unity that can be easily achieved by attenuating $I_2$. In this case, Eqn. Vb gives:

$$\frac{\Delta\tau_m}{\Delta\tau_1} \approxeq -\frac{1}{4}\cdot\frac{\tau_2}{\tau_1}$$

$\tau_2$ may be chosen near the upper level, i.e. $\tau_2 \approxeq (6\pi\nu)^{-1}$. Usually, phase fluorimeters operate at frequencies $1\cdot10^7$–$2\cdot10^7$ Hz which gives $\tau_2 \approxeq 3\cdot10^{-9}$–$6\cdot10^{-9}$ s. Hence, for $\tau_1$ in the region 10–100 ps, $\tau_2/\tau_1$ equals to 400–40 and the time-lever obtained is equal to 100–10. This means that the measurements of $\Delta\tau_m$ with the time resolution of the particular phase fluorimeter used provides an accuracy in $\Delta\tau_1$ determination 10–100 times higher, say $\Delta\tau_m \pm 50$ ps gives $\Delta\tau_1 \pm (5\text{–}0.5)$ps.

The determination of the $\tau_1$ value by the time-lever method requires two measurements of $\tau_m$ and $\tau_m+\Delta\tau_m$ and the solution of the system of two equations of the type IV:

$$\begin{aligned} \tau_m &= \frac{\tau_2+\alpha\tau_1}{1+\alpha} \qquad \text{where } \alpha = \frac{I_1}{I_2} \\ \tau'_m &= \frac{\tau_2+\alpha'\tau'_1}{1+\alpha'} \qquad \text{where } \alpha' = \frac{I'_1}{I_2} \end{aligned} \tag{VI}$$

System VI has three unknown parameters: $\tau_1$, $\tau'_1$ and $\tau_2$. Assuming that $I_1/I'_1 = \varphi_1/\varphi'_1$ and the relation between fluorescence yield and lifetime $\varphi\tau_0 = \tau$ ($\tau_0$ is the intrinsic radiactive lifetime) is preserved in the picosecond time scale (this fact has not yet been thoroughly investigated experimentally), one of three unknown parameters of system (VI) may be excluded:

$$\tau'_1 = \tau_1\frac{\alpha'}{\alpha}$$

As to $\alpha$ and $\alpha'$ values, (or corresponding relative amplitudes of fluorescence emissions) they must be determined in additional experiments. In general, two substances producing two different emissions should be studied separately. In our particular case, when the photosynthetic object emitted both emissions, they were "resolved" by a specially designed method, as was described in the Results and Discussion of this work.

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