A TWO-QUANTASOME THEORY OF CHLOROPHYLL-a FLUORESCENCE IN GREEN PLANT PHOTOSYNTHESIS*

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1. Introduction.—The results of several experiments\textsuperscript{1–3} show that the chlorophyll-a in green plants fluoresces for a time short compared to its natural lifetime, $\tau_0 = 15.2$ nsec.\textsuperscript{1} This shortening of the lifetime has been interpreted to mean that a photon absorbed anywhere in a photosynthetic unit of pigment molecules is rapidly transferred to a trapping center.\textsuperscript{4} If the trap has a fluorescence band\textsuperscript{2} which lies principally at longer wavelengths than that of Chl-a, the mean time, $t_T$, to trap an excitation quantum should equal the Chl-a fluorescence lifetime, $\tau$. Experimental values of $\tau$ are conflicting. Direct flash measurements give $\tau = 1.7 \pm 0.2$ nsec.\textsuperscript{1} Values of $\tau$ from phase fluorimetry range between 0.6 and 1.0 nsec.\textsuperscript{5, 6} The measured steady-state fluorescence yield, $\varphi$, of Chl-a in vivo is 2.7 per cent;\textsuperscript{6} the relation\textsuperscript{1} $\tau = \varphi \tau_0$ then gives $\tau = 0.4$ nsec.

Bey and Pearlstein\textsuperscript{4} constructed a theory of the PSU in which energy is transferred via weak (dipole-dipole) resonance interactions among Chl-a molecules. They consider both two- ($D = 2$) and three-dimensional ($D = 3$) pigment arrays, and use a diffusion equation to solve the latter case. The largest trapping time predicted by the diffusion approximation, $t_T = 0.09$ nsec for $D = 3$, is less than the smallest experimental value for the fluorescence lifetime. The 0.09-nsec trapping time results from the diffusion calculation with the assumption that the pigment molecules of one PSU are packed into one quantasome, a $2 \times 10^6$ Å\textsuperscript{3}-fragment of chloroplast lamellae.\textsuperscript{5, 7}

In the previous WI theory,\textsuperscript{4} as well as in the present one, the trapping center is assumed to occupy a single lattice site. In this paper, trapping times are calculated for a single-site trap by direct solution of the differential equations from which the diffusion equation was derived.\textsuperscript{4} The new $D = 3$ formula predicts approximately threefold larger trapping times, under the same conditions, than does the old.

The new theoretical results justify an attempt to accommodate the concept of two pigment systems in green plant photosynthesis\textsuperscript{8, 9} to the notion of PSU-quantasome equivalence. That two distinct trapping times, corresponding to the two pigment systems, can exist is noted in reference 4, where a two-quantasome hypothesis is introduced. As a matter of notation, the PSU is assumed to consist of two subunits, psu\textsubscript{1} and psu\textsubscript{2}, corresponding to pigment systems 1 and 2, respectively. Possible structural realizations of psu\textsubscript{1} and psu\textsubscript{2} in terms of quantasomes are detailed, and the two-quantasome hypothesis is further developed. Following the series formulation of the two pigment systems in photosynthesis,\textsuperscript{8} each psu is assigned its own trapping center. The trapping times, $t_1$ and $t_2$, appropriate to each, are calculated from the direct-solution formulas. Using these values of $t_1$ and $t_2$, the present theory predicts that flash, phase, and yield measurements should give three different values for the fluorescence lifetime.

2. The Direct Solution.—The WI equations\textsuperscript{4} were solved for certain convenient values of $N$, the number of Chl-a molecules in the psu, with the aid of the University
of Maryland's IBM 7090 computer. The methods employed are fully described elsewhere.\(^6\) In \(D = 3\), the Chl-a's are assumed to be arrayed in a simple cubic lattice having spherical symmetry about a central trap, as in reference 4; in \(D = 2\), in a square lattice having circular symmetry about such a trap. Let \(f = F t_\tau\), where \(F\) is the pairwise transfer rate (\(F^{-1}\) is called \(t_1\) in ref. 4). A least-squares analysis of the computer values for \(f\) gives

\[
f = 0.221 N \ (D = 3),
\]

and

\[
f = 0.565 N - 1.30\sqrt{N} \ (D = 2).
\]

The computer solution shows that excitation of Chl-a in the psu decays exponentially with time constant \(t_\tau = f F^{-1}\).

Equations (1) and (2) are approximately valid for \(N > 25\). Equation (1) is accurate to within 15 per cent for \(25 < N < 50\); to within 10 per cent for \(50 < N < 100\); and to within 3 per cent for \(100 < N < 500\). Equation (2) is accurate to within 10 per cent for \(25 < N < 50\); and to within 3 per cent for \(50 < N < 400\).

For given \(N\) and \(F\), \(t_\tau\) is about twice as large in \(D = 2\) as in \(D = 3\). However, the PSU-quantasome equivalence prescribes different values of \(F\) for the two cases. \(F\) is given by

\[
F^{-1} = \tau_0 (\tilde{R}/R_0)^6,
\]

where \(\tilde{R}\) is the lattice constant, and \(R_0\) is calculated from Förster's theory.\(^4\) \(\tilde{R}\) for \(D = 2\) is less than \(\tilde{R}\) for \(D = 3\); \(t_\tau\) may be as much as an order of magnitude smaller in \(D = 2\) than in \(D = 3\) (Table 1). Since the concentration of Chl-a in量子somes is inversely proportional to \(\tilde{R}^3\), a given \(t_\tau\) can be achieved by \(D = 3\) at a lower concentration than by \(D = 2\). If lower concentration leads to less concentration quenching,\(^11\) \(D = 3\) leads to higher photosynthetic efficiency than \(D = 2\).

Equation (2) provides an estimate of the effects of quantasome shape and trap location on the \(D = 3\) value of \(t_\tau\). Thus, if the quantasome is flattened, or if the trap is nearer the surface than the center, the present \(D = 3\) results for \(t_\tau\) are increased by no more than a factor of two.

<p>| (\text{TABLE 1} ) | (\text{TRAPPING TIMES FOR PSU}_1) AND (\text{PSU}_2) |
|-------------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>(\text{psu})</th>
<th>(N)</th>
<th>(D)</th>
<th>(\tilde{R}(\AA))</th>
<th>(F\text{Ge}/\text{s})</th>
<th>(t_\tau\text{(nsec)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>400</td>
<td>3</td>
<td>17</td>
<td>310</td>
<td>0.29</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>3</td>
<td>27</td>
<td>19</td>
<td>1.2</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>110</td>
<td>3</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>3</td>
<td>34</td>
<td>4.9</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>29</td>
<td>13</td>
<td>1.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| 3. Two Pigment Systems.—Current conceptions of the two pigment systems\(^8,\)\(^9\) require that \(\text{psu}_1\) contain the majority of the Chl-a and little of the accessory pigment; and that \(\text{psu}_2\) contain less Chl-a and most of the accessory pigment. In \(\text{psu}_1\), light energy is absorbed predominantly by Chl-a; in \(\text{psu}_2\), photons are absorbed by both Chl-a and accessory pigment. In each \(\text{psu}_1\), absorbed energy is transferred to the trap only through the weakly interacting Chl-a's.

In Table 1, values of \(F\) and \(t_\tau\) are given for both \(\text{psu}_1\) and \(\text{psu}_2\), and for both \(D = 3\) and \(D = 2\). The value\(^4,\)\(^12\), \(N_1 = 400\), for \(\text{psu}_1\) is probably in error by no more than \(\pm 25\) per cent. The \(N\)-value for \(\text{psu}_2\), \(N_2\), is considerably more uncertain, so two possible values of \(N_2\) are included in the table. The \(F\)-values are...
calculated from (3), using \( R_0 = 70 \, \text{Å} \).\(^{13}\) The intramolecular vibrational-relaxation rate, \( \nu_m \), for Chl-a in vivo is probably \( \sim 10^4 \) to \( 10^4 \, \text{GeV/s} \). The WI approximation is quite good for psu\(_1\), since \( F < < \nu_m \) in all cases. For psu\(_1\) and \( D = 3 \), WI is valid, at least as a first approximation. WI is clearly inadequate for psu\(_1\) if \( D = 2 \); in this case \( L_1 \) of Table 1 is an upper limit.\(^4\)

There are four readily apparent realizations of the two-psu assumption in terms of quantasomes. If both psu's occupy a single quantaosome, three combinations are possible: \( D_1 = D_2 = 2; D_1 = 2, D_2 = 3; \) and \( D_1 = 3, D_2 = 2 \). (The subscripts 1 and 2 correspond to psu\(_1\) and psu\(_2\), respectively.) If each psu occupies a separate quantaosome, the combination \( D_1 = D_2 = 3 \) is also possible. The latter is not possible for a single quantaosome within the context of "random" orientations implicit in the present WI theory. A TQH is necessary if \( D_1 = D_2 = 3 \) (hereinafter abbreviated \( D = 3 \)).

4. Predictions of \( D = 3 \) and TQH.—The three experiments cited in the Introduction have several common aspects. A purely exponential decay is assumed in determining \( \tau \) from the data. The exciting light is restricted to wavelengths shorter than 4360 Å, and hence is absorbed predominantly by the Soret band of Chl-a, rather than by accessory pigment. Putting \( N = N_1 + N_2 \), psu\(_1\) receives \( N_1/N \), and psu\(_2\) \( N_2/N \), of all absorbed quanta. The incident light intensities are well below that required to saturate photosynthesis, so that essentially all quantaosomes have operative traps. The experiments are performed under physiological conditions; Chl-a fluorescence (peaked at 6850 Å) induces a much larger detector response than does trap fluorescence (peaked at about 7300 Å).\(^2\) One may therefore neglect the effect of trap fluorescence on the observed lifetimes.

Now let us assume \( D = 3 \) and TQH are correct, and predict the outcomes of these experiments. Denoting \( t_i \) of psu\(_1\) by \( t_1 \), and of psu\(_2\) by \( t_2 \), the total fluorescence yield under the above conditions is \( \varphi = (N t_1 + N t_2)/N \). From (1), \( t_i = 0.221 N_i/F \), for \( i = 1 \) or 2; \( F = \tau_0^{-1}R_0^6N_1^2/V^2 \) from (3) and the fact that \( N, R_0^6, V \), the quantaosome volume. Thus,

\[
\varphi = 0.442V^2/N R_0^6.
\] (4)

Equation (4) has the property that \( \varphi \) is independent of the apportionment of Chl-a's between the two psu's, which is not the case if \( D = 2 \). In fact, \( \varphi \) depends only on the three quantities \( V = 2 \times 10^6 \, \text{Å}^3, N = N_1 + N_2 = 500, \) and \( R_0 = 70 \, \text{Å} \). Furthermore, each psu contributes equally to \( \varphi \), regardless of the value of \( N_2/N_1 \). With the values of \( V, N, \) and \( R_0 \) given, \( D = 3 \) and TQH predict \( \varphi = 3.0 \) per cent, in good agreement with the result (2.7%) of Latimer et al.\(^5\)

In both phase and flash fluorimetry the exciting light is modulated with an envelope, \( G(t) \). In the former, \( G(t) = e^{i\omega t}, \) a sinusoidal modulation, where \( \omega \) is the angular modulation frequency. In the latter, \( G(t) \) is a pulse, zero for all \( t \), except over a short time interval, \( \sigma \). If the response of the fluorescent sample to a \( \delta \)-function pulse is \( f(t) \) (true response), then the detector response\(^1\) is

\[
F(t) = \int_0^t G(s)f(t - s)ds.
\] (5)

If the true response is a pure exponential decay of lifetime \( \tau \), \( f(t) = (1/\tau)e^{-t/\tau} \).

(For convenience, \( f(t), F(t), \) and \( G(t) \) are normalized to unit area above the time axis.) Since \( \varphi \propto \int_0^\infty f(t)dt \), \( D = 3 \) and TQH predict
The equal normalization (0.5) of the two terms follows from the equal contribution to the yield of the two psu's.

The results of both phase and flash fluorimetry are evaluated with the help of (5). For a single exponential decay, the "phase lifetime," $\tau_p$, and the "flash lifetime," $\sigma_f$, are both equal to the time constant of that decay. With the $f(t)$ of (6), phase fluorimetry measures the average of the phase shifts produced by $t_1$ and $t_2$ (for small shifts; in this case, $\sim 10^{-2}$). Thus,

$$\tau_p = 0.5 (t_1 + t_2).$$

Brody and Rabinowitch used (5) and a second-moment analysis to evaluate their results. Following their method, and using (6), one finds

$$\sigma_f = 0.87 t_2 [1 - 0.67(t_1/t_2) + (t_1/t_2)^2]^{0.5}. \tag{8}$$

For $t_1/t_2 = 0.2$, $\sigma_f = 0.83 t_2$, and $\tau_p = 0.60 t_2$. In this case, $D = 3$ and TQH predict $\sigma_f$ to be 38 per cent greater than $\tau_p$. If $t_1/t_2 = 0.1$, $(\sigma_f - \tau_p)/\tau_p = 53\%$.

Referring to Table 1, if $D = 3$, $t_1 = 0.29$ nsec, and $t_2$ ranges between 1.2 and 2.2 nsec, for $N_2$ between 100 and 50 Chl-a's. The corresponding theoretical ranges of $\tau_p$ and $\sigma_f$ are 0.7-1.2 nsec and 1.0-1.8 nsec, from (7) and (8), respectively. For $D = 3$, best agreement with experiment is obtained if $N_2 = 65$; then the theoretical values, $\tau_p = 1.0$ nsec and $\sigma_f = 1.5$ nsec, agree with the experimental values, $\tau_p = 0.8$ nsec and $\sigma_f = 1.7$ nsec, to within estimated errors. The theoretical difference between phase and flash-moment measurements, 0.5 nsec, is, however, definitely smaller than the corresponding experimental difference, 0.9 nsec. The flash measurements were performed on Chlorella; the phase, on bean leaves and other species. The $N$-values are based on Chlorella, the quantosome volume, $V$, on spinach. A more critical comparison of theory and experiment may be possible only if all quantities are derived for a single species.

If $D_1 = 2$ rather than 3, psu makes a negligible contribution to $\varphi$ regardless of the exciting light wavelength. Then, when Chl-a alone absorbs, $\varphi = 1.5$ per cent (all from psu) and $\tau_p = \sigma_f = t_2$. In this case, there is a yield-discrepancy of 1.5 per cent (50% of the observed value) from the Latimer result, and a completely anomalous discrepancy between experimental values of $\tau_p$ and $\sigma_f$. A steady-state yield measurement integrates both prompt (<$\tau_0$) and delayed (> $\tau_0$) light emissions. A large delayed-light yield can possibly explain the $D_1 = 2$ discrepancy between prompt and steady-state yields. Delayed light cannot, however, account for the prompt-lifetime anomaly, which arises if $D_1 = 2$. The correlation between theoretical and experimental values of $\varphi$, $\tau_p$, and $\sigma_f$ is thus only possible if $D_1 = 3$, not 2. The earlier conclusion, based on spectral arguments, that $D = 2$ is inappropriate, is confirmed for psu, without recourse to spectral arguments.

$D_2 = 2$ is not so easily excluded. For the particular choice, $N_2 = 45$, the theoretical fluorescence yield is 2.6 per cent, with psu, contributing 3/5 of the total. $D_1 = 3$, $D_2 = 2$ (one or two quantasomes) with $N_2 = 45$ predicts $\varphi = 2.6\%$, $\tau_p = 0.9$ nsec, and $\sigma_f = 1.3$ nsec, giving almost as good agreement with experiment as $D = 3$ and TQH.

Regardless of whether $D_2 = 2$ or = 3, close correlation between experiment and
the present theory occurs simultaneously for the three quantities, \(\varphi\), \(\tau_p\), and \(\sigma_f\). Furthermore, in this theory, it apparently never happens that two of the theoretical quantities simultaneously correlate with experiment, unless all three are so correlated. If, for a certain choice of \(N\) and \(D\)-values in each psu, any pair of the theoretical \(\varphi\), \(\tau_p\), and \(\sigma_f\) agree with experiment, then the third quantity necessarily also agrees. Since all three quantities are related to \(t_1\) and \(t_2\) in the theory, the third correlation serves as an independent check of the theory's validity. This fact increases the reliability of the already reliable theoretical value of \(\varphi\). On this basis, one concludes that the prompt and the steady-state fluorescence yields of Chl-a are very nearly equal.

5. Discussion.—Several notions of the present theory contrast with earlier viewpoints. First, it will be noted that the present theory is a theory only of Chl-a fluorescence, peaked at 6850 Å in vivo, and not of "trap" fluorescence, peaked at about 7300 Å. It is assumed that fluorescence spectra cannot distinguish Chl-a fluorescence emitted by psu₁ from that emitted by psu₂. All Chl-a is assumed to be "activated" (capable of fluorescing); a molecule incapable of emitting dipole radiation is also incapable of transferring energy through dipole-dipole interactions. The lifetime of Chl-a fluorescence in psu's with operative traps is determined by the mean time for energy transfer to those traps.

The theory predicts that the observed fast decay should be the weighted sum of two exponential decays. Two lifetimes arise because Chl-a occurs in two different concentrations in vivo. In psu₁, which has the higher concentration, energy transfer is faster than in psu₂ because the greater proximity of Chl-a's leads to higher pairwise transfer rates than in psu₂. In psu₂, Chl-a appears in a lower concentration probably because the Chl-a in psu₂ is "diluted" by accessory pigment. Each Chl-a in psu₂ acts as a "trap," although perhaps not uniquely, for light energy absorbed by a certain number of accessory-pigment molecules. Energy is then transferred among Chl-a's to a trapping center, as in psu₁.

The two-quantaosome theory is consistent with all series-formulation interpretations of Chl-a fluorescence changes in vivo. The theory does, however, revise the notion, based on observed fluorescence changes, that system I Chl-a is actually nonfluorescent. When the traps of psu₁ and psu₂ are operative, the present calculations show that psu₁ has an intrinsic fluorescence yield \((k_1/\tau_0)\) of 2 per cent, psu₂ \((k_2/\tau_0)\) about 10 per cent. As already noted, the two psu's contribute equally to the actual yield (2.7%) when Chl-a alone absorbs; the higher intrinsic yield of psu₂ is offset by the lower optical absorption cross section of its Chl-a. Since psu₂ contains a significant amount of Chl-a, it is difficult to excite psu₂ alone. If the incident light is absorbed by accessory pigment, however, the observed yield comes almost entirely from psu₂. This results in a fourfold increase in fluorescence yield over the Latimer value, in agreement with Duyssen's conclusions. Other fluorescence changes, notably those occurring during photosynthetic induction, are explained just as satisfactorily on the present basis (intrinsic yield of psu₁ about 0.2 that of psu₂) as on the earlier one (intrinsic yield of psu₁ = 0).

Summary.—1. The mean time for transfer of electronic excitation energy to a single-site, operative trap in a photosynthetic unit is calculated by direct solution of the weak-interaction equations. Both two- and three-dimensional arrays of pigment molecules are considered.
2. Following reference 4, the PSU is assumed to consist of two subunits, psu₁ and psu₂, corresponding to the two pigment systems of green plant photosynthesis. If each subunit has its own trapping center, and the concentration of chlorophyll-a is higher in one psu than in the other, two trapping times, t₁ and t₂, occur.

3. The two-quantasome hypothesis of reference 4, that each psu is embodied in a separate quantasome (chloroplast lamellar fragment), is shown to be a necessary adjunct of the assumption that both psu’s contain three-dimensional pigment arrays. The trapping times for such arrays are t₁ = 0.29 nsec, if psu₁ contains 400 Chl-a molecules; and t₂ = 1.2–2.2 nsec, if psu₂ contains 100–50 Chl-a’s.

4. The relation of these trapping times to the fluorescence lifetime of Chl-a in vivo is discussed. The theory explains the anomaly in the values of this lifetime as measured by steady-state fluorescence yield, phase fluorimetry, and direct flash (with second-moment analysis) experiments. Theoretical values of yield, phase, and flash lifetimes are 0.46, 1.0, and 1.5 nsec, respectively. The corresponding experimental values are 0.41, 0.8, and 1.7 nsec.

5. Two- and three-dimensional pigment arrays are compared in their abilities to correlate theory and experiment. It is shown that agreement of the theory with more than one of the three experiments simultaneously is only possible if psu₁, at least, is three-dimensional.

6. The theory calculates two quantities, t₁ and t₂, and correctly predicts from them three independent experimental results. The third correlation verifies the internal consistency of the theory.

7. The theoretical value of the total Chl-a fluorescence yield is the most reliable quantity calculated, because it is independent of the (relatively unknown) apportionment of Chl-a’s between the two pigment systems.

8. The present theory is completely consistent with interpretations of Chl-fluorescence yield changes based on the series formulation of photosynthesis. However, the theory predicts that, rather than being actually nonfluorescent, the Chl-a of psu₁ has an intrinsic yield of 2 per cent, about one fifth that of psu₂.

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Abbreviations: WI, weak interactions; PSU, photosynthetic unit; TQH, two-quantasome hypothesis; Chl, chlorophyll; nsec, nanosecond \((10^{-9}\text{ sec})\); Gc/s, gigacycle per sec \((10^{9}\text{ sec}^{-1})\).

*This work is contained in a thesis submitted to the University of Maryland in partial fulfillment of the requirements for an advanced degree in physics.
4 Bay, Z., and R. M. Pearlstein, these PROCEEDINGS, 50, 1071 (1963).
MATHEMATICS: W. LEIGHTON

Consider the differential equation

\[ y'' + p(x)y = 0, \quad (1) \]

where \( p(x) \) is of class \( C' \) on the interval \( I : x_0 \leq x < \infty \). If \( p(x) \) is a nondecreasing function on \( I \), it is known that all solutions of (1) are bounded on \( I \). If \( p(x) \to +\infty \) as \( x \to \infty \), it is known that there exists at least one nonnull solution \( y_0(x) \) of (1) with the property that

\[ \lim_{x \to \infty} y_0(x) = 0. \]

It has been an open question whether or not all solutions have this property. This note provides an affirmative answer to this question.

Note that all solutions of (1) are oscillatory on \( I \) and that it follows from a result due to Osgood that the relative maxima of any solution form a sequence in which the terms decrease monotonely as \( x \) increases.