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ENERGY TRANSFER IN PHOTOSYNTHESIS

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SUMMARY

The results of recent spectroscopic measurements on chlorophyll *a* in bilayers and lipid vesicles stimulated a re-examination of energy transfer in photosynthesis. The Förster resonance transfer mechanism is believed to be applicable under reasonable assumptions despite recent criticism. The Förster parameter was newly determined to be $R_0 = 65$ Å; previous uncertainty due to unknown transition moment orientation can be avoided by assuming the black film dichroic result to be applicable in vivo. The effect due to the still incompletely known thylakoid structure and chlorophyll aggregation is discussed qualitatively. Agreement with experimental fluorescence lifetimes and quantum efficiencies may be obtained with a reasonable choice of parameters.

I. INTRODUCTION

Chlorophyll *a* and its auxiliary pigments absorb light energy and transfer 97% of it to a reagent center, where water is split (System II) and the reducing agent NADPH is produced (System I). In a dark reaction CO_2 is then incorporated into sugar. About 3% of the absorbed light is re-emitted as fluorescence. Emerson and Arnold's flashing light experiments have shown that there are about 2500 chlorophyll molecules per O_2 released, and there is evidence that 8 photons are required per O_2 released, which then implies that we have 300 chlorophyll *a* molecules per reaction center (50 for bacteria). A detailed and elementary discussion of this subject may be found elsewhere[1,2].

The decay time and yield of fluorescence depends either on the efficiency of energy uptake by the reaction center[3,4], or on the time required for light energy to reach the reaction center[2,5–7], or possibly on both. Bay and Pearlstein and Pearlstein[6,7] discussed the second possibility using the Förster[8,9] theory of energy transfer between adjacent pigment molecules by dipole–dipole resonance interaction. Using somewhat extreme values, Robinson[3] was critical of applying Förster's theory, which assumes negligible back transfer of excitation from acceptor to donor molecule. This will hold if the lifetime forthermal degradation is short compared to the Förster transfer time between nearest neighbors. At a two-dimesional chlorophyll *a* separation of 11 Å both times are comparable[3], and we are dealing with a boundary situation

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where the application of Forster's theory (R^{-6} rate) and Perrin's theory (R^{-3} rate) are both doubtful[10]. Thus, it is rather important to calculate R_0 in Förster's theory[8] with the highest possible accuracy. The calculation was previously done from absorption and fluorescence data of chlorophyll *a* in ether[2,5,8–10]. *In vivo* data are difficult to estimate due to the presence of several pigments, and absorption band shifts due to interaction of pigment molecules with each other and with other molecules of the medium[11]. The present paper is motivated by four new developments: (1) Recent bilayer (black film) experiments[12,13] showed that the red transition moment of chlorophyll *a* makes an angle $\beta_R = 36.5°$ with the plane of the bilayer. It will be argued that the same is true *in vivo*, and the consequences of this assumption are investigated in detail. (2) Recent experiments by the author[14] determined the fluorescence and absorption parameters of chlorophyll *a* in lipid vesicles. This data should provide, in combination with the black film experiments[12,13], a better model system than ether solution spectra, for a new calculation of R_0 in Förster's theory. (3) Montroll[15] derived expressions for the mean number of steps the excitation takes in a random walk to reach the reaction center. This makes computer calculations[3,7] unnecessary and gives roughly the same answers. Equal jump time to each nearest neighbor was assumed. (4) Much recent work[16,17] indicates that chlorophyll *a* aggregation is important and may in fact account for most of the changes of *in vivo* spectra compared to solution spectra. This has an important effect on energy transfer to the reaction center.

II. THE MODEL

Many different models have been proposed for the structure of the chloroplast thylakoids[18–25]. We shall assume a simple fluid lipid bilayer model[22,23], in which the globular proteins have little influence on energy transfer between chlorophyll molecules. The latter are assumed to be in the lipid bilayer (Fig. 1), anchored at the membrane–water interface by the hydrophilic cyclopentane ring (V). Analogous to the black film dichroism results[12,13] we shall assume that the red transition moment makes an angle of $\beta = 36.5°$ with the membrane surface. The red transition moment lies along the diagonal of the conjugated porphyrin ring (I to III in Fig. 2). From blue dichroism in black films and the knowledge that the red and blue transition moments are perpendicular[26] it follows that the porphyrin head makes an angle $\gamma = 48°$ with the membrane surface[12,13]. We believe the same angular arrangement is present *in vivo*, since it provides the simplest explanation for the weak dichroism in all but the 695-nm absorption band[21,26]. We do not necessarily believe the bilayer model assumed (Fig. 1) to be the correct one. The difference between the assumed model and those advocated by Calvin[24] or Kreutz[21], is only important for the comparatively small transfer across the plane of the membrane, to be discussed in section VI. The major energy transfer to the reaction center is in either case a two-dimensional problem. The still disputed point is whether to believe a lipid thickness of 40 Å obtained from electron microscopy[23], or a 21-Å thickness obtained from X-ray scattering[21]. No use was made of the quantasomes[5,18,27] introduced by Park on the basis of electron microscopy; they may be artifacts[18].

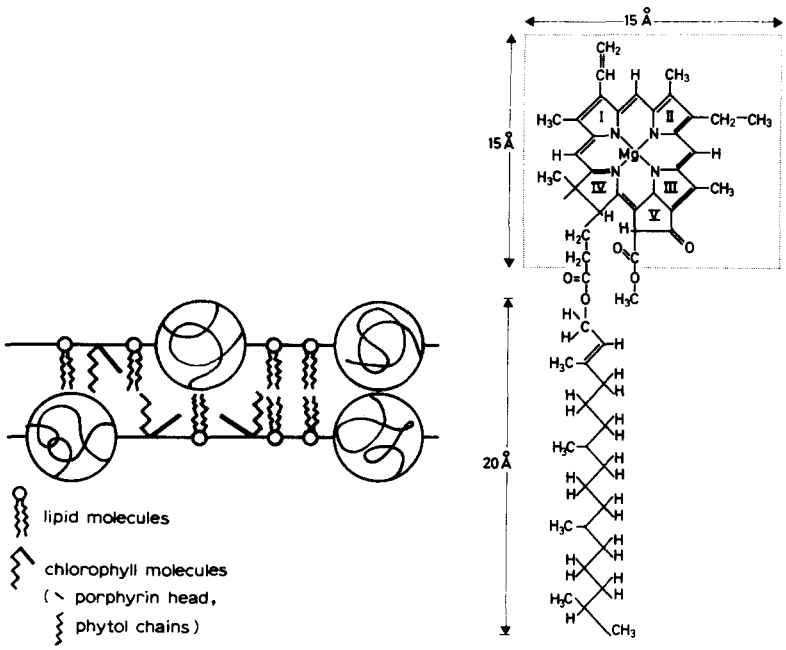

Fig. 1. Lipid bilayer model. According to Muelhethaler[23] the lipid molecules form a bilayer 40 Å thick. The coiled up spherical proteins (60 Å diameter) are immersed about 20 Å into the lipid. We have also shown chlorophyll molecules with their porphyrin head at an angle of 48° to the membrane surface and their phytol chains perpendicular to the surface.

Fig. 2. Structure of chlorophyll *a*. The molecule is believed to be anchored by the group V (cyclopentane ring) to the water–lipid interface; the red transition moment points from ring I to III and makes an angle of 36.5° with the membrane surface.

III. THE PAIRWISE ENERGY TRANSFER RATE (F'RSTER'S THEORY)

This pairwise energy transfer rate by dipole–dipole resonance interaction between two molecules distance R apart may be written in the form[8–10]

$$\frac{1}{\tau_J} = \frac{1}{\tau_0}\left(\frac{R_0}{R}\right)^6 \tag{1}$$

Here

$$R_0^6 = \frac{9K^2(\ln 10)^2 c\tau_0}{16\pi^4 n^2 N'^2 \nu_0^2} J\nu \tag{2}$$

Assuming a mirror image between red absorption and fluorescence band the overlap integral takes the form[8,9]

$$J_\nu = \int_0^\infty \varepsilon(\nu)\varepsilon(2\nu_0 - \nu)\,\mathrm{d}\nu \tag{3}$$

Here ε is the extinction coefficient ($l \cdot mole^{-1} \cdot cm^{-1}$) on a wave number scale (cm^{-1}), n the index of refraction, c the velocity of light, $N' = 6.023 \cdot 10^{20}$ molecules $\cdot$ mmole^{-1}, and ν_0 is the mean wave number of the absorption and fluorescence peak.

$$K^2 = (2\pi)^{-2} \iint d\psi \, d\psi' (\cos\phi_{ad} - 3\cos\phi_a \cos\phi_d)^2 \tag{4}$$

becomes an average over dipole directions for the energy acceptor (a) and donor (d), with ϕ_{ad} the angle between them and ϕ_a, ϕ_d the angle each one makes with the line connecting them. K^2 can vary between 0 and 4 and has the value 2/3 for a completely random arrangement[8,9]. Many expressions in the literature differ from Eqn 2 by a factor of π or 2π and are in our opinion in error (see also ref. 27). Förster obtained the value $R_0 = 80$ Å ($J_\nu = 2.1 \cdot 10^{12}$ cm$^3 \cdot$ mmole^{-2}) for chlorophyll *a* at low concentration in ethyl ether[8,9]. More recent estimates[2,5,10] use the shorter natural lifetime $\tau_0 = 15.2$ ns, and obtain $R_0 = 70$ Å under the assumption of three-dimensional random dipole directions[2,6,10].

Using the model outlined in Section II and Fig. 1, where the red transition moment is free to rotate, while maintaining an angle of β with the plane of the membrane we obtained for an average over dipole directions (Eqn 4)

$$K^2 = \sin^4\beta + \tfrac{5}{4}\cos^4\beta \tag{5}$$

Using the black film result[12,13] $\beta = 36.5°$ one finds $K^2 = 0.647$, which is quite close to the completely random value of 2/3. We shall neglect for the present, transfer between chlorophyll *a* molecules across the plane of the bilayer (see Section VI).

The overlap integral J_ν (Eqn 3) was re-evaluated using spectra of chlorophyll *a* in lipid vesicles recently obtained by the author[14]. For comparison the calculations were also performed on spectral data from chlorophyll *a* in ether and ethanol, where our data is in close agreement with previous work[14,29]. Let us assume a Gaussian line profile, which gives a good fit to the fluorescence and the red absorption band down to 0.1 of peak height. Thus

$$\varepsilon(\nu) = \varepsilon_m \exp\{-\tfrac{1}{2}\alpha^2(\nu - \nu_a)^2\} \tag{6}$$

and from Eqn 3

$$J_\nu = \varepsilon_m{}^2 (\sqrt{\pi}/\alpha) \exp\{-\alpha^2 \nu_s{}^2/4\} \tag{7}$$

where ε_m is the extinction at the absorption maximum ν_a (cm^{-1}), ν_s the Stoke shift, and $\alpha = (2 \ln 2)^{\frac{1}{2}}/\nu_{\frac{1}{2}}$. For the half width at half peak height $\nu_{\frac{1}{2}}$ the high energy side was taken for the fluorescence band and the low energy side for the absorption band. Both agree within 10% from each other and also from the slightly wider halfwidths on the low energy side in fluorescence and high energy side in absorption. The relevant values together with the calculated J_ν (Eqn 7) are given in Table I. R_0 (Eqn 2) was then calculated using an index of refraction for the lipid[6] of $n = 1.45$, a lifetime[10] $\tau_0 = 15.2$ ns and $K^2 = 0.647$.

IV. MEAN CHLOROPHYLL SPACING AND EXCITATION TRAPPING

Based on the dimensions of a quantasome, Bay and Pearlstein[5] used a mean chlorophyll *a* separation *in vivo* of 11 Å under the assumption of a two-dimensional

TABLE I

FLUORESCENCE AND ABSORPTION PARAMETERS, THE OVERLAP INTEGRAL J_ν (EQN 6), AND THE FÖRSTER ENERGY TRANSFER PARAMETER R_0 (EQN 2) FOR CHLOROPHYLL *a*

Parameter	*Ether*	*Ethanol*	*Lipid vesicle*
Absorption max., ν_a (cm^{-1})	15149	15056	14945
Fluorescence max., ν_f (cm^{-1})	14988	14872	14846
Stoke shift, ν_s (cm^{-1})	161	184	100
Halfwidth, $\nu_{\frac{1}{2}}$ (cm^{-1})	188	232	260
Extinction coefficient ε_m ($l \cdot mole^{-1} \cdot cm^{-1}$)	85100	69400	62000
J_ν (10^{12} $cm^3 \cdot mmole^{-2}$)	1.59	1.35	1.43
R_0 (Å)	66.0	64.4	65.1

arrangement and 17 Å in a three-dimensional arrangement. Neglecting for the moment chlorophyll aggregation effects, we would prefer a value of 15 ± 1 Å for the mean two-dimensional separation within one plane. This corresponds to an available surface area of 200 $Å^2$ to 250 $Å^2$ per porphyrin ring, which is in agreement with several estimates[30–32,21] and is consistent with chlorophyll making up 21% of the lipid-soluble fraction of thylakoid membranes[21,27]. The differences between 15 Å and 11 Å is important. Using 11 Å and $R_0 = 70$ Å one obtains (Eqn 1) a nearest neighbor transfer rate of $1/\tau_J = 4.4 \cdot 10^{12}$ s^{-1}, which is of the same magnitude as the vibrational relaxation rate ($3 \cdot 10^{12}$ s^{-1})[33]. Thus Robinson's criticism[3,10] of the application of Förster's theory is justified. However, with a mean spacing of 15 Å and $R_0 = 65.1$ Å (Table I) the nearest neighbor transfer rate is $0.44 \cdot 10^{12}$ s^{-1}, which is sufficiently below the vibrational relaxation rate to justify the application of Förster's theory. Now let us assume we have per reaction center N equally spaced chlorophyll *a* molecules in a plane; effects due to chlorophyll aggregation will be considered in the next section. Further we shall assume that the reaction center permanently traps and utilizes the excitation energy with high efficiency, once it arrives there. This may or may not be true[1–6,10,33]. Thus the fluorescence efficiency and decay time depend on the mean trapping time

$$\langle \tau_T \rangle = \langle n \rangle \tau_J \tag{8}$$

wher $\langle n \rangle$ is the mean number of steps before trapping in a random walk. This problem has been discussed in two dimensions for a square lattice by Pearlstein[7] and Robinson[3]; and Knox[34] has treated it for square and triangular lattices by direct machine inversion of the transfer matrix. More recently Montroll[15] has solved the problem in terms of an asymptotic series in $1/N$. For the special case of equal probability for a random walker to go to any nearest neighboring point on a given step, he finds for a square lattice

$$\langle n \rangle = \pi^{-1} N \ln N + 0.19506\, N \tag{9}$$

plus higher order terms which we shall neglect. In the region of interest Montroll's expression is in close agreement with machine computations. For example for $N = 324$,

a reasonable value for plants, we obtain $\langle n \rangle = 659$ steps from Eqn 9 compared to 670 steps from machine computation by Robinson[3]. For $N = 50$, a likely value for bacteria, the corresponding numbers are 72 and 74 steps, respectively.

Using $N = 300$ and a nearest neighbour transfer rate of $0.44 \cdot 10^{12}$ s^{-1}, the mean trapping time (Eqn 8) is $\langle \tau_T \rangle = 1.37$ ns, and the corresponding fluorescence efficiency $\phi = 100 \langle \tau_T \rangle / \tau_0 = 9\%$. These values are considerably larger than previous estimates[5] and closer to *in vivo* experimental values of fluorescence lifetimes, which range from 0.4 to 2 ns, and fluorescence efficiencies of 3 to 6%[1,5,33]. In fact the calculated values are somewhat large in view of the fact that the calculation assumes all traps open, a situation which corresponds to the minimum values of both $\langle \tau_T \rangle$ and ϕ.

V. THE EFFECT OF CHLOROPHYLL AGGREGATION

Let us now take into account that the chlorophyll molecules are not located on a lattice corresponding to the mean spacing. A proper treatment using the correct chlorophyll distribution, even if it were known, would seem too difficult mathematically. The red absorption band of chlorophyll *a in vivo* may be separated into at least three bands with maxima at 673, 683 and 695 nm[1,2,11,17,21,33]. There is strong evidence that this multiplicity of peaks is due to chlorophyll aggregation, rather than different interactions with lipids of proteins[1,17,21,33]. There is general agreement that the 683-band is due to a dimer[21,33] and the 695-nm band may be a stacked chlorophyll polymer[21,35]. Whether the 673-nm band is due to a different chlorophyll dimer[21], or represents the monomer[33] is an important unanswered question. The suggestion that the 673-nm band represents a dimer as well comes from the solution spectra shift to 663 nm in many solvents[29], which may then be taken to represent the monomer[21]. In general about 20% of the red absorption is in the 695-nm band; the ratio of 673-nm to 683-nm absorption may vary from 3:1 to 1:3 depending on plant and growing condition[11,17].

For simplicity let us now assume that all the chlorophyll is in dimer form. Placing 150 dimers per reaction center on a square lattice with twice 225 Å^2 area per dimer, one obtains a mean dimer spacing of 23.5 Å instead of the 15-Å monomer spacing (Section IV). The excitation requires on the average 268 steps to reach the trap (Eqn 9 with $N = 150$). The overlap integral (Eqn 7) should not change too much; thus if we use again $R_0 = 65.1$ Å and $\tau_0 = 15.2$ ns, we obtain a nearest neighbor transfer rate of $0.30 \cdot 10^{11}$ s^{-1}, and a mean trapping time of 9 ns (Eqn 8). This trapping time is too large by a factor of about 10, probably since the natural lifetime of chlorophyll *a* dimers is smaller than the monomer lifetime of 15.2 ns corresponding to a smaller fluorescence efficiency[33,36].

VI. ENERGY TRANSFER BETWEEN TWO LAYERS

The transfer of energy from one layer to the next nearest layer may be important since it might represent the possibility of energy transfer from System II to System I. If we take the bilayer model (Fig. 1) with a membrane thickness[23] of 40 Å, and assume the porphyrin center is on each side (15/2) sin 48° (Å) inside the membrane surface (Figs 1 and 2), then the effective thickness for energy transfer between two layers is $d = 29$ Å. On the basis of the thylakoid model by Kreutz[21] one finds

$d=76$ Å and from the model by Calvin[24] $d=162$ Å. The problem of the exact structure of the thylakoid membrane probably still needs further clarification.

The energy transfer rate between two layers by the Förster mechanism is

$$W_a = C \int_0^\infty \gamma\rho(\rho^2+d^2)^{-3}d\rho = C\gamma_a/4d^4 \tag{10}$$

where $C=2\pi\sigma\tau_0{}^{-1}R_0{}^6$ and $\sigma=$density of chlorophyll monomer or dimer per unit area, depending on the assumptions made regarding chlorophyll aggregation. ρ is the distance in the plane of the membrane. The angular factor γ is the K^2 given by Eqn 4, and

$$\gamma_a = \tfrac{2}{3}(\sin^2\beta+\tfrac{1}{2}\cos^2\beta)^2 \tag{11}$$

Taking[12,13] $\beta=36.5°$ for the angle the red transition moment makes with the plane of the membrane we find $\gamma_a=0.687$ compared to an angular factor of $\gamma_i=0.647$ for transfer within one plane and an average value of 2/3 for a three-dimensional random arrangement of chlorophylls. The ratio of across-plane to in-plane energy transfer is then

$$W_a/W_i = (\gamma_a/\gamma_i)(r_0/d)^4 \tag{12}$$

An upper limit for this ratio comes about from the assumption that all chlorophyll is in dimer form (Section V), corresponding to a mean dimer spacing of $r_0=23.5$ Å, and the smallest possible choice for the transfer plane separation of $d=29$ Å. In this case the ratio is 0.46 and the across-plane transfer rate is relatively large. However for $d=50$ Å across-plane transfer rate is already down to 5% of the in-plane transfer.

VII. CONCLUSION

We have followed through the assumption that the red transition moment of chlorophyll *a in vivo* makes an angle of 36.5° with the membrane surface, as observed[12,13] by dichroism in black lipid bilayers. This assumption is further strengthened by the weak red dichroism observed *in vivo*. Together with new spectroscopic measurements on chlorophyll *a* in lipid vesicles, this enabled us to calculate a more precise value (65 Å) for the Förster energy transfer parameter, which is in the range of previous estimates. Recent criticism of using the Förster theory is rejected on the basis of previous choices for the chlorophyll nearest neighbor distance. Aggregation effects will further improve the conditions for applying Förster's theory to energy transfer *in vivo*. With a reasonable choice of parameters one may fit the fluorescence lifetime and efficiency data *in vivo*. However, a detailed comparison with experiments is difficult due to present uncertainty about thylakoid structure and chlorophyll aggregation.

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