ORIENTATION OF EMITTING DIPOLES OF CHLOROPHYLL *A* IN THYLAKOIDS

CONSIDERATIONS ON THE ORIENTATION FACTOR IN VIVO

GYÖZÖ I. GARAB, JÓZSEF G. KISS, AND LÁSZLÓ A. MUSTÁRDY, *Institute of Plant Physiology, Biological Research Center, Hungarian Academy of Sciences, H-6701 Szeged, Hungary*

MICHEL MICHEL-VILLAZ, *Laboratoire de Biophysique Moleculaire et Cellulaire, Department de Recherche Fondamentale, Centre d'Etudes Nucléaires de Grenoble, 85X, F 38041, Grenoble, France*

ABSTRACT Orientation angles of five emitting dipoles of chlorophyll *a* in thylakoids were estimated from low temperature fluorescence polarization ratio spectra of magnetically oriented chloroplasts. A simple expression is given also for the evaluation of data from linear dichroism measurements. It is shown that the Q_y dipoles of chlorophylls lie more in the plane of the membranes and span a larger angular interval than was previously thought. Values for the orientation factor are calculated using various models corresponding to different degrees of local order of the Q_y dipoles of chlorophylls in the thylakoid. We show that the characteristic orientation pattern of the Q_y dipoles of chlorophylls in the membrane, i.e., increasing dichroism toward longer wavelengths, may favour energy transfer between the antenna chlorophylls as well as funnel the excitation energy into the reaction centers.

INTRODUCTION

Polarization spectroscopy of oriented chloroplasts has contributed considerably to our knowledge of the molecular structure of photosynthetic membranes (for a review see reference 1). In studying the Q_y dipoles of chlorophylls, extensive investigations have revealed a characteristic orientation pattern, namely that chlorophyll *b* tilts out of the plane of the membrane (2, 3). The corresponding dipoles of chlorophyll *a* lie rather more in the plane of the membrane than out, and the dipoles tend to lie more in the plane of the membrane with increasing wavelength (4). The smallest angles with respect to the plane (i.e., the largest angles with respect to the normal of the membrane) are constrained by the transition dipoles of the reaction centers of the two photosystems (5, 6). The orientation angles of the different red absorption and emission dipoles of chloroplasts, however, are not yet known. (For the red dipoles in the antenna and the reaction center, calculations are usually based on idealized membrane planes resulting in an angle of ~60°, with respect to the normal of the membrane.)

A thorough analysis of the shape of chloroplasts in algae, the mechanism of magnetic orientation, and an evaluation of measurements of saturation-polarization ratios have recently

---

Dr. Kiss' permanent address is the Depertment of Oto-Rhino-Laryngology, University Medical School, H-6701 Szeged, Hungary.

been published by Knox and Davidovich (7). These authors concluded that the dipoles emitting at 685 nm constrained an angle of ~75° with the normal of the membrane.

One of the aims of the work presented in this paper was to evaluate our low-temperature fluorescence-polarization ratio spectra to provide a resolution of the orientation angles of at least five emitting dipoles (8, 9). In our calculations, which are based on the kind and degree of magnetic orientation of chloroplasts as observed by light microscopy, we took into account both the characteristic geometry of thylakoids and the imperfect orientation of the chloroplast population. Because the fluorescence polarization ratio may change from sample to sample, we took special care to estimate the different parameters of the experimental material under conditions as close as possible to those in which polarization measurements were carried out.

After determining the orientation angles of the different emitting and absorbing dipoles, the value of the orientation factor (proportional to the rate of energy transfer between chlorophylls in vivo) was estimated in model calculations supposing different degrees of local order.

PLANT MATERIAL AND EXPERIMENTAL METHODS

Seedlings of maize (*Zea mays* KSC 360 L) were grown in the greenhouse for 8–12 d. Chloroplasts were isolated from the mesophyll of the first leaves as described (10) and were suspended and diluted in the isolation buffer (0.3 M sucrose, 0.05 M phosphate, pH 7.2, and 0.01 M KCl) or in a glycerol-buffer (2:1 vol/vol) mixture.

Low temperature (−140°C) fluorescence-polarization measurements with oriented chloroplasts were carried out in a set-up described earlier (11). To avoid large reabsorption and/or scattering artifacts, the absorbance of the samples was adjusted to ~0.05–0.1 at 678 nm.

For measurements at room temperature, a chloroplast suspension in a 1-mm cuvette, set in the electromagnet, was excited with naturally polarized light focused onto the front side of the cuvette. Blue spectral bands of an HBO 200 high-pressure mercury arc lamp were selected by a liquid filter (5-cm cuvette containing 0.5 M $CuSO_4$ solution). Fluorescence was observed from the front side of the cuvette in a direction perpendicular to the magnetic field. The intensity of the polarized fluorescence measured with an RCA 31034-a photomultiplier (RCA Electro-Optics & Devices, RCA Solid State Div., Lancaster, Pa.) equipped with an interference filter (Oriel 680.5-nm, 10-nm halfbandwidth Oriel Corp. of America, Stamford, Conn.), was recorded with polaroid sheets in positions transmitting vertically (F_v) and horizontally (F_h) polarized light. For the oriented samples, the fluorescence polarization ratio (FP) F_v/F_h corresponded to the polarization ratio PR = $I_\perp/I_\parallel$ (7) or to $FP = I_\parallel/I_\perp$ (12), when related to the direction of magnetic field or to the alignment of the membranes (see below), respectively. In this paper $I_\parallel$ will denote the polarized fluorescence intensity emitted in a plane parallel, $I_\perp$ in a plane perpendicular to the idealized membrane plane.

To monitor the orientation of chloroplasts, a microscope was fitted into the electromagnet and the same field strength (1.2 T = 12 kG) was applied as with FP measurements.

For electron microscopy, chloroplasts in isolation medium or in glycerol buffer were fixed in 2% glutaraldehyde for 4 h with the magnetic field off and on. They were then dehydrated, embedded, sectioned, and stained by conventional methods (13). Micrographs, with magnifications between 10,000 and 30,000, were prepared using a JEOL 100 B electron microscope (JEOL LTD, Tokyo, Japan).

ORIENTATION OF CHLOROPLASTS IN A MAGNETIC FIELD

The manner and actual degree of orientation of chloroplast population in suspension was monitored using the light microscope set in the electromagnet. It may be clearly seen in Fig. 1 that the "equatorial plane" of the lens-shaped chloroplasts tends to be perpendicular to the

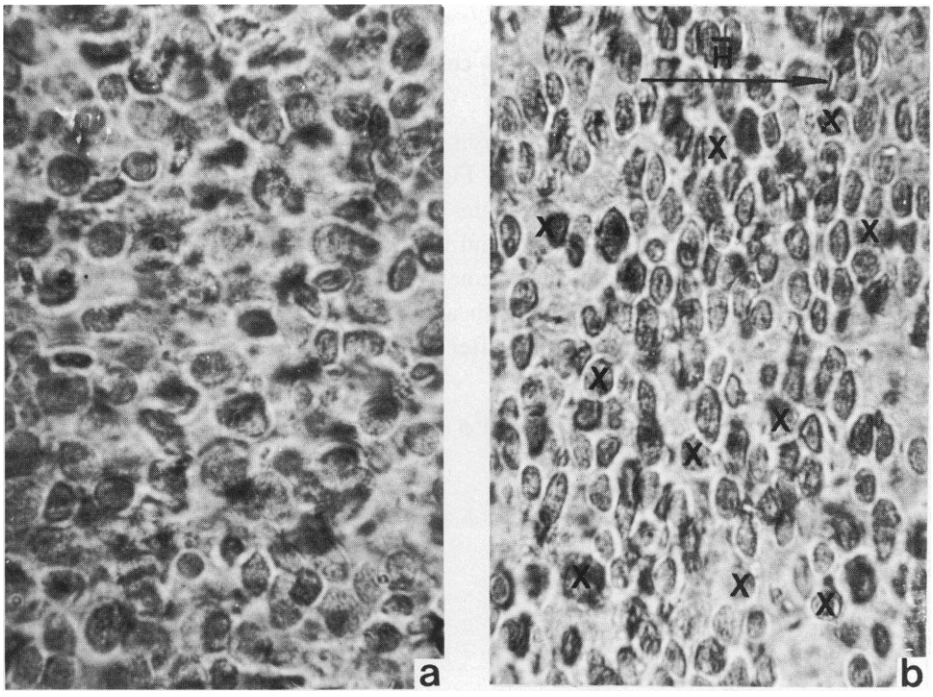

FIGURE 1 Chloroplast suspension in the light microscope at 0 (*a*) and 1.2 T (*b*) magnetic field strengths. x, chloroplasts nonoriented in the magnetic field. The arrow indicates the direction of the magnetic field vetor $\overline{H}$.

magnetic field. This observation is in accord with earlier suggestions (14) and experimental results (15, 16). It was observed, however, that the orientation of the chloroplast population was never perfect at the field strengths (up to 1.4 T) applied in these experiments. A statistical evaluation of micrographs showed that ~70% of the total whole chloroplasts in a suspension were oriented by the magnetic field. In our experience the imperfect orientation may arise from plastids that are attached to each other, or from swollen chloroplasts. It was also observed that, when chloroplasts were gradually disrupted into fragments with a homogenizer, the *FP*, measured at room temperature, dropped gradually to 1.0 from an initial level of about 1.35. This can be explained by a decrease in the degree of orientation, which in turn, can be accounted for by a low torque due to the small size of fragments or to sphericity of the particles. The chloroplast suspension was examined in the light- and electron microscope and it was found that, during the usual isolation procedure, ~10% of the chloroplasts were broken into fragments.

Idealized and Realistic Membrane Patterns

As a first approximation, dichroism data may be interpreted in terms of an idealized system: 100% orientation and membrane sheets that are oriented perpendicular to the magnetic field vector. With dipoles of angle ϕ to the normal of the membrane, the fluorescence intensities parallel and perpendicular to the membrane are proportional to

$$I_{\parallel} = \tfrac{1}{2} \sin^2\phi \qquad (1)$$

and

$$I_{\perp} = \cos^2\phi, \tag{2}$$

respectively.

The intensity of fluorescence originating from the nonoriented chloroplasts and particles is proportional to $I_{\parallel} = I_{\perp} = 1/3$. It is shown in Fig. 2 *a* that a contribution from this type of fluorescence can dramatically change the values of *FP* expected for an idealized system.

For a given angle ϕ between the dipoles and the normal of the membrane, *FP* values also depend on the percentage of fluorescence originating from the marginal part of the thylakoids (Fig. 2 *b*). Margins were approximated with a hemitorus (Fig. 3). For a system of such geometry, the corresponding intensities—after averaging on $\lambda \in [0, 2\pi]$ and $\gamma \in [-\pi/2, \pi/2]$—are proportional to

$$I_{\parallel} = 3/8 \sin^2\phi + 1/4 \cos^2\phi \tag{3}$$

and

$$I_{\perp} = 1/4 \sin^2\phi + 1/2 \cos^2\phi, \tag{4}$$

respectively.

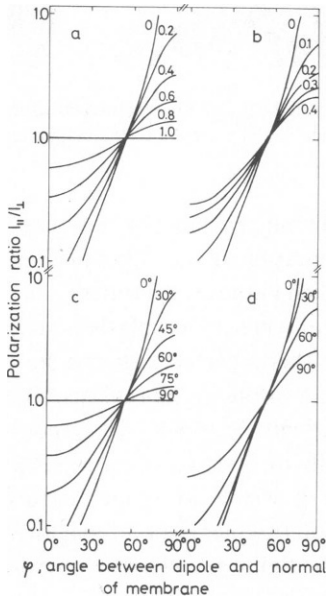

FIGURE 2

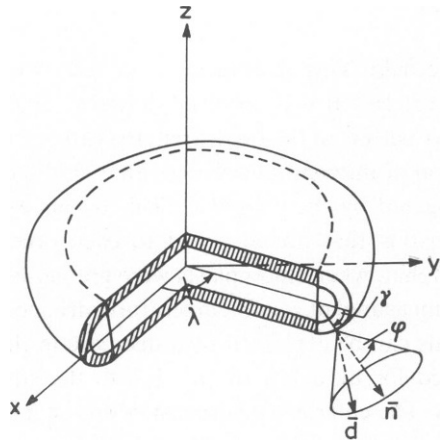

FIGURE 3

FIGURE 2 *FP* as a function of orientation angle between the emitting dipole and the normal of membrane. Idealized oriented planar membrane sheets with various proportions of nonoriented membranes (*a*) and marginal surfaces (*b*). (*c*) Oriented membranes having a spherical section geometry, characterized by angle δ, and (*d*) membranes of $\delta \in [0, \delta_m]$ in oriented chloroplasts of δ_m sphericity (see Fig. 4).

FIGURE 3 Illustration of the marginal part of the thylakoids, oriented parallel to the *x-y* plane, containing dipoles ($\bar{d}$) oriented at angle ϕ with respect to the normal ($\bar{n}$) of the membrane. The fluorescence intensities $I_{\parallel}$ and $I_{\perp}$ were measured with polarizers aligned along the *y* and *z* axis, respectively.

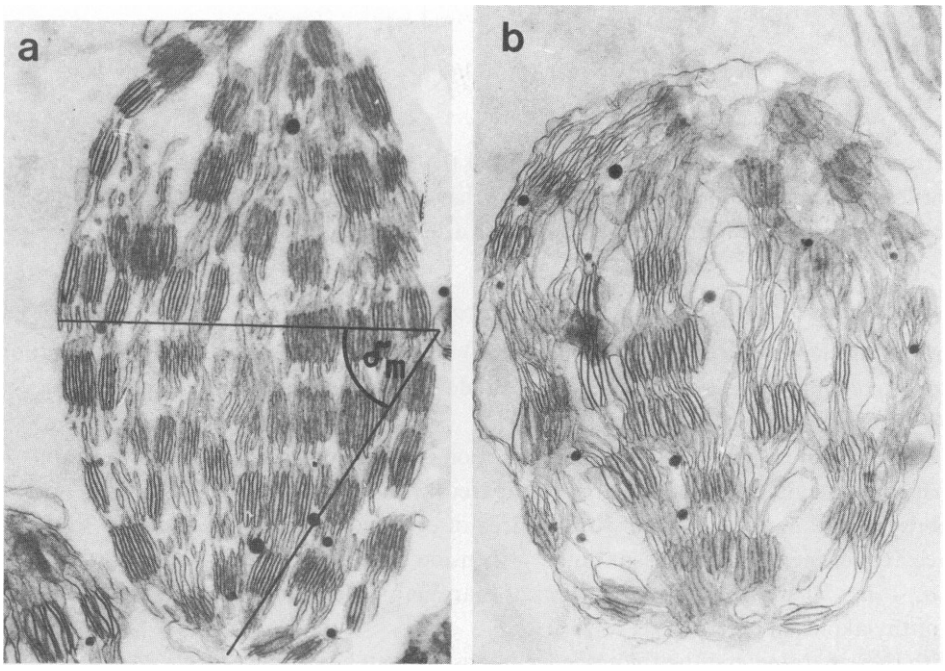

FIGURE 4 Typical electron micrograph of chloroplasts fixed in phosphate buffer with 0 T (*a*), and in a 2:1 mixture of glycerol and buffer with 1.2 T magnetic field strength (*b*). Determination and meaning of δ_m is also illustrated.

After examining numerous electron micrographs of isolated chloroplasts, we concluded that the geometry of thylakoid lamellae could be approximated fairly well by spherical sections (Fig. 4). For this case a shape parameter b (7) can be defined as $b \equiv \langle \cos^2\mu \rangle = \frac{1}{3}(1 + \cos\delta + \cos^2\delta)$, where the average of $\cos^2\mu$ is calculated for $\mu \in [0, \delta]$ (δ is the angle of sphericity of the spherical section).

$$I_\parallel = \tfrac{1}{4}(1 + b)\sin^2\phi + \tfrac{1}{2}(1 - b)\cos^2\phi \tag{5}$$

and

$$I_\perp = \tfrac{1}{2}(1 - b)\sin^2\phi + b\cos^2\phi, \tag{6}$$

respectively.

Eqs. 5 and 6 are consistent with the corresponding expressions of Knox and Davidovich (cf. Eqs. 15 and 24 in reference 7).

Moreover, it was found that, in chloroplasts characterized by angle δ_m, lamellae of angle δ were present with equal probabilities between 0 (at the equatorial) and δ_m (at the envelope). Figs. 2 *c* and *d* show the effect of sphericity on *FP* (ϕ) with single lamellae of angle δ, and with oriented chloroplasts of angle δ_m, respectively. For the latter case the corresponding intensities are

$$I_\parallel = \tfrac{1}{12}(5 + \cos\delta_m) - \tfrac{1}{4}(1 + \cos\delta_m)\cos^2\phi \tag{7}$$

and

$$I_{\perp} = \tfrac{1}{6}(1 - \cos\delta_m) + \tfrac{1}{2}(1 + \cos\delta_m)\cos^2\phi, \tag{8}$$

respectively.

Here, as in all our calculations, we have used the assumption that fluorescence intensity is proportional to membrane surface area. We have also taken into account that the same number of lamellae are found at equal intervals around any $\delta \in [0, \delta_m]$.

Interpretation of FP and Linear Dichroism

The resulting fluorescence intensities ($I_{\parallel}$ and $I_{\perp}$) may be described as a linear combination of the corresponding intensities obtained for a random suspension and those expressed in Eqs. 3 and 4; 7 and 8.

Weighting factors are as follows: a_n, proportion of nonoriented chloroplasts; a_m, proportion of marginal membrane surface area compared with the total surface area of oriented membranes; and a_o, proportion of surface area of oriented nonmarginal lamellae to the total surface area of membranes present in the suspension. Accordingly, $a_m = (1 - a_n)\,a'_m$ and $a_o = 1 - a_n - a_m$, where a'_m is the proportion of marginal surface compared to the total surface area of thylakoids in chloroplasts.

With these parameters, the fluorescence intensities can be expressed as

$$I_{\parallel} = \tfrac{1}{3}a_n + \tfrac{3}{8}a_m + \tfrac{1}{12}a_o(5 + \cos\delta_m) - \tfrac{1}{4}[\tfrac{1}{2}a_m + a_o(1 + \cos\delta_m)]\cos^2\phi \tag{9}$$

$$I_{\perp} = \tfrac{1}{3}a_n + \tfrac{1}{4}a_m + \tfrac{1}{6}a_o(1 - \cos\delta_m) + \tfrac{1}{2}[\tfrac{1}{2}a_m + a_o(1 + \cos\delta_m)]\cos^2\phi. \tag{10}$$

With a given set of parameters a_n, a'_m, and δ_m, which can be determined by a microscopic examination of the material, the angle ϕ between the emission dipole and the normal of the membrane may be determined from the *FP* exhibited by the corresponding emission band.

A common method of interpretation of the dichroism of absorbance is the determination of the orientation parameter, $S \equiv (A_{\parallel} - A_{\perp})/3A$, from the spectra of linear dichroism ($LD \equiv A_{\parallel} - A_{\perp}$) and absorption ($A$), respectively. (For an idealized membrane system, i.e., 100% orientation and membrane sheets oriented perpendicularly to the magnetic field vector, we have $S = (1 - 3\cos^2\phi)/2$. According to the relationships $A_{\parallel} = 3AI_{\parallel}$ and $A_{\perp} = 3AI_{\perp}$, the corrected values of the orientation parameter, S, can be calculated by using Eqs. 9 and 10 to yield

$$S = \frac{1 - 3\cos^2\phi}{2}(1 - a_n)\left[\frac{1}{4}a'_m + (1 - a'_m)\frac{1 + \cos\delta_m}{2}\right]. \tag{11}$$

The experimentally determined value of the orientation parameter of an absorption dipole (as with *FP*) depends not only on ϕ, but also on the degree of orientation, the proportion of marginal part, and on the mean sphericity of chloroplasts.

When fluorescence or absorption at a given wavelength cannot be attributed to a single spectroscopic band, but, as is usually the case, bands overlap each other, the polarization ratio or linear dichroism are composite. Thus, for example, the *FP* with emission bands contribu-

ting to the fluorescence at wavelength λ may be described as

$$FP(\lambda) = \frac{\sum_{i-1}^{k} c_i I_{\parallel}(\phi_i)}{\sum_{i-1}^{k} c_i I_{\perp}(\phi_i)}, \qquad I(\lambda) = \sum_{i-1}^{k} c_i I(\lambda_i), \tag{12}$$

where c_i is the relative intensity of the ith bands, $I(\lambda)$ is the fluorescence intensity, and $I(\lambda_i)$ denotes the spectral distribution of the ith band.

According to Eq. 12 a constant value of *FP* is expected with a single fluorescence band. An overlap between different bands with different orientation of dipoles, however, results in a more complex *FP* spectrum. On the other hand, a structured *FP* spectrum may help to reveal the composite character of fluorescence emission (8, 9).

Similar considerations may be applied to linear dichroism spectra. The composite character of the broad absorption band is reflected in deviations of linear dichroism spectra from the spectral distribution of absorbance that result in a wavelength-dependent orientation parameter (4).

A complication in the interpretation of *FP* spectra would arise if the different fluorescence bands originated from different structural entities with different correction factors (a_n, a_m,

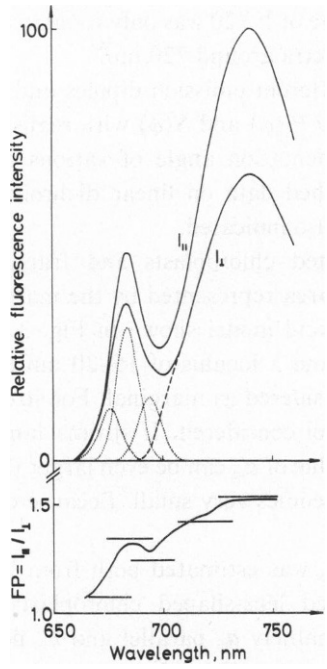

FIGURE 5 Low-temperature polarized fluorescence intensity and (*FP*) spectra of oriented mesophyll chloroplasts of maize (upper and lower part, respectively). Bands represented by dotted lines were obtained by deconvolution of the emission of spectrum $I_{\perp}$. Thin constant lines in the *FP* spectra correspond to the polarization ratios obtained for the different bands emitting with maxima at around 675, 685, 695, 720, and 735 nm, respectively.

δ_m). This case, and other possible factors, e.g., local variations of polarizability around stacked regions (17), different orientation of spectrally identical dipoles from different pigment-protein complexes, etc. cannot be considered here because there are no reliable data.

Estimation of Orientation Angles of Chlorophyll a-Emitting Dipoles

Because of overlapping emission bands, the low-temperature *FP* spectra of oriented chloroplasts exhibited a composite character (Fig. 5). Because the emissions between 680 and 690 nm, and 730 and 760 nm were dominated by the fluorescence bands F 685 and F 735, respectively, the measured *FP* values were almost constant and characterized the corresponding dipoles reasonably well. In other spectral regions, however, where the overlap of bands was large, the "true" *FP* values of the hidden bands could not be directly determined. As an approximation, a simple deconvolution into Gaussian components was applied between 660 and 710 nm for both polarized fluorescence spectra ($I_\parallel$ and $I_\perp$). The maximum and halfbandwidth of the fluorescence band F 685 were determined from the derivative of the logarithmic spectrum. The relative intensity of this band was iterated with the assumption that both the shorter and longer wavelength overlapping bands (named F 675 and F 695, respectively) could be characterized with the same value of halfbandwidth (12 nm) as was found for F 685. *FP* values of the minor bands were obtained from the ratio of the corresponding band intensities. This method was not applied at longer wavelengths where, because of vibration of the shorter wavelength bands, a considerable distortion could be expected. Therefore, the *FP* value of F 720 was only roughly estimated from the small plateau regularly observed in the *FP* spectra around 720 nm.

The ϕ angles between the different emission dipoles and the normal of membranes were estimated using Eqs. 9 and 10. *FP* (ϕ) and $S(\phi)$ with realistic values of the parameters, are shown in Fig. 6, where the orientation angle of various dipoles, estimated from our *FP* measurements and from published data on linear dichroism and the polarization ratio of absorbance changes (2–6), are also indicated.

The proportion of nonoriented chloroplasts and fragments, a_n, was about 0.4. The percentage of the total surface area represented by the marginal surface, a'_m, was calculated according to the granum thylakoid model shown in Fig. 3. With a diameter of 500 nm, a membrane thickness of 7 nm, and a loculus of 15–20 nm (Fig. 3), about 20% of the total surface of thylakoid may be considered as marginal. For stroma thylakoids, the value of this parameter depends on the model considered. If stroma lamellae are thought of as narrow bridges connecting grana, the value of a'_m can be even larger than with grana. In contrast, with plastid-size disk lamellae, a'_m becomes very small. Because of these uncertainties, we used a value of $a'_m = 0.2$.

The sphericity parameter, δ_m, was estimated both from light- and electron micrographs. Two main axes of the oriented lens-shaped chloroplasts were measured on numerous micrographs such as Fig. 1 *b*, namely a_i, parallel and b_i, perpendicular to the field vector. Taking the mean value of the parameter $\delta_{mi} = 2 \arctan a_i/b_i$ results in an angle of 55° (68% of chloroplasts can be characterized by angle δ_m between 47 and 63°). A similar value of δ_m was also obtained from an analysis of electron micrographs of isolated plastids.

From electron micrographs of chloroplasts in 67% glycerol (Fig. 4 *b*) we found that, due to intensive swelling and a loosening of the ultrastructure, the mean of δ_m in 67% glycerol increased to ~70°. This is in good agreement with the observation that, upon addition of

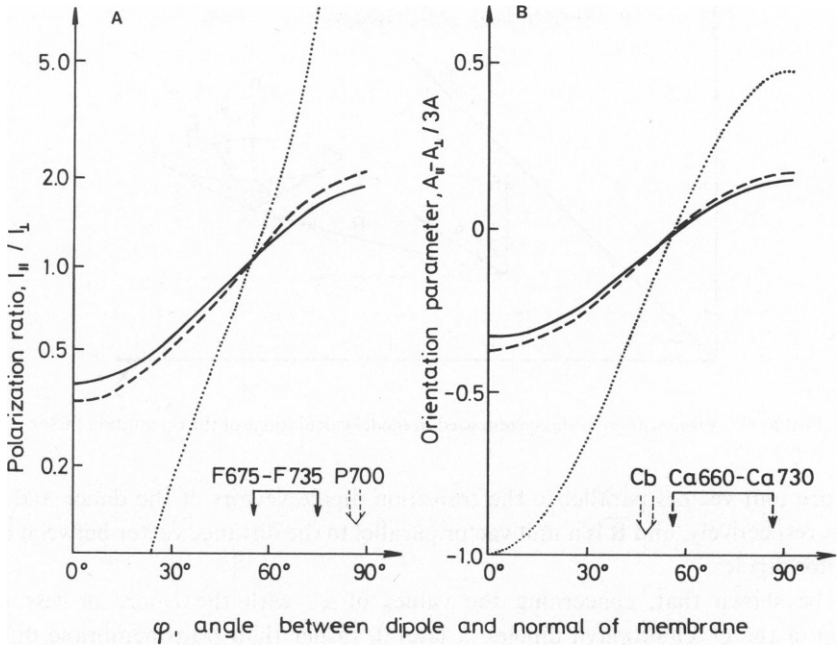

FIGURE 6 Polarization ratio (*A*) and orientation parameter (*B*) as a function of the dipole-angle with respect to the normal of membrane. Dotted, dashed, and solid lines correspond to an idealized orientation- and membrane-pattern, chloroplast oriented in phosphate buffer, and in a 2:1 mixture of glycerol and buffer, respectively. Set of parameters (a_n, a'_m, δ_m) were (0, 0, 0), (0.4, 0.2, 55°) and (0.4, 0.2, 70°), respectively. The orientation-angles of different emitting dipoles of chlorophyll *a* were estimated from our low-temperature fluorescence polarization measurements, that of absorbing dipoles of chlorophyll *a* and *b* and of P-700 were estimated from data in the literature (2–6). In this latter case it was supposed that orientability and structure of the samples used were similar to those in our experiments.

glycerol to a chloroplast suspension, the room temperature *FP* at 680 nm decreased to 1.30 from an initial value of 1.38. It should be noted that, upon cooling, *FP* at 680 nm remains constant over a wide temperature range (down to −140°C) if the sample is glassy (free of cracks or "clouds").

On the other hand, a comparison of electron micrographs of chloroplasts in 67% glycerol, fixed at 0 and 1.2 T, showed that the magnetic field did not cause any appreciable change in the ultrastructure of the plastids.

The results of our calculations, depicted in Fig. 6, show that: (*a*) in accordance with recent estimates of the mean orientation angle of chlorophyll *a* (7, 18), the Q_y dipoles lie more in the plane of the membrane and (*b*) they span a larger angular interval than was previously thought.

The Orientation Factor with Different Degrees of Local Order

The orientation factor (κ^2), determining the dependence of the Förster type of resonance interaction (19) between two electric dipoles after their mutual orientation, may be defined as

$$\kappa^2 = [(\bar{\mathbf{A}}, \bar{\mathbf{B}}) - 3\,(\bar{\mathbf{A}}, \bar{\mathbf{R}})\,(\bar{\mathbf{B}}, \bar{\mathbf{R}})]^2 \qquad |\bar{\mathbf{A}}| = |\bar{\mathbf{B}}| = |\bar{\mathbf{R}}| = 1. \tag{13}$$

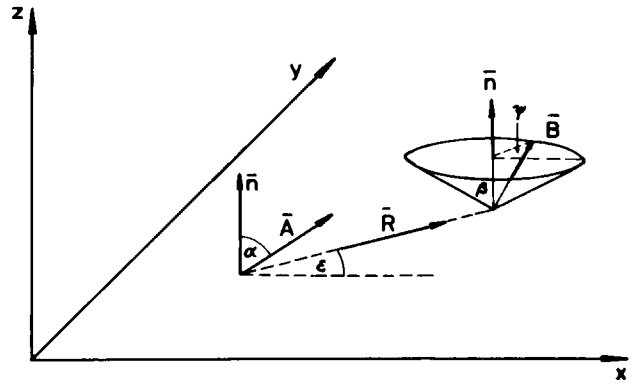

FIGURE 7 Visualization of the system used in model calculations of the orientation factor.

$\bar{\mathbf{A}}$ and $\bar{\mathbf{B}}$ are unit vectors parallel to the transition dipole vectors of the donor and acceptor molecules, respectively, and $\bar{\mathbf{R}}$ is a unit vector parallel to the distance vector between the donor and acceptor dipoles.

It can be shown that, concerning the values of κ^2, with the (more or less in-plane) orientation of the Q_y chlorophyll dipoles, a lateral, rather than transmembrane diffusion of excitation is preferred. (If complete optimalization of κ^2 was reached in vivo, energy transfer should occur only between uniformly oriented dipoles and the direction of energy transfer would cross the membrane with the same angle as the orientation angle of the dipoles.)

As an approximation we calculated the average values of κ^2 for the lateral diffusion of excitation energy along the membrane plane. It is now well established that the chlorophyll molecules are fixed on proteins. The distances between the pigment-protein complexes, however, do not exclude energy-transfer interactions (20). Moreover, experimental evidence showing the existence of energy-transfer processes between photosynthetic units or between pigment-protein complexes (for a review, see 21) supports the idea that a lateral diffusion of excitation along the membrane plane may be important in vivo.

In Fig. 7 we present a donor emission-dipole vector $\bar{\mathbf{A}} = (\sin\alpha, 0, \cos\alpha)$ oriented with an angle α with respect to the normal of the membrane, and a set of acceptor dipoles oriented with angle β, $\bar{\mathbf{B}} = (\sin\beta\cos\psi, \sin\beta\sin\psi, \cos\beta)$. For the lateral diffusion of excitation energy, the distance vector can be given as $\bar{\mathbf{R}} = (\cos\epsilon, \sin\epsilon, 0)$.

From values of the polarization ratio or linear dichroism obtained with oriented samples, α and β may be estimated satisfactorily. Regarding the values of angles ϵ and ψ, however, assumptions must be made concerning the local organization of the pigments. Values of the orientation factor, as a function of α and β, were calculated in three different models assuming different degrees of local order.

In the first model, corresponding to a low degree of local order, the excitation energy of $\bar{\mathbf{A}}$ (α) is transferred to an acceptor $\bar{\mathbf{B}}$ (β) with the same probability, regardless of its position, characterized by $\epsilon, \psi \in [0, 2\pi]$. In other words, in this model, the local order is so low that the acceptor dipoles are oriented with angle β, but otherwise they are arranged at random around the donor molecule. The orientation factor, after averaging on ϵ and ψ, can be expressed as

$$\langle \kappa^2 \rangle_{\epsilon,\psi} = \cos^2\alpha \cos^2\beta + \tfrac{9}{4} \sin^2\alpha \sin^2\beta. \tag{14}$$

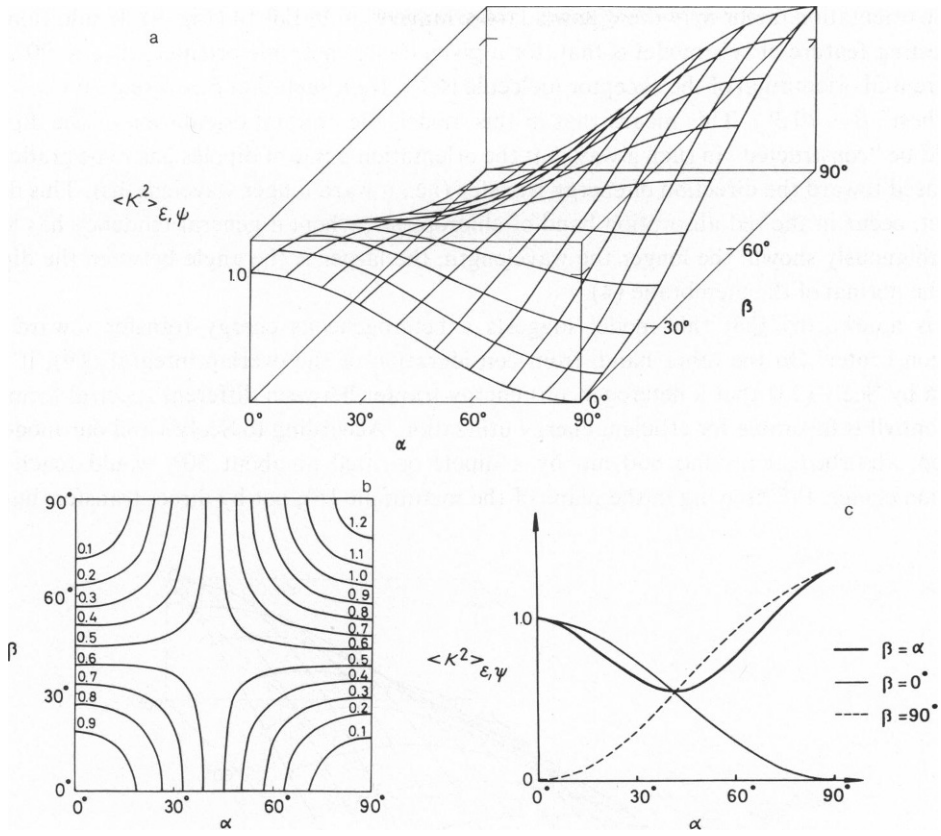

FIGURE 8 Orientation factor in a "loosely organized system" as a function of the orientation angles α and β of donor and acceptor dipoles, respectively. *a*, three-dimensional; *b*, contour plot; *c*, characteristic sections of the $\langle \kappa^2 \rangle_{\epsilon,\psi}$ surface defined by Eq. 14.

This model is analogous to the one described by Tweet et al. (22), in which the donor and the acceptor dipoles were constrained to make a constant angle with the normal to the membrane, but were free to rotate through their azimuthal angle about the vertical axis.

The dependence of $\langle \kappa^2 \rangle_{\epsilon,\psi}$ on α and β is shown in Fig. 8 *a* and *b*. The orientation factor can range from 0 to 1.25; if the donor is not in the plane of the membrane, to assure the highest possible rate of energy transfer, the acceptor should make an angle 0° or 90° with the normal of the membrane. In more restricted models, higher local orders that influence the flow of excitation energy in photosynthetic units can be arbitrarily assumed.

In our second model, it is assumed that the acceptor dipoles are positioned at a favourable azimuthal angle, but otherwise the probability of transfer is the same for any $\epsilon \in [0.2\pi]$. In other words, ψ is supposed to be optimalized at each ϵ, but a symmetrical diffusion of excitation is allowed along the x-y plane around the donor molecule. In this case,

$$\kappa^2_{\epsilon(\psi)} = (\cos\alpha \cos\beta + \sin\alpha \sin\beta \sqrt{4 - 3\sin^2\epsilon})^2 \tag{15}$$

and

$$\langle \kappa^2 \rangle_{\epsilon(\psi)} = \cos^2\alpha \cos^2\beta + 0.77 \sin 2\alpha \sin 2\beta + \tfrac{5}{2} \sin^2\alpha \sin^2\beta. \tag{16}$$

The orientation factor may then show a larger range than in Eq. 14 (Fig. 9). In addition, an interesting feature of this model is that, for a given donor molecule oriented at $\alpha \neq 90°$, the preferential orientation of the acceptor molecule is $\beta = f(\alpha)$, such that $\beta > \alpha$ (e.g., for $\alpha = 60°$ the "best" $\beta = 70.3°$). This means that in this model, the optimal orientation of the dipoles should be "constructed" in such a way that the orientation angle of dipoles has to be gradually increased toward the direction of energy transfer (i.e., toward longer wavelengths). This does, in fact, occur in the red absorption band of chloroplasts, where a general tendency has been unambiguously shown: the longer the wavelength, the larger is the angle between the dipole and the normal of the membrane (4).

It is noteworthy that this model suggests a heterogeneous energy transfer toward the reaction center. On the other hand, from consideration of the overlap integral (19), it was shown by Seely (23) that a heterogeneous energy tranfer between different spectral forms of chlorophyll is favorable for efficient energy utilization. According to Seely's and our model, a photon, absorbed at around 660 nm by a dipole oriented at about 60°, would reach the reaction center, P-700, lying in the plane of the membrane (5), not by direct transfer, but by

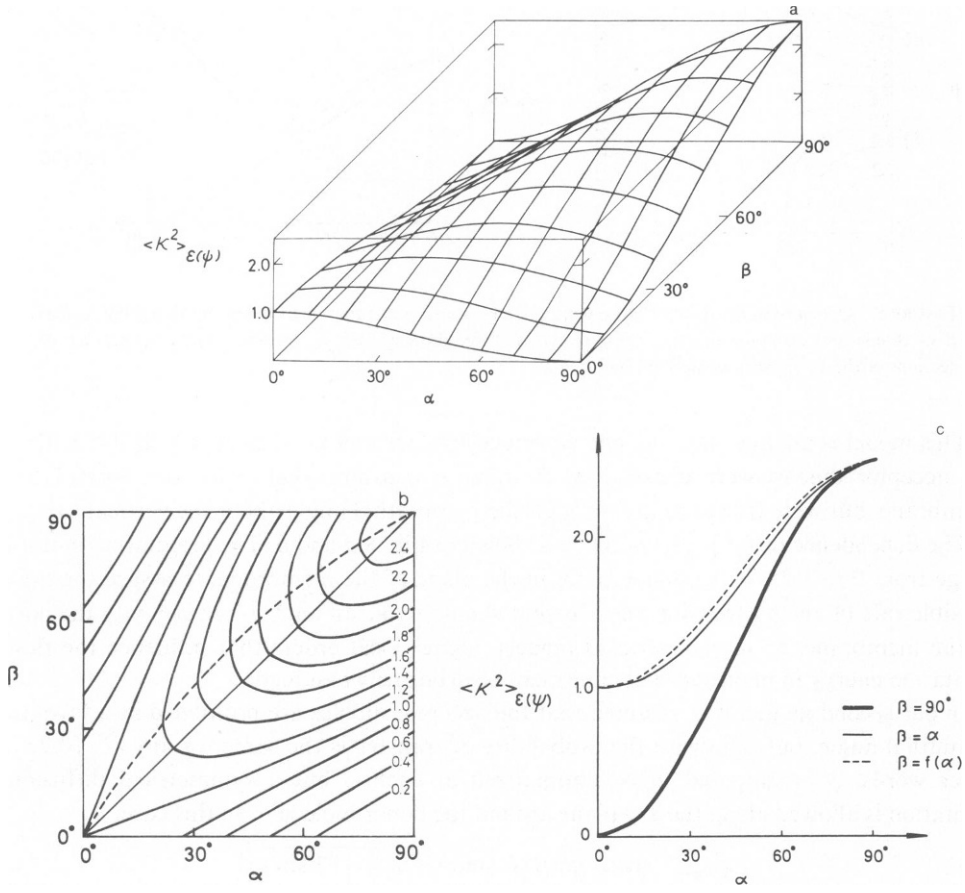

FIGURE 9 Orientation factor in a "moderately organized system" *a*, *b*, and *c*, as in Fig. 8, but using Eq. 16. Dashed line in the contour plot shows $\beta = f(\alpha)$.

passing through several absorption bands between 660 and 700 nm, with a gradually increasing orientation angle of the corresponding dipoles (with respect to the normal of the membrane).

The same type of transport of excitation energy is advantageous in our third model also. In this model we supposed a very high degree of local order: the molecular organization is good enough for $\overline{\mathbf{A}}$ (α) to find a $\overline{\mathbf{B}}$ (β) with an optimal position. In this case there would be linear transport along the x axis (Fig. 7); the orientation factor may be expressed as:

$$\langle \kappa^2 \rangle_{\epsilon(\psi)\mathrm{lm}} = (\cos\alpha \cos\beta + 2\sin\alpha \sin\beta)^2, \tag{17}$$

and the corresponding plots are shown in Fig. 10.

The models described above are limited by the conditions for validity of dipole-dipole approximation of resonance-energy transfer, i.e., transfer over distances not < 2.5 nm (24). At distances < 2.5 nm, which may often be the case when there is a high local concentration of chlorophylls, a more complex picture must be considered. The dipole-dipole approximation would have to be corrected, a procedure recently carried out by Chang (25). Although such a correction (taking into account the possible shorter distances and the relative orientation of Q_y dipoles of chlorophylls) was not attempted, some qualitative statements can be made: (*a*) if

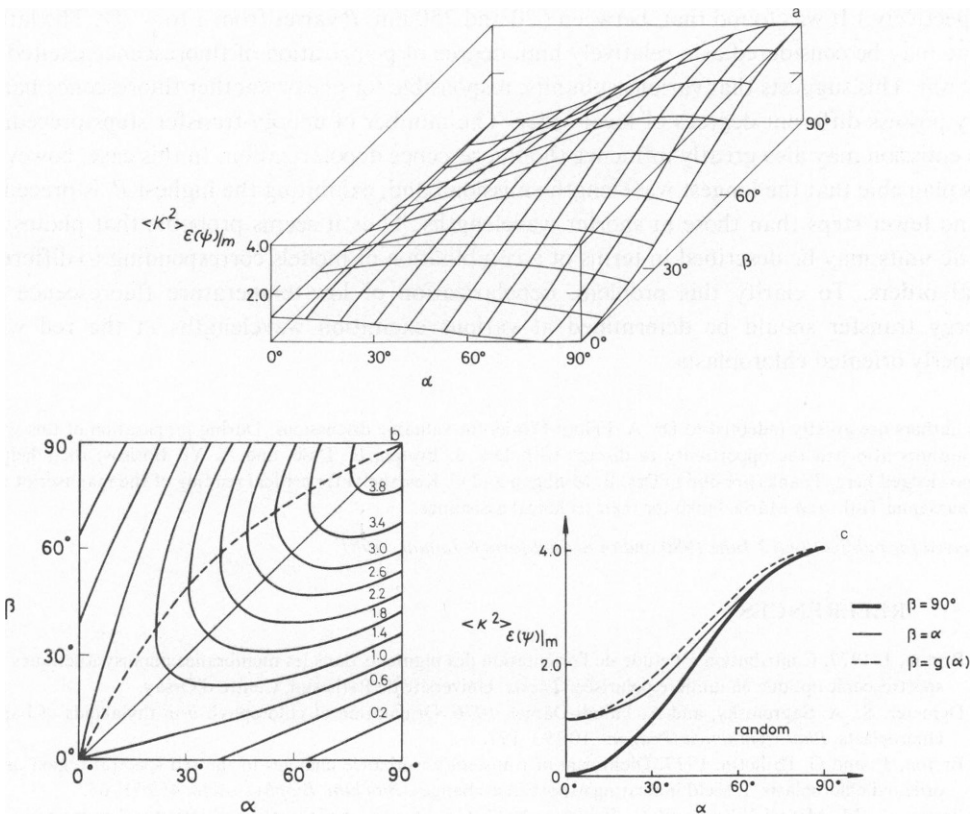

FIGURE 10 Orientation factor in a "system of high local order." *a*, *b*, and *c* as in Fig. 8, but using Eq. 17. Dashed lines in the contour plot represent $\beta = g(\alpha)$.

one compares the two coplanar arrangements (cf. reference 26, Fig. 3; also cited in reference 27) and relates them to the mostly in-plane orientation of chlorophyll *a* dipoles, it is clear that, when the dipoles lie in the $\overline{\mathbf{R}}$ direction, a lateral diffusion of excitation can be more efficient than with the other type of coplanar array, where transfer would occur in the transmembrane direction. (*b*) Furthermore, values of the orientation factor, and hence the significance of the relative orientation of dipoles in determining the energy transfer rate, may be even larger at shorter distances than in the strict validity range of the dipole-dipole approximation of the Förster type of energy transfer.

ORIENTATION FACTOR IN SITU

It is difficult to decide which of these models is applicable to chlorophylls in thylakoids. This question might be answered by an investigation of depolarization by energy transfer in chloroplasts oriented in such a way that the contribution of orientational anisotropy to the degree of polarization is minimized (27).

In such an arrangement, i.e., when thylakoids are oriented perpendicularly to the direction of polarized excitation and observation, values of $P = (F_v - F_h)/(F_v + F_h)$ have been determined as a function of the wavelength of the low-temperature emission (8). (F_v and F_h denote the fluorescence intensities measured with vertically or horizontally polarized light, respectively.) It was found that, between 670 and 750 nm, P varies from 1 to ~ 5%. The latter value may be considered as a relatively high degree of polarization of fluorescence excited at 442 nm. This suggests that various subunits, responsible for one or another fluorescence band, may possess different degrees of local order. The number of energy-transfer steps preceding the emission may also greatly influence the fluorescence depolarization. In this case, however, it is plausible that the longest wavelength emission band, exhibiting the highest P, is preceded by no fewer steps than those at shorter wavelengths. Thus it seems probable that photosynthetic units may be described in terms of a combination of models corresponding to different local orders. To clarify this problem, depolarization of low-temperature fluorescence by energy transfer should be determined at various excitation wavelengths in the red with properly oriented chloroplasts.

The authors are greatly indebted to Dr. A. Faludi-Dániel for valuable discussions. During preparation of this work the authors also had the opportunity to discuss with Drs. J. Breton, R. Dale, and A. Yu Borisov; their help is acknowledged here. Thanks are due to Drs. B. Mulligan and C. Keszthelyi for critical reading of the manuscript and to Zsuzsanna Tóth, and Mária Jankó for their technical assistance.

Received for publication 12 June 1980 and in revised form 6 January 1981.

REFERENCES

1. Breton, J. 1977. Contribution a l'étude de l'orientation des pigments dans les membranes photosynthétiques par spectroscopie optique en lumière polarisée. *Thesis.* Universite de Paris-sud, Centre d'Orsay.
2. Demeter, S., A. Sagromsky, and Á, Faludi-Dániel. 1976. Orientation of chlorophyll *b* in thylakoids of barley chloroplasts. *Photosynthetica (Prague).* 10:193–197.
3. Breton, J., and G. Paillotin. 1977. Dichroism of transient absorbance changes in the red spectral region using oriented chloroplasts. I. Field indicating absorbance changes. *Biochim. Biophys. Acta.* 459:58–65.
4. Breton, J., M. Michel-Villaz, and G. Paillotin. 1973. Orientation of pigments and structural proteins in the photosynthetic membrane of spinach chloroplasts: a linear dichroism study. *Biochim. Biophys. Acta.* 314:42–56.

5. Breton, J., E. Roux, and J. Whitmarsh. 1975. Dichroism of chlorophyll *a* absorption change at 700 nm using chloroplasts oriented in a magnetic field. *Biochem. Biophys. Res. Commun.* 64:1274–1277.
6. Mathis, P., J. Breton, A. Vermeglio, and M. Yates. 1976. Orientation of the primary donor chlorophyll of photosystem II in chloroplast membranes. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 63:171–173.
7. Knox, R. S., and M. A. Davidovich. 1978. Theory of fluorescence polarization in magnetically oriented photosynthetic systems. *Biophys. J.* 24:689–712.
8. Garab, Gy. I., and J. Breton. 1976. Polarized light spectroscopy on oriented spinach chloroplasts: fluorescence emission at low temperature. *Biochem. Biophys. Res. Commun.* 71:1095–1102.
9. Vasin, Yu.A., and V. N. Verkhoturov. 1979. Issledovanie polarizacii fluorescencii Chlorelly i chloroplastov goroha orientirovannyh v magnitnom pole. *Biofizika.* 24:260–263.
10. Garab, Gy. I., G. Horváth, and Á. Faludi-Dániel. 1974. Resolution of fluorescence bands in greening chloroplasts of maize. *Biochem. Biophys. Res. Commun.* 56:1004–1009.
11. Garab, Gy. I., C. Sundqvist, L. A. Mustárdy, and Á. Faludi-Dániel. 1980. Orientation of short wavelength and long wavelength protochlorophyll species in greening chloroplasts. *Photochem. Photobiol.* 31:491–494.
12. Geacintov, N. E., F. van Nostrand, and J. F. Becker. 1974. Polarized light spectroscopy of photosynthetic membranes in magneto-oriented whole cells and chloroplasts: fluorescence and dichroism. *Biochim. Biophys. Acta.* 347:443–463.
13. Mustárdy, L. A., E. Machowicz, and Á. Faludi-Dániel. 1976. Light-induced structural changes of thylakoids of normal and carotenoid deficient chloroplasts of maize. *Protoplasma.* 88:65–73.
14. Geacintov, N. E., F. van Nostrand, J. F. Becker, and J. B. Tinkel. 1972. Magnetic field induced orientation of photosynthetic systems. *Biochim. Biophys. Acta.* 267:65–79.
15. Clement-Metral, J. 1975. Direct observation of the rotation in a constant magnetic field of highly organized lamellar structures. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 50:257–260.
16. Sadler, D. M. 1976. X-ray diffraction from chloroplast membranes oriented in a magnetic field. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 67:289–293.
17. Michel-Villaz, M. 1976. Fluorescence polarization: pigment orientation and energy transfer in photosynthetic membranes. *J. Theor. Biol.* 58:113–129.
18. Paillotin, G., and J. Breton. 1977. Orientation of chlorophylls within chloroplasts as shown by optical and electrochromic properties of the photosynthetic membrane. *Biophys. J.* 18:63–69.
19. Förster, T. 1965. Delocalized excitation and excitation transfer. *In* Modern Quantum Chemistry. Istambul Lectures. O. Sinanoglu, editor. Academic Press, Inc. New York. III: 93–137.
20. Armond, P. A., L. A. Staehelin, and C. J. Arntzen. 1977. Spatial relationship of photosystem I, photosystem II, and the light-harvesting complex in chloroplast membranes. *J. Cell Biol.* 73:400–418.
21. Williams, W. P. 1977. The two photosystems and their interactions. *In* Primary Processes of Photosynthesis. J. Barber, editor. Elsevier North-Holland, Amsterdam, The Netherlands. 99–147.
22. Tweet, A. G., W. D. Bellamy, and G. L. Gaines, Jr. 1964. Fluorescence quenching and energy transfer in monomolecular films containing chlorophyll. *J. Chem. Phys.* 41:2068–2077.
23. Seely, G. R. 1973. Effects of spectral variety and molecular orientation on energy trapping in the photosynthetic unit: a model calculation. *J. Theor. Biol.* 40:173–187.
24. Shipman, L. L., and D. L. Housman. 1979. Förster transfer rates for chlorophyll *a*. *Photochem. Photobiol.* 29:1163–1167.
25. Chang, J. C. 1977. Monopole effects on electronic excitation interactions between large molecules. I. Application to energy transfer in chlorophylls. *J. Chem. Phys.* 67:3901–3909.
26. Knox, R. S. 1975. Excitation energy transfer and migration: theoretical consideration. *In* Bioenergetics of Photosynthesis. Govindjee, editor Academic Press Inc., New York. 183–221.
27. Breton, J., J. F. Becker, N. E. Geacintov. 1973. Fluorescence depolarization study of randomly oriented and magneto-oriented spinach chloroplasts in suspension. *Biochem. Biophys. Res. Commun.* 54:1403–1409.