Why Does the Light-Gradient Photovoltage from Photosynthetic Organelles Show a Wavelength-dependent Polarity?

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ABSTRACT The light-gradient photovoltage from photosynthetic organisms and organelles is thought to arise from the primary charge separation in the reaction centers. The current explanation of the effect is the stronger excitation of the membrane side of a vesicle facing the light source than the one on the opposite side. Together with the known orientation of reaction centers, this explanation predicts unequivocally the polarity of the photovoltage. However, a polarity opposite to the one expected has often been reported. A dependence of the polarity on the wavelength has been published but no explanation was given (Gräber, P., and H.-W. Trissl. 1981. *FEBS Lett.* 123:95–99).

Here we report on a theoretical treatment of light propagation and interference in pigmented and nonpigmented multilayers. A model calculation is carried out for a pair of membranes, demonstrating the wavelength-dependent light distribution as well as the relative photovoltage and its polarity. When the membranes contain no chromophores or when the absorption coefficient is low, the predicted polarity is opposite to that expected from a simple macroscopic absorption behavior. The model is tested by comparing new photovoltage data obtained at 532 nm as well as in the blue and red absorption bands of chlorophyll in chloroplasts. It is concluded that outside the main absorption bands the amplitude and polarity of the photovoltage is determined by the ratio of the refractive indices of the membrane and the medium.

INTRODUCTION

In the photosynthetic reaction center (RC), an intrinsic membrane protein complex, the primary reactions occur between a series of cofactors. The transmembrane organization of these cofactors makes the light-induced primary charge separation reactions electrogenic. The electric events are currently detected either by intrinsic probes or by external electrodes. An external electrode method based on the light-gradient effect was first reported in 1972 by Fowler and Kok (1). These authors gave the following generally accepted interpretation of the photovoltage observed under subsaturating flash illumination (1): "Due to the high pigment content, there is a light gradient over the individual chloroplast vesicle, i.e., the trapping centers facing the light source receive more quanta than those in the back, so that the particle becomes unevenly charged."

In the succeeding years further details of light-gradient effects have been reported from different laboratories (2–8). It is undoubted that the time-resolved light-gradient photovoltage is directly related to the primary charge separation and charge stabilization. Also, it is generally accepted that the photovoltage has its origin in the slightly different number of charge separations in the two antiparallel membranes of a vesicular structure. However, the sign of this difference and, hence, the polarity of the photovoltage remained unclear.

For example, due to the movement of the electron from the inside to the outside of the thylakoid membrane, the electrode near the light source should become negative compared to the electrode at a more distant location. Although this is the polarity originally found by Fowler and Kok (1, 3) as well as by Witt and Zickler (2), subsequent studies have shown that the polarity critically depends on the wavelength of the excitation (5, 8), on the flash duration (6), and also on the structural state of vesicular samples (5, 6). Under experimental conditions similar to those of Fowler and Kok (3) and Witt and Zickler (2), a 60-μs flash at 689 nm applied to intact chloroplasts elicits a signal with negative polarity as expected from the above-mentioned scheme of transmembrane charge separation (6). A positive polarity, however, is observed upon excitation of the same suspension of chloroplasts with a ruby laser flash (λ = 694 nm) of either 60-ns or 30-ps duration (6). Such a positive polarity has been consistently observed when the excitation wavelength was outside or on the flank of the main absorption bands of the pigments, whereas the normal negative polarity was found upon excitation close to the red and blue absorption maxima of chlorophyll in vivo (5, 6).

Here we present new data on the light-gradient photovoltage from pea chloroplasts, which were obtained by excitation with a picosecond flash in the red absorption band between 680 and 718 nm, in the blue absorption band at 436 nm, and at 532 nm, a region of low absorption. The photovoltage showed a positive polarity at 532 nm and for $\lambda > 690$ nm, whereas it showed a negative polarity at 436 nm and between 680 and 690 nm. This puzzling behavior of the light-gradient photovoltage polarity will be explained in the present work by a calculation of the light intensities across

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thylakoid membranes taking into account reflection and interference effects due to the difference in the refractive index between membranes and their aqueous surrounding.

It will be shown that the experimentally found wavelength dependence of the light-gradient photovoltage can be well simulated using reasonable values for the parameters.

MATERIALS AND METHODS

The set-up for measuring the light-gradient photovoltage was essentially the same as described before (9). One excitation source was a Nd-YAG-driven dye laser (Continuum, models YG 601 CD-10 and PTL 10) delivering flashes of ~8-ps duration (FWHM). The contrast ratio (peak pulse with respect to residual pulses) was better than 10^3. The other excitation source was a Nd-YAG-driven Raman cell (Quantel, France) filled with pressurized H_2. It delivered flashes of 25-ps duration (FWHM) at 436 and 682 nm. The components of the detection electronics had an upper limiting frequency of more than 6 GHz.

Broken chloroplasts from 14-day-old peas were prepared as described (10). After the last washing step they were resuspended in a medium containing 100 mM sorbitol, 10 mM NaCl, 5 mM $MgCl_2$, 10 mM Tricine (*N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine) (pH 7.8) and 30% (v/v) ethylene glycol. They were stored in liquid nitrogen until use. If necessary, the chloroplasts were diluted with this medium. The chlorophyll *a* and *b* content was determined according to a previous study (11). From this we determined an apparent molar decadic absorption coefficient of chlorophyll (Chl) *a* + *b* in pea thylakoid membranes of ~53,000 M^{-1} cm^{-1} at 680 nm (12).

Light-gradient experiments were performed in a microcoaxial cell with a 100-μm spacing of the electrodes. The concentration of the chloroplast suspension in the cell was adjusted to yield an optical density of OD = 0.2 at excitation wavelengths $\lambda < 710$ nm. At $\lambda > 710$ nm smaller optical densities had to be used, because the chloroplasts could not be concentrated more. All photovoltage data were normalized to the chlorophyll concentration which implies that the photovoltage is virtually proportional to the membrane concentration in the capacitor. We found that, for excitation at low energies and for wavelengths selected over the visible range, the light-gradient photovoltage was proportional to the Chl-concentration up to optical densities of ~0.3.

For the purpose of this study the experimental conditions were chosen so that the photovoltage was due to photosystem I (see Discussion). This was achieved by using conditions for which the thylakoid membranes form grana stacks (5 mM $MgCl_2$), by adding the photosystem II inhibitor DCMU (50 μM final concentration) and by applying a short preillumination (duration of 200 ms) at 532 nm ($\Delta\lambda$ = 7 nm) 200 ms prior to the ps flash. As was shown in an earlier study, PS II does not contribute to the photovoltage signal under these conditions (13). Photosystem I activity was held constant by the presence of 200 μM phenazine methosulfate and 10 mM ascorbate. At each wavelength the photovoltage was measured as a function of the flash energy which yielded a saturation curve. The energy was expressed as the number of photons/flash, and the initial slopes were taken as data points (Fig. 5).

THEORY

Light distribution

Let us consider a system of layers, *i*, characterized by their interfaces at the positions z_{i-1} and z_i, and their refractive indices, n_i, as shown in Fig. 1. A light beam of wavelength λ and intensity I_0 shall enter this system perpendicular to the plane of the layers, i.e., in the *z* direction. At each interface *i*, part of the light wave will be reflected if $n_{i+1} \neq n_i$. In the following we will recall a standard treatment of light propagation in thin layers to describe the variation of the light intensity along the *z* axis.

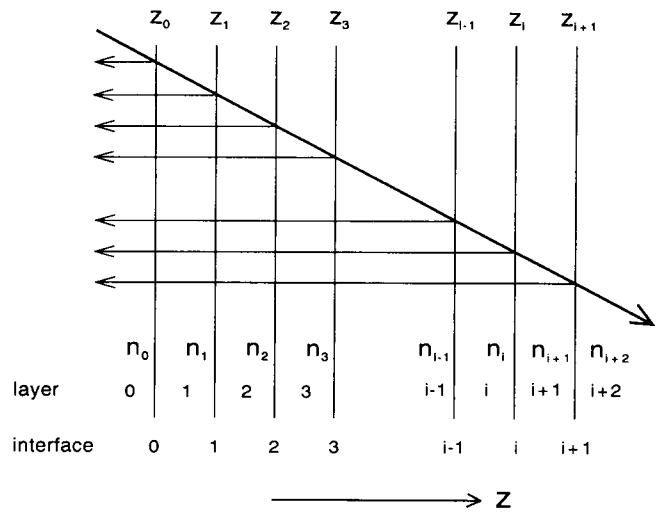

FIGURE 1 Definition of a system of layers with incident and reflected light beams, numbering of the interfaces, layers, and refractive indices. For a clearer presentation the incoming light enters the system at an angle $\neq 90°$.

Neglecting the time dependence of the light wave, the electric field vector of a wave propagating in the *i*th layer toward positive *z* can be written in a general fashion

$$E_i^t(z) = a_i \cdot \exp[i(k_i z + \Phi_i)] \quad (1)$$

and for a wave propagating in the reverse direction (negative *z*)

$$E_i^r(z) = b_i \cdot \exp(-i(k_i z + \Theta_i). \quad (2)$$

In these equations the wave vector k_i is given by

$$k_i = 2\pi n_i/\lambda, \quad (3)$$

whereas the phase shifts Φ_i and Θ_i, and the amplitude factors a_i and b_i are determined by the respective boundary conditions. For simplicity we take the amplitude of the incoming light wave (layer 0) as $a_0 = 1$ and $\Phi_0 = 0$.

First we consider the waves propagating to the right starting with

$$E_0^t(z) = \exp(ik_0 z) \quad (4)$$

From this one can calculate all $E_i^t(z)$ successively by using the boundary conditions

$$E_i^t(z_{i-1}) = T_{i-1} \cdot E_{i-1}^t(z_{i-1}). \quad (5)$$

The transmission coefficient for the *i*th interface, T_i, can be calculated from the corresponding reflection coefficient, R_i, which is given by the difference of the refractive indices on the interface according to the following equations

$$T_i = 1 + R_i, \quad R_i = (n_i - n_{i+1})/(n_i + n_{i+1}). \quad (6)$$

The amplitudes and the phase shifts in Eq. 1 are given by

$$a_i = T_{i-1} \cdot a_{i-1} \quad \Phi_i = \Phi_{i-1} + (k_{i-1} - k_i) \cdot z_{i-1}. \quad (7)$$

Next we consider the reflected waves propagating to the left. The wave reflected at the interface i and propagating in the layer i, $E^r_{i,i}(z)$, can be calculated by using the boundary condition

$$E^r_{i,i}(z_i) = R_i \cdot E^t_i(z_i) \tag{8}$$

which gives for the amplitude and phase in Eq. 2

$$b_i = R_i \cdot a_i \quad \Theta_i = -2k_i z_i - \Phi_i. \tag{9}$$

These waves continue propagating to the left through the layers $j < i$. In the following, multiple reflections are neglected, i.e., reflected waves shall pass the further interfaces without reflection. This approximation is justified, when the reflection coefficients are of the order of some percentage which makes the amplitudes of multiple reflected waves extremely small. The boundary condition for the wave reflected on interface i and propagating in the layer j ($j < i$)

$$E^r_{i,j}(z_j) = E^r_{i,j+1}(z_j) \tag{10}$$

gives, for the phase Θ_j,

$$\Theta_j = \Theta_{j+1} + (k_{j+1} - k_j) \cdot z_j \tag{11}$$

taking into account that, for $j = i$, Θ_i and b_i are given by Eq. 9.

Having calculated all partial waves propagating in the ith layer in this manner one can write for their superposition

$$E_i(z) = E^t_i(z) + \sum_j E^r_{j,i}(z) \tag{12}$$

and one finally obtains for the intensity in the layer i, $I_i(z)$:

$$I_i(z) = |E_i(z)|^2. \tag{13}$$

From the above treatment it can be seen that two factors are of particular importance: the distances in the layer system and the refractive indices. In transparent (nonabsorbing) media the refractive index is a real number and independent of the wavelength, whereas in absorbing media it is complex and wavelength-dependent as follows.

$$n(\lambda) = n'(\lambda) + i \cdot n''(\lambda) \tag{14}$$

The imaginary part $n''(\lambda)$ is related to the molar decadic absorption coefficient, $\epsilon(\lambda)$, and the concentration of absorbing molecules, c, by

$$n''(\lambda) = 1/4\pi \cdot \lambda \cdot \ln(10) \cdot \epsilon(\lambda) \cdot c \tag{15}$$

The real part, $n'(\lambda)$, consists of a constant value, n, the value found far from an absorption band, plus a wavelength-dependent term which calculates from the Kronig-Kramers transform (14, 15):

$$n'(\lambda) = n - \frac{2\lambda^2}{\pi} P \int_0^\infty \frac{n''(u)}{u(\lambda^2 - u^2)} du \tag{16}$$

where P stands for the Cauchy principal value of the integral.

With this set of equations it is possible to calculate the light intensity for an arbitrary number of layers of variable thickness, whether they are absorbing or transparent. The following examples shall demonstrate the effect of wavelength

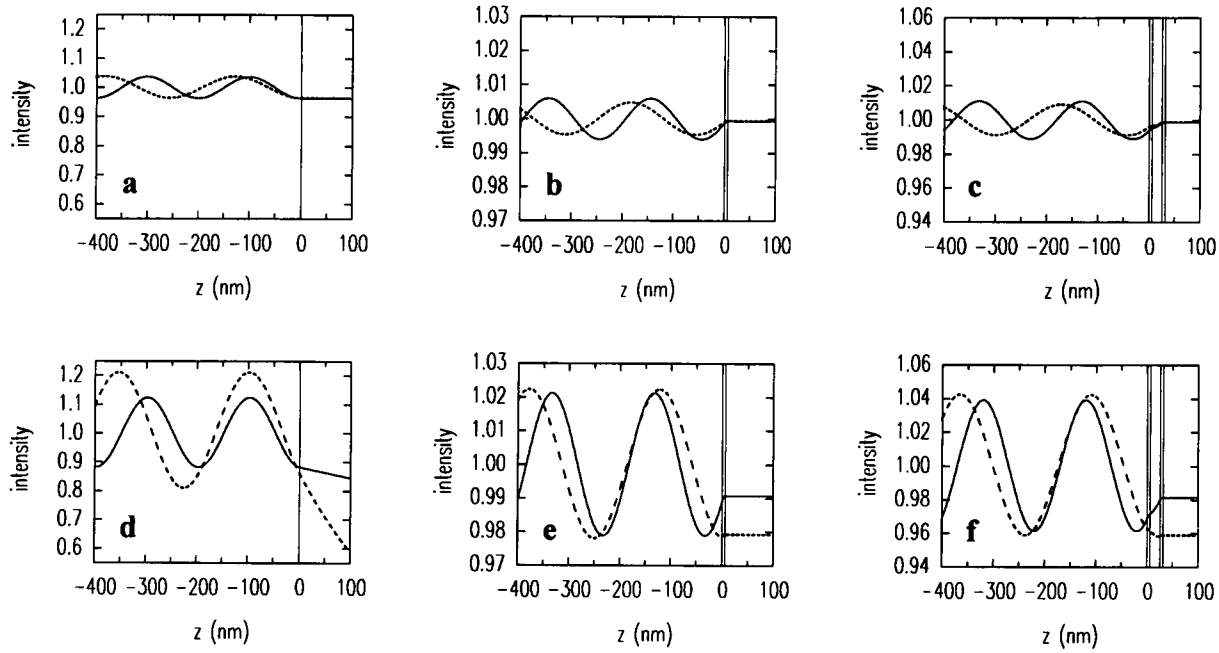

FIGURE 2 Modulation of the incident light intensity in the proximity of a single interface (*a*, *d*), a single membrane (*b*, *c*), and a pair of membranes (*c*, *f*) calculated for wavelengths of λ = 532 nm (*solid lines*) and λ = 680 nm (*dashed lines*). Nonpigmented cases (*a*, *b*, and *c*) and pigmented cases (*d*, *e*, and *f*). Parameters: $n_0 = n_2 = n_4 = 1.33$; $n_1 = n_3 = 1.38$; [Chl] = 0.3 M.

and absorption on the intensity variation along the z direction for different wavelengths and for chlorophyll as the chromophore.

In a first example we consider a single planar interface separating an aqueous and a membraneous medium, the latter having a higher refractive index (Fig. 2). For the nonpigmented case the light intensity in front of the layer oscillates on the order of about 5% of I_0 displaying an intensity minimum at the interface (Fig. 2 *a*). For the pigmented case the light intensity in front of the layer also oscillates, however, more strongly at wavelengths matching an absorption peak (e.g., 680 nm) than at wavelengths lying within absorption minima (e.g., 532 nm; Fig. 2 *d*). Further calculations show, that a higher absorption always increases the amplitude of the oscillation found in front of the interface and shifts the phase of the interference pattern. Within the absorbing layer the intensity decreases exponentially according to Lambert-Beer's law (Fig. 2 *d*).

In the second example we consider a single thin membrane surrounded by an aqueous medium. The membrane is assumed to be either nonpigmented (Fig. 2 *b*) or pigmented (Fig. 2 *e*). A comparison of Figs. 2 *a* and 2 *b* shows that the addition of a second interface leads to a strong decrease of the amplitude of the oscillation, and to a shift of the oscillation pattern in front of the membrane, creating an intensity minimum at about −50 nm. This minimum is still found when the membrane is slightly absorbing (532 nm), but may disappear when it is strongly absorbing (680 nm; Fig. 2 *e*). This is an important fact, as it becomes conceivable that a second membrane positioned in this minimum could be exposed to less intensity than a membrane behind. This would explain the unexpected polarity of the light-gradient photovoltage found for excitation wavelengths in regions of low absorption.

Therefore, in a third example we consider a system of two thin membranes in aqueous surroundings, separated by a distance comparable to their thickness. Again the membranes are assumed to be either nonpigmented (Fig. 2 *c*) or pigmented (Fig. 2 *f*). By comparing Figs. 2 *b* and 2 *c* as well as Figs. 2 *e* and 2 *f* it can be seen that, for the two cases of nonpigmented and pigmented membranes, the interference pattern does not change significantly by adding a second membrane. When the two membranes contain pigments (like thylakoid membranes) Fig. 2 *f* demonstrates that, depending on the wavelength, the membrane facing the light source may be exposed to higher or lower intensities compared to the rear membrane.

The calculation carried out for two pigmented membranes (Fig. 2 *f*) can be regarded as the minimal model system which accounts for a thylakoid vesicle, e.g., two antiparallel photosynthetic membranes (Fig. 3). The theory clearly predicts an inversion of the difference of light intensities (and consequently also of the light-gradient photovoltage) when changing the wavelength from a strongly absorbing to a slightly absorbing region. The absolute value of this intensity difference depends on the concentration of pigments in the membranes as well as on the thickness of the membranes and their distance. The effect of the most important parameters will be demonstrated in the next section.

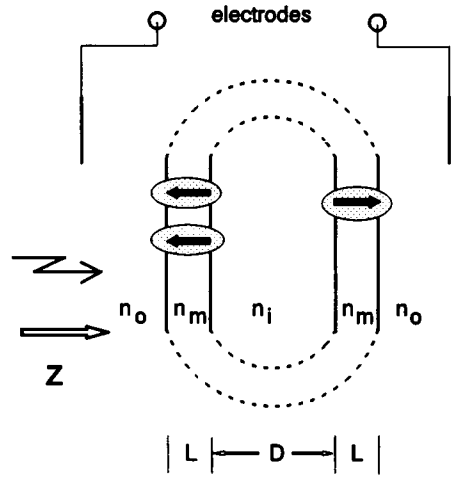

FIGURE 3 Schematic drawing of a photosynthetic vesicle (thylakoid) illuminated by light entering from the left in the z direction. The refractive indices of the different layers are indicated as well as the direction of light-induced electron transfer within the photosynthetic reaction centers.

Light-gradient photovoltage

With subsaturating excitation flashes the light-gradient photovoltage from a two membrane system, V, may be assumed to be proportional to the difference of the light intensities in the middle of the two membranes (Fig. 3) and the absorption cross section of a photosynthetic unit

$$V(D, L) \sim [I(1/2L) - I(3/2L + D)] \cdot N \cdot \sigma \qquad (17)$$

using the definition for the membrane thickness, L, the separation distance of the membranes, D, the number of absorbing antenna pigments per RC, N, and the absorption cross section of one pigment, σ. For $D \ll \lambda$ one can obtain the following equation as a sufficient approximation for the light-gradient in a vesicle $\Delta I = I_1(1/2L) - I_3(3/2L + D)$ as follows.

$$\Delta I = \frac{1}{1 + \dfrac{8\pi \cdot L \cdot n' \cdot n''}{\lambda \cdot n_0}} \cdot \left\{ \frac{4}{3} \cdot \left(\frac{2\pi}{\lambda}\right)^3 n_0 \cdot n' \cdot n'' \cdot [(3/2L + D)^3 - (L/2 + D)^3] - \left(\frac{2\pi}{\lambda}\right)^2 \cdot (n'^2 - n_0^2 - n''^2) \cdot [(3/2\,L + D)^2 - (L/2 + D)^2] \right\} \qquad (18)$$

In Fig. 4 is shown the wavelength dependence of the theoretical light-gradient calculated for different parameter sets. The thickness of the model membranes was fixed at the generally accepted value for biological membranes of $L = 5$ nm

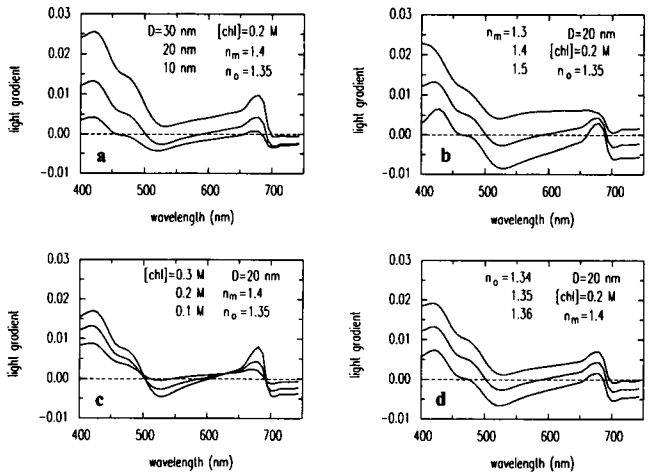

FIGURE 4 Wavelength dependence of the theoretical light-gradient photovoltage between two pigmented membranes calculated for different parameter sets. (*a*) Variation of distance, *D*, between the membranes. (*b*) Variation of the refractive index of the membrane, n_m. (*c*) Variation of the chlorophyll concentration, [chl]. (*d*) Variation of the refractive index of the suspension medium, n_0. Other parameters: n_i = 1.33; *L* = 5 nm; molar absorption coefficient of the Q_y band of chlorophyll (*a* + *b*) in thylakoid membranes 53 000 M^{-1} cm^{-1}.

(16, 17), although one should be aware of a biological membrane not being a very well defined quantity (see Discussion). The refractive index of the medium between the two membranes (e.g., lumen) was fixed to n_i = 1.33, but the refractive index of the suspension medium was allowed to be different ($n_i \neq n_o$). To give a realistic example for the wavelength dependence of the light gradient we used a typical absorption spectrum of pea thylakoids. To demonstrate the influence of the parameters involved, we calculated whole action spectra at otherwise fixed parameters and varied the separation distance of the membranes, *D* (Fig. 4 *a*), the refractive index of the membrane, n_m (Fig. 4 *b*), the chlorophyll concentration in the membrane, [Chl] (Fig. 4 *c*), and the refractive index of the suspension, n_0 (Fig. 4 *d*). The chlorophyll concentration (Chl *a* + Chl *b*) was introduced in Eq. 15.

From Fig. 4 it can be seen that the light-gradient is positive near the absorption maxima for the chosen combinations of parameters. The presence and extent of a region where the gradient is inverted depends strongly on the parameters. The polarity is most sensitive to the distance between the two membranes of a vesicle, the refractive indices, and the concentration of absorbing pigments.

RESULTS AND DISCUSSION

The polarity of the light-gradient photovoltage has been a puzzle for almost a decade. We report here that the principles of propagation and interference of electromagnetic waves determine both the polarity and the amplitude of the signal.

Our theory predicts that the basic reasons for the unexpected (positive) polarity found in wavelength regions of small absorption are the small geometrical dimensions of the membranes and the difference between the refractive index of the membrane and of the suspension medium. The effect of this difference in the refractive indices dominates over the absorption within a single thylakoid membrane in some regions of the spectrum.

This property is clearly verified for the photovoltage signals recorded from broken chloroplasts upon picosecond excitation at various wavelengths. In Fig. 5 are given the experimental photovoltage amplitudes as a function of the excitation wavelength. These amplitudes were normalized to the number of absorbed photons per RC as well as to the chloroplast concentration (see Materials and Methods). It is evident from Eq. 17 that the wavelength dependence of these normalized amplitudes differs from $\Delta I(\lambda)$ only by a factor which is constant for a given wavelength. The latter was used to adjust the data points to the calculated spectrum at 532 nm. Fig. 5 shows a negative polarity of the light-gradient photovoltage at λ = 532 nm and for λ > 690 nm, whereas a positive polarity occurs at 436 nm and between 680 and 690 nm. The data as well as the theoretical curve show further that the light-gradient effect induced at 436 nm is approximately two times stronger than the one induced at 680 nm, although the absorption at both wavelengths is comparable.

The detection of a light-gradient photovoltage from chloroplasts of higher plants is an indirect measurement of the light intensity which is effectively absorbed by the light-harvesting pigments inside the thylakoid membrane. Thus the photovoltage must be proportional to the molar absorption coefficient of the pigments (n'' or σ) and to the light intensity to which each pigment is exposed. What has been demonstrated by the above calculation is that, for a pair of membranes separated by distances smaller than the wavelength, the local field at the entrance site can be either larger or smaller than the one at the exit site. The present treatment was derived for the small (compared to the wavelength) unit

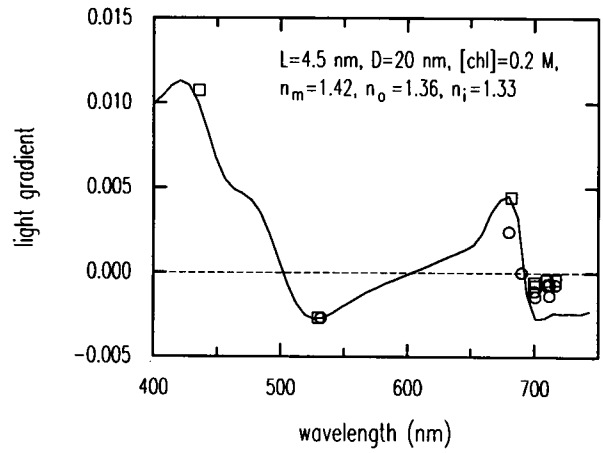

FIGURE 5 Wavelength dependence of the light-gradient photovoltage of stacked thylakoid membranes. The experimental data (○, □) were normalized to the number of absorbed photons/reaction center as described in the text. The solid line represents a best fit calculated as relative intensity difference for the membrane system shown in Fig. 3 with parameters stated in the figure.

of closely spaced thylakoid membranes and not for larger units like chloroplasts or whole cells. These latter cases have been treated by light-gradient theories for macroscopic organelles and tissues in which also multiple scattering occurs (18–20). However, these theories of macroscopic scattering effects are principally not able to explain the typical polarity inversion occurring in photovoltage light-gradient experiments for two reasons. First, the theories are not designed to calculate light-distributions over subwavelength dimensions but the total wavelength-dependent penetration of light in scattering material. Second, the corresponding intensity measurements are made with detectors that are large compared to the wavelength. All these spectra display only positive polarities. In contrast, the light-gradient photovoltage represents an intrinsic probe for the light intensity distribution on dimensions much smaller than the wavelengths of visible light and, due to its differential nature, can display positive or negative polarities. As the scattering of large multilamellar membraneous structures is based on the same principles of wave propagation and interference as the present light-gradient theory, it is therefore not totally surprising to find a similar wavelength dependence of scattered light in both cases.

The light-gradient theory developed here has been formulated for pairs of membranes exhibiting opposite orientation of the RC and a close spacing between them. It was implicitly assumed that the intensities calculated for each membrane cause excited states which remain localized there until their trapping by the photosynthetic charge separation occurs (i.e., transfer of excitation energy between parallel membranes is excluded). This must not necessarily be true for all paired photosynthetic membranes. Chloroplasts contain two types of photosystems which are heterogeneously distributed between stroma and grana membranes (Fig. 6). Photosystem II is almost exclusively localized in the appressed membranes of grana, whereas PS I is restricted to stroma-exposed membranes. It has been suggested that the close approach of two PS II-containing membranes allows for fast transfer of excitation energy between them so that the initially present asymmetry of excitons is lost before they are trapped (excitonic short-circuit). Accordingly, a PS II photovoltage is absent in such a system (13). It is worth noting that it is well possible to detect a PS II photovoltage if the distance between the membrane pairs is increased under destacking conditions (13).

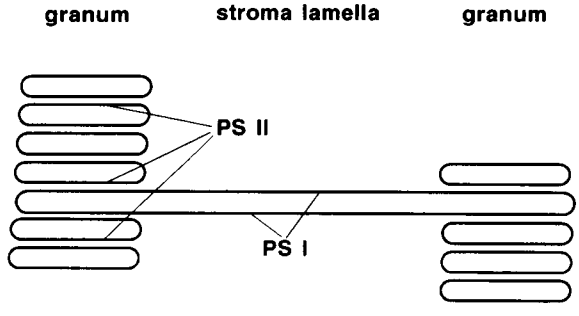

FIGURE 6 Schematic view of the thylakoid membrane structure and the distribution of the two photosystems (PS I and PS II) in chloroplasts of higher plants. The stacking and the concomitant separation of the photosystems is conserved at millimolar concentrations of divalent cations. Only in the absence of these cations the membranes destack and allow for mixing (26).

Under the experimental conditions chosen in the present study the photovoltage signals originate from PS I only. The membranes containing PS I (stroma lamellae) are supposed to be pairwise-coupled at a separation distance of $D > 20$ nm (21, 22). Hence, the calculations based on the model in Fig. 3 (stroma lamellae) are well adapted to describe PS I-light-gradient photovoltage data.

We tried to determine a combination of parameters which gives the best fit of the theoretically calculated light gradient and the normalized photovoltage data. A satisfying fit could be achieved with the parameter values stated in Fig. 5. According to Fig. 5, the distance should be around 20 nm, a value that is well compatible with electron microscopy results (17). For the thickness of the membrane a value of $L = 4.5$ nm was used. Electron microscopy graphs of stroma membranes are consistent with this value (22).

In order to fit the photovoltage amplitudes in the whole spectral range the chlorophyll concentration must be assumed rather high, namely approximately 200 mM (Fig. 5). However, this value is well compatible with the chlorophyll concentration within light-harvesting complexes. For instance, referring to Kühlbrandt and Downing (23) the dimensions of an LHC II trimer in the thylakoid membrane may be described by a cylinder of 6 nm in diameter and 6 nm in height. If 15 chlorophyll molecules bind to the monomer, the chlorophyll concentration in the cylinder calculates to be 440 mM which is in the same order of magnitude as the result from the light-gradient experiments.

It was found during optimization that the fit could be improved by choosing the refractive index of the suspension medium slightly higher than the refractive index of the lumen, namely $n_o = 1.36$ and $n_i = 1.33$. However, a similar good fit could be achieved using $n_o = n_i$ 1.33 and a higher value for D (40 nm). It should be mentioned here that the application of dielectric constants as well as refractive indices to thin membranes is ambiguous, since these quantities refer essentially to macroscopic media (16). This could be the reason for the relatively small value of the refractive index of the membrane resulting from the fit in Fig. 5 as compared to literature data (16, 24). Furthermore, the present theory is based on the assumption of infinitely thin interface regions. This, however, is not the case for biological protein-containing membranes, in which the interfacial regions in the z axis may extend over several nanometers (polar head groups of lipids; intrinsic proteins protruding into the aqueous phase). Therefore, this simple model must be considered as a first approximation to the problem.

Despite the good qualitative agreement between our measurements and the photovoltage predicted by the model calculations, the possibility of extracting quantitative values for the parameters involved should not be overestimated for the following two reasons. First, the theory only treats the case

of perpendicular penetration of light through the membranes. In the real case all orientations of the membrane lamellae with respect to the z direction occur and an appropriate averaging over three space directions is required. Second, under experimental conditions light-gradient photovoltages depend, in addition to the interference patterns in one thylakoid described here, also on the macroscopic light distribution between the different photosynthetic organelles in the sample (25). This light distribution is a composite of light-gradients, optical density, organelle concentration, and light scattering in the measuring cuvette. The influence of these effects on the light-gradient photovoltage is presently not well understood and may be the subject of further refinements.

We conclude, that the simple model applied in our calculation is able to explain the observation of a negative light-gradient in small vesicles. Certainly, the more complicated structure of the real photosynthetic organelles will call for a more sophisticated treatment. However, the good general agreement between our experimental data and the theoretical predictions gives confidence that the "mystery" of the "wrong" polarity of the light-gradient photovoltage is over.

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