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DETERMINATION OF CHLOROPHYLL PORPHYRIN RING ORIENTATION IN BLACK LIPID MEMBRANES BY PHOTOVOLTAGE SPECTROSCOPY

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SUMMARY

Photovoltage spectroscopy with polarized light has been used to investigate the structure of black lipid membranes formed from spinach chloroplast extracts The photovoltage action spectrum of the black lipid membranes is similar to the absorption spectrum of the membrane-forming solution, with a red and principal blue peak. The magnitudes of these peaks have been found to depend on the direction of polarization of the exciting light. This is apparently a direct consequence of the dichroism of the membrane. The polarized light photovoltage data have been used to obtain information on the orientation of chlorophyll in the membrane

INTRODUCTION

The chlorophyll bilayer lipid membrane separating two aqueous phases has been proposed as a model system for the study of the primary processes of photosynthesis of green plants. Various properties of black lipid membranes in the dark have been measured, *e g.* bifacial tension, water permeability, thickness, resistance, and dielectric breakdown[1]. Recently, light-excitable properties such as fluorescence[2], absorbance[3,4], and photovoltage effects[5] have been investigated.

With Fe^{3+} in one aqueous phase, the light-induced electromotive force of the black lipid membrane has been found to depend on the wavelength of illuminating light A "photo-emf action spectrum" can be obtained by scanning the visible wavelengths[6].

This paper concerns the finding that the magnitudes of the peaks of the photo-emf action spectrum depend on the direction of polarization of the exciting light. This appears to be a direct consequence of the absorption properties of the chlorophyll in the membrane, and may be used to determine the orientation of the chlorophyll porphyrin ring in the black lipid membranes

MATERIALS AND PROCEDURE

The black lipid membranes-forming solution was prepared from chloroplasts isolated from fresh commercial spinach by a procedure described in detail elsewhere[7].

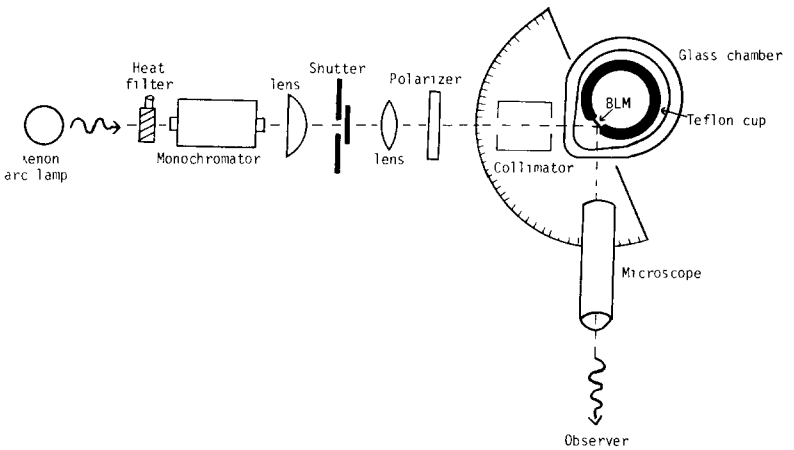

Fig. 1. Schematic diagram of optic set-up (top view).

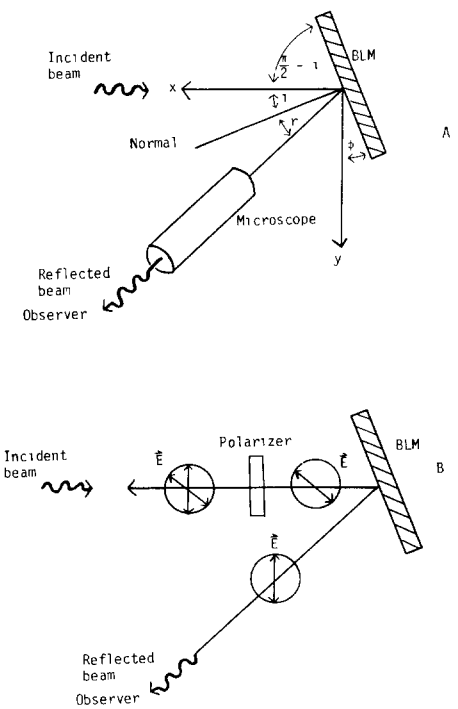

Fig 2 A Schematic diagram illustrating the determination of the orientation of the membrane relative to the direction of propagation of the incident light (top view) i, angle of incidence; r, angle of reflection. $i = r = \phi$ B. Schematic diagram illustrating the establishment of the direction of polarization of the incident light with the electric vector vibrating parallel to the plane of incidence (top view)

Briefly, the chloroplasts were then broken by osmotic shock, the chlorophyll and lipids extracted with light petroleum–methanol and dissolved in an *n*-butanol–dodecane solution. The membrane was formed in 0.1 M acetate buffer, pH 5, across a circular aperture of 2 mm diameter in a Teflon beaker set inside a glass cup. After the membrane had reached the black stage, $FeCl_3$ was added to the inner chamber to bring the Fe^{3+} concentration to 1 mM. The open-circuit potential difference across the membrane was monitored by a Cary 31 electrometer *via* a calomel electrode in the aqueous phase on each side of the black lipid membranes.

The black lipid membranes were excited with light from a 1000 W D.C. xenon arc lamp (Hanovia, Type 976 C) which was passed through a heat filter, a visible grating monochromator (Bausch and Lomb, Model 5), a plano-convex lens, a shutter, a converging lens, a polarizer, and a collimator (Fig 1). During formation, the membrane was observed with dim green light (525 nm).

After application of the membrane-forming solution across the aperture, the membrane thinned first to a thickness of less than 1 μm. At this stage, if the membrane is observed at an angle with the normal equal to the angle of incidence of illuminating light, interference fringes are seen (Fig. 2a). In this way, the orientation of the membrane relative to the direction of propagation of the incident light was determined

Most of the light reflected from the membrane is polarized with the direction of vibration of the electric vector parallel to the plane of the membrane, *i.e.* perpendicular to the plane of incidence (Fig. 2b). The direction of polarization of the illuminating light was varied, by rotating the polarizer about the direction of propagation, until the direction for which the interference fringes were observed to have a minimum intensity. This established the direction of polarization of the incident light with the electric vector vibrating parallel to the plane of incidence

When the thickness of the membrane has fallen much below 1000 Å, destructive interference give rise to the optically "black" appearance[7]. When the "black" membrane is illuminated with exciting light, an electromotive force (open-circuit voltage) is generated across it, with the side in contact with Fe^{3+} becoming more negative than the other side. The magnitude of this "photo-emf" is dependent upon the wavelength of the exciting light, and a "photo-emf action spectrum" can be obtained by scanning the visible wavelengths[6].

THEORETICAL CONSIDERATIONS

Before presenting the results and discussing their significance, a consideration of some aspects of theoretical background upon which the present interpretation is based is in order. First, it is assumed that the black lipid membrane is a lyotropic liquid crystalline system with smectic structure. In a smectic structure the molecules are arranged in layers, with their long axes parallel to each other and approximately normal to the plane of the layers. The molecules can move in two directions in the plane and can rotate about one axis[8]. In the black lipid membrane, each transition moment is probably restricted to any direction which lies on a conical surface making an angle of θ with the normal, N, as depicted in Fig. 3

The orientation of each transition moment, M, is given by the angle θ between M and the normal N, to the membrane. The components of M along the two directions, y and z, of polarization of the incident light are M_y and M_z, where

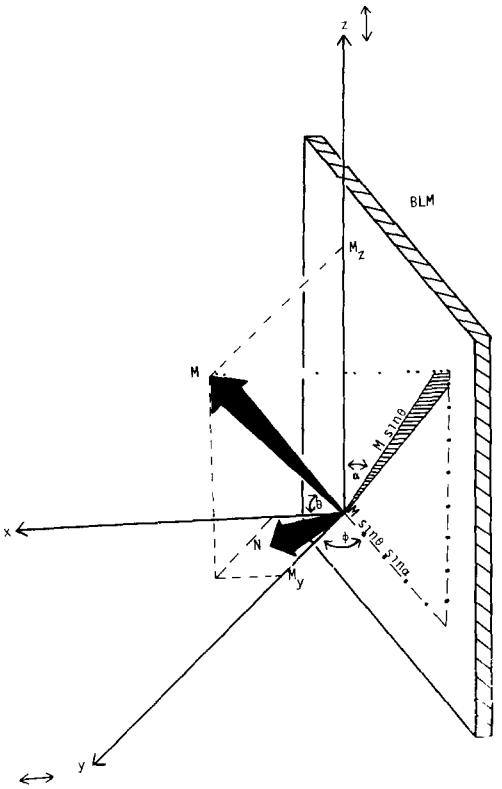

Fig. 3 Schematic diagram of the transition dipole moment, M, of either the red or the principal blue chlorophyll absorption band (see text) x, the direction of propagation of the exciting light.

$$M_y = M \sin\theta \sin\alpha \cos\phi + M \cos\theta \sin\phi \tag{1}$$

$$M_z = M \sin\theta \cos\alpha \tag{2}$$

The dichroic ratio, D, which is defined as the ratio of the absorbance for monochromatic light polarized in the y-direction (*i.e.* horizontally polarized) to that for the light polarized in the z-direction (*i.e.* vertically polarized)[9], is given by

$$D = \frac{A_y}{A_z} \tag{3}$$

If the absorption band for light polarized in the y-direction has the same shape as the absorption band for light polarized in the z-direction, then

$$\frac{A_y}{A_z} = \frac{I_y}{I_z} \tag{4}$$

where I_y and I_z are the integrated intensities for the bands[10]. Since the integrated intensity of an absorption band is proportional to the square of the transition dipole moment,

$$\frac{A_y}{A_z} = \frac{I_y}{I_z} = \frac{M_y^2}{M_z^2} \tag{5}$$

Dichroism of chlorophyll black lipid membranes has been attributed to orientation of chlorophyll molecules in the membrane[4,11,12]. The dependence of the magnitudes of the blue and red peaks in the photo-emf action spectrum on the direction of polarization of the exciting light is apparently a direct consequence of the dichroism of the membrane. If so, then ratios of emf magnitudes of each peak for the stated two directions of polarization of incident light should allow calculation of the orientations in the membrane of the transition dipole moments of the blue and red absorption bands. For each peak the ratio of photo-emfs, $(E_{hv})_y/(E_{hv})_z$ varied less than 8% with a 4-fold increase in light intensity.

The red or principal blue peak absorbance of a chlorophyll–lipid bilayer has been reported to be at the largest of the order of 0.010[3,4,11], which implies a value of about 0.023 for the fraction, ρ, of the light intensity incident on the membrane which is absorbed. Therefore, the absorbance, $-\log(1-\rho)$, is approximated by $\rho/2.30$ with an error of less than $\pm 2\%$. If the photo-emf, E_{hv}, is proportional to the amount of light energy absorbed by the membrane, then the ratio of the emf magnitudes for horizontally polarized light to vertically polarized light for the red peak or the principal blue peak gives the ratio of the absorbances for these directions of polarization,

$$\frac{(E_{hv})_y}{(E_{hv})_z} = \frac{\rho_y}{\rho_z} = \frac{M_y^2}{M_z^2} \tag{6}$$

Since each transition moment is probably restricted to any direction which lies on a conical surface making an angle of θ with the normal, M_y and M_z must be integrated over all α,

$$\frac{(E_{hv})_y}{(E_{hv})_z} = \frac{\frac{1}{2\pi}\int_0^{2\pi} M_y^2 \, d\alpha}{\frac{1}{2\pi}\int_0^{2\pi} M_z^2 \, d\alpha} \tag{7}$$

$$\frac{(E_{hv})_y}{(E_{hv})_z} = \cos^2\phi + 2\,\mathrm{ctn}^2\phi \sin^2\phi \tag{8}$$

Since the orientation, ϕ, of the membrane is known, Eqn 8 can be used to calculate the direction, θ, of each transition moment from the magnitudes of the polarized light induced photo-emf peaks. Since there are both chlorophyll *a* and chlorophyll *b* in the spinach chloroplast[13], the value for θ thus obtained is actually an average over both types of chlorophyll present in the membrane.

Polarized absorption and fluorescence measurements with chlorophyll have shown that the two transition moments responsible for the red and principal blue absorption bands are perpendicular to each other and lie in the plane of the porphyrin ring[14,15]. The orientations, θ_R and θ_B, of the red and principal blue dipole moments with respect to the normal, *N*, to the membrane then supply enough information that the orientation of the chlorophyll porphyrin ring in the membrane can be calculated.

With the assumptions that the red and principal blue dipole moments are mutually perpendicular and lie in the plane of the porphyrin ring, it may be shown that the angle, β, between the plane of the porphyrin ring and the normal to the membrane is given by the expression

$$\cos^2 \beta = \cos^2 \theta_R + \cos^2 \theta_B \tag{9}$$

RESULTS AND DISCUSSION

A typical photo-emf action spectrum of a black lipid membrane, obtained by the method outlined in the experimental section, is shown in Fig. 4. The photovoltage spectrum shows a slight dependence on the direction of wavelength scan, with peaks shifting about 5 nm and each peak magnitude changing by a factor of about 1.2. The peaks in the action spectrum are shifted to the red from the peaks for the bulk solution absorption spectrum. Otherwise, the photo-emf spectrum bears a strong resemblance to the absorption spectrum for chlorophyll *a*, with peaks at 430 and 660 nm. Other researchers have observed a red shift in the red and blue peaks from the chlorophyll absorption spectrum in bulk to the absorption spectrum in chlorophyll black lipid membranes[3,4] It is likely that this is responsible for the red shift in the photo-emf action spectrum

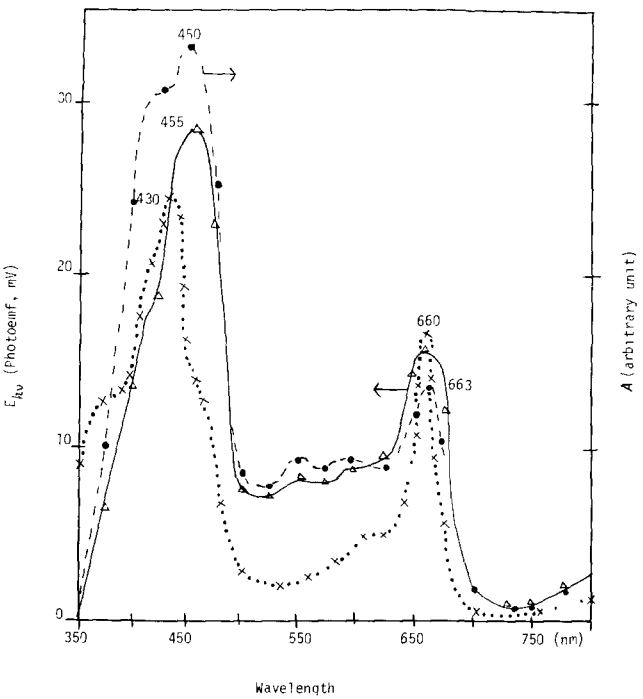

Fig. 4 ·····, absorption spectrum of spinach chloroplast extract membrane-forming solution in bulk – – –, photo-emf action spectrum of spinach chloroplast extract black lipid membranes, scan from 350 to 800 nm. ——, photo-emf action spectrum of spinach chloroplast extract black lipid membranes, scan from 800 to 350 nm The action spectra have been corrected to show emf per unit incident light intensity.

TABLE I

ORIENTATIONS IN THE SPINACH CHLOROPLAST EXTRACT CHLOROPHYLL BLACK LIPID MEMBRANE OF THE TRANSITION DIPOLE MOMENTS RESPONSIBLE FOR THE CHLOROPHYLL RED AND PRINCIPAL BLUE ABSORPTION BANDS, AND ORIENTATION OF THE CHLOROPHYLL PORPHYRIN RING

These values are averaged over the chlorophyll *a* and chlorophyll *b* present in the membrane

Absorption peak	$(\overline{E_{hv}})y$ / $(\overline{E_{hv}})z$	θ (°)	*"Average" angle between transition moment and plane of the membrane* $(\pi/2-\theta)$ (°)	*"Average" angle between plane of porphyrin ring and plane of membrane* $(\pi/2-\beta)$ (°)
Blue peak (455 nm)	0.69	69±2	21±2	45±5
Red peak (660 nm)	1.08	52±2	38±2	

The photo-emf blue peak is of greater magnitude for the incident light polarized perpendicular to the plane of incidence than for it polarized parallel to it. The situation is opposite for the red peak. From the photo-emf peak values for horizontally and vertically polarized light, the orientation θ of each transition moment can be calculated with the aid of the equations developed above. The results are summarized in Table I. The principal blue transition moment was calculated to make an angle of 21 ± 2 ° with the plane of the membrane; the red transition moment, an angle of 38 ± 2 °. From these angles, an angle (averaged over the chlorophyll *a* and chlorophyll *b* in the membrane) of 45 ± 5 ° is calculated for that between the plane of the porphyrin ring and the plane of the membrane.

These results can be compared with values for chlorophyll porphyrin ring orientation obtained from polarized absorption spectroscopy on artificial chlorophyll membranes by other researchers. For chlorophyll-egg lecithin black lipid membranes, Cherry *et al.*[11] found angles of 48 ° for chlorophyll *a* and 51 ° for chlorophyll *b*. Steinemann *et al.*[12] found angles of 44 ± 3 °, 46 ± 3 °, and 49 ± 5 ° for chlorophyll *a*–phosphatidyl ethanolamine, chlorophyll *a*–dioleoyl-phosphatidyl choline, and chlorophyll *a*–phosphatidyl serine membranes, respectively. They found 42 ± 4 ° for chlorophyll *b*–dioleoyl-phosphatidyl choline membranes. It is interesting to note that Calvin[16], while discussing energy reception and transfer in photosynthesis, has speculated about the orientation of chlorophyll in the lamella and suggested that the porphyrin rings lie in a characteristic pattern, namely at an angle about 45° to the stacking axis.

The polarized light-induced emf in black chlorophyll–lipid membranes is apparently a sensitive technique for investigating the orientation of chlorophyll in a bilayer lipid membrane. It requires only one membrane and a simple optic and electronic set-up. Furthermore, the existence of the phenomenon of light-induced emf in artificial chlorophyll–membranes suggests that a similar phenomenon may occur *in vivo* in the chloroplast thylakoid membrane during the process of transduction of light energy into chemical energy.

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