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PHOTOVOLTAGES IN SUSPENSIONS OF MAGNETICALLY ORIENTED CHLOROPLASTS

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Summary

The photovoltage of suspensions of magnetically oriented chloroplasts using polarized light of 680 nm has been measured. The magnitude of the photo e.m.f. depends on the polarization of the light and on its direction of propagation with respect to the oriented thylakoid planes. This photo e.m.f. is qualitatively attributed to the Dember effect which arises when inhomogeneous light absorption gives rise to a gradient of positive and negative charges along $\vec{x}$, where $\vec{x}$ is the direction defined by the propagation vector of the light and which is also the direction joining the two electrodes. The photovoltage obtained with the planes of the oriented thylakoids parallel to $\vec{x}$ depends on the plane of polarization of the incident light and shows that (1) the magnitude of the photovoltage depends on the absorption coefficient (which itself is polarization dependent) and thus on the magnitude of the charge gradient produced by the inhomogeneously absorbed light, and (2) a charge gradient within the planes of the thylakoids can give rise to the macroscopic photovoltage. While our experimental observations are basically in agreement with those previously reported (Fowler, C.F. and Kok, B. (1974) Biochim. Biophys. Acta 357, 308—318 and Witt, H.T. and Zickler, A. (1973) FEBS Lett. 37, 307—310) for unoriented chloroplasts, their interpretation of the origin of this effect in terms of a transmembrane potential must be modified in view of our results obtained with oriented chloroplasts. The macroscopically observed photovoltage of oriented chloroplasts is due to the creation of charge gradients

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either parallel or perpendicular to the thylakoid planes by a flash of light, the diffusion of these charges and to differences in the mobilities of the negative and positive charges. This interpretation in terms of the Dember effect is completely consistent with the existence of a transmembrane electric field as proposed by Fowler and Kok, as well as by Witt and Zickler. However, macroscopic measurements of the photovoltage using either oriented or unoriented chloroplast suspensions cannot prove that a transmembrane voltage exists, as previously claimed.

Introduction

In a photosynthetic membrane, shortly after a photon has been absorbed by the antenna pigments and the excitation energy is transferred to a reaction center, a proton is transported across the thylakoid membrane from the outside of the thylakoid to the region inside [1,2]. This charge separation gives rise to an electric field across the membrane and has been the subject of intensive investigations during the last decade [3—5]. In fact, it has been postulated that changes in the absorption of membrane pigments observed immediately following the absorption of a short pulse of light are due to the Stark effect caused by the interaction between the electric dipole moment of the membrane pigment molecules and the transmembrane electric field [6,7].

Recently, Fowler and Kok [8] discovered that when a suspension of chloroplasts is illuminated by a brief (μs) flash of red light, a transient photo e.m.f. is produced if the two electrodes are spatially separated along the direction of the excitation light beam. They attribute this photovoltage to the transmembrane electric field and have derived an equation in which the electric field contribution of each thylakoid in a granum stack was summed to produce the net voltage which is experimentally observed. In their model, reaction centers are arranged in such a way that charges move perpendicular to the membrane planes. The donors are located on the inside of the thylakoids and the acceptors on the outside. Therefore, in the paired membrane, electrons move to the outside of each membrane during illumination and the inside of the thylakoid becomes positively charged. This charge separation produces an electrical field whose direction is perpendicular to the membrane plane. Futhermore, the absorption of light within each granum stack is inhomogeneous, producing more charge separation within those membranes which are closer to the light source, and less charge separation within those membranes which are on the opposite side (further from the light source). This gives rise to a net dipole of the particles. According to Fowler and Kok [8], those membranes which are oriented with respect to the electrodes such that there is a non-zero component of the electric field along a direction parallel to the electrodes, contribute to the macroscopically observed photo e.m.f.

A schematic representation of oriented grana stacks which contribute (Fig. 1A) and which do not contribute (Fig. 1B) to the macroscopically observed e.m.f., according to this model, are shown in Fig. 1. In Fig. 1B, the electric field due to the dipoles always points in a direction which is perpen-

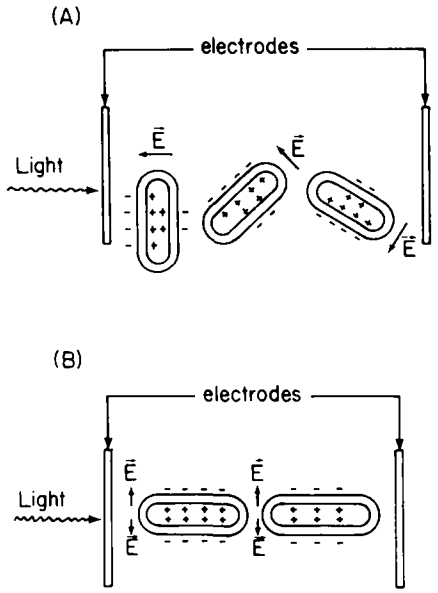

Fig. 1. Idealized representation of thylakoid membranes and the transmembrane electric field ($\vec{E}$) which gives rise to a resultant field. A. Membrane orientations which contribute to the macroscopic photo e.m.f. in the context of the Fowler and Kok [8] model; B. Membrane orientations which do not contribute to the photo e.m.f. since the electric field points in a direction parallel to the planes of the electrodes (no attempt is made to show charge gradients due to inhomogeneous light absorption in this schematic representation).

dicular to the direction of the light source and the planes of the electrodes; thus no photo e.m.f. is expected in this case.

In order to verify these predictions of the Fowler and Kok model we have measured the photo e.m.f. using chloroplasts which are magnetically oriented. Maximum orientation of the chloroplasts can be achieved at magnetic field strengths of approx. 13 kG. The orientation of the chloroplasts is such that the membrane planes are perpendicular to the magnetic field direction [9,10]. Using polarized light, the photo e.m.f. can be measured for various polarizations and propagation vector (relative to the membrane planes) of the light beam.

We find that an e.m.f. is produced even when the orientation of the chloroplasts is of the type shown in Fig. 1B; furthermore, when the chloroplasts are oriented as shown in Fig. 1B, the magnitude of the photo e.m.f. depends on the polarization of the incident light.

On the basis of these experiments, it is concluded that while the macroscopically observed photo e.m.f. is consistent with a transmembrane electrical field, these photo e.m.f. measurements do not necessarily prove that the electric field is directed across the membrane. The macroscopically observed photo e.m.f. is attributed to the Dember effect, a well known effect in solid state physics [11,12].

In the Dember effect the light is nonuniformly absorbed within the sample which gives rise to a gradient of positive and negative charges (these charges arise because of the usual charge separation process across the membranes

[1—8]) along a direction defined by the propagation vector of the light, which is also the direction joining the two electrodes. A photo e.m.f. develops across these electrodes if the diffusion coefficients and thus the mobilities $\mu+$ and $\mu-$ of the positive and negative charge carriers respectively, are different. (Note that the diffusion coefficients D are related to the mobilities by the expression $D = (kT/e)\mu$, where e is the electronic charge, k is Boltzmann's constant and T is the absolute temperature). The photo e.m.f. goes to zero as the initial charge gradient produced by a flash of light is destroyed by the thermal diffusion of the carriers.

Consistent with a Dember effect mechanism, we show that there is a photo e.m.f. even when the planes of the membranes are aligned parallel to the vector joining the two electrodes (configurations of Fig. 1B). Since, in this configuration, the dipoles and, thus, the electric field are oriented parallel to the planes of the electrodes, the photo e.m.f. predicted by the Fowler and Kok model should be zero.

It should be emphasized that the Dember mechanism does not in any way contradict the existence of a potential across the membranes [1—8]. The appearance of charge gradients is consistent with a vectorial charge separation across the membranes, but these charge gradients and therefore the Dember potentials, can lie either within the planes of the thylakoid membranes, or perpendicular to these planes, depending on the direction of propagation of the light with respect to the orientation of the thylakoid planes. Our findings thus indicate that the model proposed by Fowler and Kok [8] and by Witt and Zickler [13,14], in which they relate the macroscopically observed photo e.m.f. to the transmembrane electric field, must be modified. Such measurements, while consistent with the existence of such a transmembrane field, do not provide direct evidence that such a field perpendicular to the planes exists (dipole model of ref. 8).

Experimental methods

Spinach chloroplasts were prepared by blending de-veined market spinach in a Waring blendor for 8—10 s in a medium of 0.4 M sucrose/0.004 M NaCl/ 0.02 M Tris buffer, pH 8 at 5°C. The resulting suspension was filtered through 16 layers of cheese cloth and centrifuged at $2000 \times g$ for 1 min. The supernatant was discarded and the upper fraction of the pellet was resuspended in fresh medium to which 10^{-4} M benzylviologen was added. The preparation procedures were carried out in a cold room at 4°C and the chloroplasts stored in the dark at 0°C for less than 5 h before use in the experiments. All of the experiments were performed at room temperature.

The apparatus was designed to fit into the 6 cm gap between the pole pieces of an electromagnet which produced a maximum field of 13 kG. The light sources and electronic equipment were kept at least 2 m from the magnet to minimize the effects of stray fields. Excitation light was obtained from either a Xenon flash lamp with a duration of 8—10 μs and an intensity (wavelength $\lambda > 640$ nm) of 10^7 ergs $\cdot$ cm^{-2} $\cdot$ s^{-1}, or a photographic flash lamp with a risetime of 40 μs and a falltime of approx. 200 μs. The intensity of excitation with this flash was approx. 10^{10} ergs $\cdot$ cm^{-2} $\cdot$ s^{-1}. The flash was

directed onto the sample using a 7 mm diameter quartz light pipe. A red cut-off filter (CS 2-64), a 680 nm interference filter, and an HN-22 sheet polaroid were placed in the excitation beam.

The cuvette used to hold the spinach chloroplast suspension was made by cementing a glass tube (2 cm inside diameter × 1 cm length) between two glass plates coated with tin oxide. Copper wire leads were cemented to the tin oxide coating with lacquer impregnated with silver. The wire leads were electrically insulated, and shielded with copper braid to reduce the electrical noise pick-up. The signal was fed into an oscilloscope plug-in amplifier (model 1A7A, Tektronix, Inc.). The e.m.f. produced by the flash was either displayed directly on the oscilliscope and photographed, or fed into a signal averager and averaged over several flashes. This signal-averaging system consisted of a Biomation 610 transient recorder coupled to an Intertechnique Didac 800 signal averager. The sample holder was designed and built so that it could be rotated 90°; thus, the direction of the magnetic field was either parallel to the excitation beam or perpendicular to it.

Results

In comparing the magnitude of the photo e.m.f. for different membrane orientations and polarization vectors of the exciting light, it was found convenient to compare the magnitude of the photo e.m.f. for the oriented samples with that of unoriented chloroplasts. Typical results for several different orientations (shown schematically) for the oriented membranes, are shown in Fig. 2.

Referring to Fig. 2, A and B, the Fowler and Kok model predicts that a smaller e.m.f. should be observed for the oriented samples than for the unoriented suspensions. It is actually observed that the e.m.f. is larger if the polarization vector is perpendicular to the magnetic field, i.e., parallel to the membrane plane (Fig. 2A). On the other hand, with the polarization vector parallel to the magnetic field (Fig. 2B), but perpendicular to the membrane, the photo e.m.f. is decreased when the chloroplasts are oriented.

With the orientation shown in Fig. 2C, the Fowler and Kok model predicts a larger photo e.m.f. for the oriented than for the unoriented chloroplasts. The opposite is actually observed, there is an approximately 20% decrease in the photo e.m.f. when the magnetic field is switched on.

In order to verify further that the applied magnetic field (which is produced by passing a large current through the coils of the electromagnet) does not influence the measurements, the following experiments were performed. The relaxation time of the orientation of the chloroplasts can be as long as several minutes in a highly viscous environment [9]. We have thus added Ficoll polymer to a suspension of chloroplasts to increase the viscosity, and measured the photo e.m.f. within several seconds after shutting off the magnetic field. The photo e.m.f. anisotropy was essentially the same as the field on value, since there was insufficient time for an observable decay of the orientation after the field was shut off. This experiment demonstrates that there was no influence of the applied magnetic field on the photo e.m.f. measurements themselves. Furthermore, addition of approx. 10^{-5} M 3(3,4-

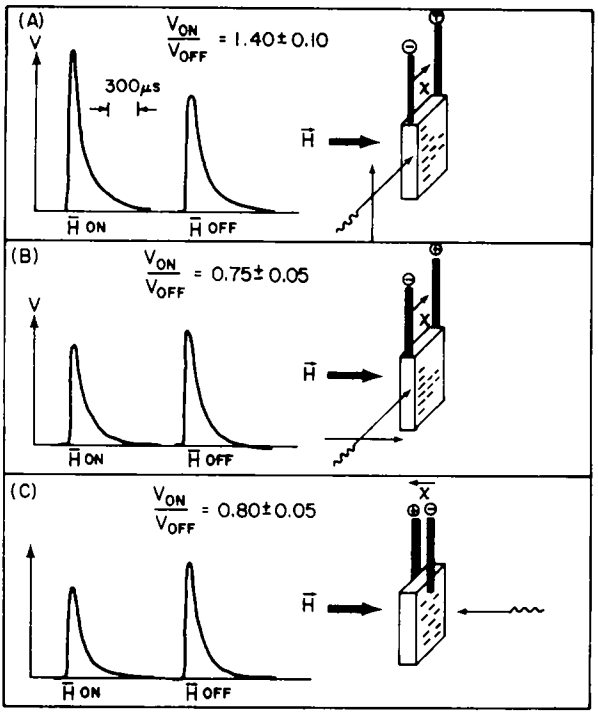

Fig. 2. Photo e.m.f. (V) of suspensions of magnetically oriented chloroplasts. The excitation wavelength is 680 nm and the orientations of the polarization vector of the light, the propagation vector of the light, the magnetic field $\vec{H}$, and the vector $\vec{x}$ joining the two electrodes are also shown. The membrane planes are represented by rectangular slabs which orient with their normals parallel to $\vec{H}$. The illuminated electrode al ways exhibits a negative polarity (see text). The magnetic field value ("$\vec{H}$ on") was 13 kG. The charge density gradients are also schematically indicated. The V_{on}/V_{off} values represent the ratios of the photo e.m.f. with the magnetic field on and off, and represent average values for four different chloroplast preparations. The signals shown represent an accumulation of ten traces each for one of these samples. Chlorophyll concentration: 40 μg/ml. The intensity of the flash corresponded to 4—5 hits/trap (this was established by comparing the intensity dependence of the photo e.m.f. in our experiments with the one reported by Fowler and Kok [8]).

dichlorophenyl)-1,1-dimethylurea to the chloroplast suspension eliminated the photo e.m.f., in agreement with the results of Fowler and Kok [8].

Discussion

The results shown in Fig. 2 indicate that the Fowler and Kok model cannot account for the observations using polarized light and magnetically oriented chloroplasts and thus must be modified. Since the transmembrane electrical field in Fig. 2, A and B is perpendicular to the vector $\vec{x}$ joining the two electrodes, there should be no photo e.m.f. in these two cases, assuming that the membrane planes are oriented perfectly with respect to the magnetic field $\vec{H}$. However, since the chloroplasts are somewhat curved rather than perfectly flat objects, a photo e.m.f. of zero is not expected in Fig. 2, A and B, but the photo e.m.f. is expected to decrease when the chloroplasts are oriented by the magnetic field, at least within the context of the Fowler and Kok model.

Instead, it is observed that there is a significant change in the photo e.m.f. which is produced by simply changing the orientation of the polarization with respect to the membrane plane. Generally, there are changes in the amount of light absorbed by chloroplast suspensions when the magnetic field is switched on. Typically, the changes in the amount of transmitted light are of the order of 1—15% depending on the wavelength [9,18]. In principle, since the absorbance of light depends on the polarization vector of the light, more photons are absorbed in the configuration of Fig. 2A, while less photons are absorbed in the configuration of Fig. 2B when the magnetic field is switched on. The light intensities used in our experiments corresponded to about 4—5 hits/trap. According to Fowler and Kok, the photo e.m.f. tends to decrease as the absorbed light intensity increases. If this were the cause of our effect, the photo e.m.f. should decrease in Fig. 2A and increase in Fig. 2B when the magnetic field is switched on. Since exactly the opposite effect is observed, we conclude that the small changes in the number of photons absorbed when the magnetic field is switched on do not account for the results shown in Fig. 2, A and B. With the polarization vector oriented parallel to the plane of the membrane (Fig. 2A), the photo e.m.f. is nearly twice as large as that observed when the polarization vector is oriented perpendicular to the plane of the membrane (Fig. 2B). The results shown in Fig. 2C further support the contention that the photo e.m.f. is not directly related to the transmembrane potential since the photo e.m.f. actually decreases when the chloroplasts are oriented and in a configuration in which the contribution of the transmembrane dipoles along $\vec{x}$ should be maximum. The only property which is known to be different for the two polarization orientations shown in Fig. 2, A and B, is the absorption coefficient of the chloroplasts. From linear dichroism [9,15] and fluorescence anisotropy [16,17], experiments, it has been established that the long wavelength Q_y transition moments of chlorophyll in vivo tend to lie close to or within the membrane planes. Polarized light at 680 nm is thus more strongly absorbed when the electric vector is oriented parallel to the membrane plane, than when it is oriented perpendicular to this plane.

The results in Fig. 2, A and B, thus suggest that the photo e.m.f. is related to an absorption of light within the chloroplasts which produces an inhomogeneous distribution of charges. The stronger the absorption of light (the greater the absorption coefficient), the greater the photo e.m.f.

This dependence on the absorption coefficient suggests that the photo e.m.f. observed with suspensions of chloroplasts is a type of Dember effect [11,12]. This effect is well known in solid state physics and occurs whenever a crystal sandwiched between two electrodes is illuminated nonuniformly; furthermore, the direction of propagation of the light must be along the vector $\vec{x}$ joining the two electrodes (Fig. 2), and the mobilities $\mu+$ and $\mu-$ of positive and negative charge carriers must be different from each other.

In the absence of any externally applied electric fields, and under open circuit photovoltage measurement conditions, the current flow in a homogeneous crystal is zero. The electric field within the crystal $\vec{E}(x)$, the charge carrier density $n(x)$ at the position x, the diffusion coefficient D, and the mobility μ are related to each other by the following set of equations for

positive (n^+) and negative (n^-) charge carriers:

$$\mu^+ n^+(x) E(x) - \frac{kT}{e} \frac{dn^+(x)}{dx} = 0$$
$$\mu^- n^-(x) E(x) - \frac{kT}{e} \frac{dn^-(x)}{dx} = 0 \tag{1}$$

which give the photo e.m.f. (V):

$$V = \frac{-kT}{e} \int_{n^+(0),n^-(0)}^{n^+(L),n^-(L)} \frac{\mu^+ dn^+(x) - \mu^- dn^-(x)}{\mu^+ n^+(x) + \mu^- n^-(x)} \tag{2}$$

Upon integrating between the limits defined by the electrodes ($x = 0$ and $x = L$), the general expression for the photo e.m.f. is obtained:

$$V = \frac{kT}{e} \left[\frac{\mu^+ - \mu^-}{\mu^+ + \mu^-}\right] \ln \left[\frac{\mu^+ n^+(L) + \mu^- n^-(L)}{\mu^+ n^+(0) + \mu^- n^-(0)}\right] \tag{3}$$

The front (illuminated) electrode is defined by $x = 0$ and the back electrode by $x = L$. The photo e.m.f. disappears when the charge density at the front electrode is the same as the back electrode, or when $\mu^+ = \mu^-$.

The photo e.m.f. observed with chloroplasts is qualitatively consistent with the Dember effect. As observed by Fowler and Kok [8], V goes to zero as the light intensity is increased; this is due to the fact that the inhomogeneous distribution of charges tends to disappear as the reaction centers near the back electrode become saturated (i.e., a charge separation has occurred), as well as those at the front electrode. There can be no more than one charge pair per photosynthetic unit, and this effect thus tends to cancel out the charge inhomogeneity at very high light intensities. This is one of the major differences between the simple crystal (solid state case) and chloroplasts. Furthermore, the chloroplasts are present as particles in a suspension; thus the Dember effect appears to manifest itself within each particle, and the overall macroscopically observed effect is due to the diffusion of ions within each particle, which in turn manifests itself by a diffusion of ions within the bulk solution, and thus to an observable photo e.m.f.

As pointed out previously [8], the sign of the illuminated electrode is negative. The photo e.m.f. sign convention used in ref. 8, and which is adopted here also, is that the illuminated electrode is considered to be negative when negative ions within the sample are moving away from this electrode (Fowler, C.F., private communication). Within the context of the Dember model, the negative polarity of the photo e.m.f. indicates that $\mu^- > \mu^+$. Thus, the negative ions at the outside surface of the chloroplast are more mobile than the positive ions. It should also be noted, that the experimental results are also consistent with $\mu^+ = 0$, whereas $\mu^- \neq 0$.

According to the Dember model, the photo e.m.f. vanishes when there is no longer a charge density gradient along the direction x. This can be due to either a delocalization of negative carriers resulting from diffusion along x or to a recombination of negative and positive carriers across the membrane

[8,13,14]. The disappearance of the photo e.m.f. with time following the excitation flash depends on the viscosity of the suspension and is thus due to the diffusion of negative ions, as long as this charge delocalization time is shorter than the recombination time of charges across the membrane. The Dember model is thus completely consistent with the experimental results of Witt and Zickler [13,14] and of Fowler and Kok [8].

Conclusions

Our measurements of the photo e.m.f. using oriented chloroplasts and polarized light indicate that this phenomenon, while relate to the separation of charges within the thylakoid membranes, is not directly related to either the direction or the magnitude of the transmembrane electrical field. While our results are totally consistent with the existence of such a transmembrane electric field, they do not demonstrate its presence as proposed earlier [8,13,14].

The photo e.m.f. is qualitatively interpreted as being due to the Dember effect. This phenomenon arises because of an inhomogeneous absorption of light within a chloroplast particle which gives rise to an inhomogeneous distribution of positive and negative charges along the direction defined by the propagation vector of the light (which is parallel to the vector joining the two electrodes). An e.m.f. arises due to the diffusion of these charges, but only when the mobility of the two charge carriers is unequal.

In this paper we have limited ourselves to a qualitative interpretation of this effect. A quantitative explanation of this phenomenon should also be possible, but must take into account the saturation of the reaction centers with increasing light intensity, the discontinuous variation of the light intensity within the membrane stacks, and the fact that the photo e.m.f. is a result of phenomena produced within particles which are well separated from each other in the aqueous suspension.

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