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Two Effects of Electrical Fields on Chloroplasts[1]

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ABSTRACT

An electrical field across a suspension of *Chenopodium* chloroplasts stimulates the emission of delayed light during the time the field is on. This stimulation can be used to calculate the distance over which the electron moves in the untrapping process that gives the delayed light. An electrical field applied at the time of illumination gives a polarization to the suspension of chloroplasts that lasts for some seconds. This polarization is a new way to study delayed light and fluorescence from chloroplasts.

Several years ago, at the Gatlinburg Conference on the Photosynthetic Unit, we described the stimulation of delayed light from chloroplasts by electrical fields (2). That observation was reminiscent of one made in the 1920s by Gudden and Pohl (4) that a few kv/cm could stimulate the "phosphorescent light" from crystals by 10 to 20%. With a suspension of chloroplasts, we observed that only a few hundred v/cm could make the delayed light several times brighter. Thus, this biological analog of the Gudden-Pohl effect was a few hundred times more sensitive to the electrical field than was its simple physical counterpart. The stimulation of delayed light with 60-cycle AC was very fast, and we observed 120 bright flashes/sec. The peak in the delayed light signal was not more than 0.2 to 0.3 msec behind the peak in the voltage.

In this paper we describe a second effect of an electrical field on chloroplasts. We find that an electrical field applied at the time of illumination polarizes the chloroplasts in the sense that an electrical field, with the same polarity as the field applied at the time of illumination, gives much less stimulation of delayed light than does a field with the opposite polarity. The effect can be observed for only a few sec before it is destroyed by rotation of the chloroplasts in the suspension.

MATERIALS AND METHODS

"Broken" chloroplasts were isolated from greenhouse-grown Good King Henry (*Chenopodium* Bonus-Henricus L.) by a variation of the method of Walker (5). Leaves were kept in running water for about 1 hr, after which 50 g were ground in a Waring Blendor by means of three high speed blasts of 5 sec each. The blender contained 200 ml of the following ice-cold grinding medium: 0.02 M tris-Cl (pH 7.4), 0.45 M sucrose, 1 mM MgCl, 1 mM NaCl. The leaf homogenate was squeezed through 16 layers of cheesecloth and centrifuged in four tubes for 10 min at 1,200*g* at 1 C. The pellets were rinsed by pouring over each of them 40 ml of a resuspending medium of 1 mM MgCl and 1 mM NaCl. The resuspending medium was poured off and the pellets were combined in 3 ml of fresh suspending medium. These are called "broken" chloroplasts.

For the experiments, a volume of 0.05 ml of thick chloroplast preparation was diluted in 35 ml of distilled H_2O contained in a 3.2-cm diameter cellulose-nitrate centrifuge tube. This tube was used because when it is new and clear, it gives very low background luminescence.

The electrode assembly immersed in the suspension consisted of two sheets of platinum 2 cm wide, 4 cm long, and spaced 1.59 cm apart by Teflon spacers at the top and bottom.

CALCULATIONS

At Gatlinburg we argued that there were at least four different mechanisms producing the delayed light: (*a*) recombination of holes and electrons; (*b*) untrapping of electrons; (*c*) untrapping of holes; and (*d*) a process involving O_2.

We have now studied the effect of electrical fields on delayed light from a few msec to 5 min after illumination. Over this time range we believe that the delayed light is produced by the thermal untrapping of electrons and holes. If this is so, we would expect the intensity of delayed light at any time to be given by the equation

$$S(00) = NF \exp\left(\frac{-E}{kT}\right) \quad (1)$$

where $S(00)$ is the intensity of delayed light at the time of measurement. The first zero means no voltage was applied during illumination and the second zero means no voltage was applied at the time of measurement. N is the number of photosynthetic units that contain a free hole and a trapped electron, or the number of photosynthetic units that contain a free electron and a trapped hole, at the time of measurement. F is the appropriate frequency factor. E is the activation energy in electron volts and $k = 8.6 \times 10^{-5}$ electron volts/degree. T is the absolute temperature.

We believe that the effect of the electrical field is brought about by a change in the activation energy, E. Since the chloroplasts in suspension must be randomly arranged with respect to the electrical field, for some photosynthetic units the activation energy will be reduced and for others it will be increased. If we let $S(0+)$ be the intensity of delayed light at the time of measurement with no electrical field during illumination, the + indicates that a field was applied for the measurement, and if we let ϵ equal the change in activation energy caused by the electrical field when the voltage is applied, and if we make the simple assumption that half of the photosynthetic units are aligned with the field and half are aligned against the field, we can write the following equation (from ref. 3):

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$$S(0+) = NF\left(\frac{1}{2}\exp\left[\frac{-E+\epsilon}{kT}\right] + \frac{1}{2}\exp\left[\frac{-E-\epsilon}{kT}\right]\right) \quad (2)$$

or

$$\frac{S(0+)}{S(00)} = \cosh\frac{\epsilon}{kT} \quad (3)$$

Note: $S(0+)$ and $S(00)$ are measured at the same time in the dark.

In an experiment which we published in 1972 (1), where we had 314 v/cm across the chloroplast suspension, we found $S(0+)/S(00) = 10.1$. From equation 3 we calculated $\epsilon = 0.075$ ev. If we naïvely believe that ϵ is given by the field strength multiplied by the distance over which the electron is transferred in the untrapping act, we find that the distance has to be 2.4 μm. This distance is 160 times bigger than the size of a photosynthetic unit, which we believe to be about 150 Å. We mentioned in the introductory section that the stimulation of delayed light by electrical fields was a few hundred times larger than might be expected. Better calculations making use of the structure of the chloroplasts follow.

In the Gatlinburg paper (3) we pointed out that, in addition to a direct effect of an electrical field on chloroplasts, the delayed light might be stimulated by two other effects; namely, the suspension could be heated by the current flow or the chloroplasts could be affected directly by ions set free by the electrodes. To avoid these two difficulties we used AC voltage, which reduces ion production, and we suspended the chloroplasts in 10^{-4} to 10^{-5} M solutions, which essentially eliminated the heating effect. Under this low osmotic pressure, the outer membrane of the chloroplast is ruptured and the chloroplast expands into a spherical object, 10 to 20 μm in diameter, known as a "bleb." (See left side of Fig. 1.)

The fluorescence microscope reveals that all of the Chl fluorescence is confined to the dark patches on the outside of the bleb. The interior of the bleb shows no Chl fluorescence.

Our tentative explanation as to why chloroplasts are stimulated by such low electrical fields rests on the following assumptions. (*a*) If the wall of the bleb is an electrical insulator, electrostatic theory says that the voltage difference across the bleb is 1 1/2 times the voltage drop in the solution over a distance equal to the diameter of the bleb. (*b*) If the interior of the bleb contains ions, and is therefore an electrical conductor and thus has no voltage drop inside, then the whole voltage drop is across the walls (see right side of Fig. 1).

Although we have watched several hundred chloroplasts unfold to form blebs, we do not know what happens in the process, any more than a child knows where the rabbit was before the magician pulled it from the hat. We think that the stroma lamellae form the large sphere and that the grana discs (thylakoids) are on the outside surface of the sphere. We have not succeeded in removing the grana discs from the blebs. We think that system II photosynthetic units are in or on the grana discs and emit the delayed light.

The diameter of the blebs we have been using varies from 6 to 22 μm; the average size is 13 μm. We used this value to calculate the 1972 experiment.

The voltage across the bleb is $314 \times 13 \times 1.5 \times 10^{-4} = 0.61$ v. This voltage is across a number of membranes. If there is only one grana disc on the surface of the sphere, there are four membranes—two from the bleb and two from the grana disc. If there are two grana discs there are six membranes, etc. The voltage across a single membrane will be 0.153 or 0.102 v.

From the ratio $S(0+)/S(00)$ we found that $\epsilon = 0.075$ ev, $0.075/0.153 = 0.49$, and $0.075/0.102 = 0.73$. This means that a voltage on a suspension of chloroplasts changes the activation energy for delayed light by about one-half of the voltage across a single membrane of a grana disc.

For our new experiments where the voltage is on during the time of illumination as well as at the time of measurement, and when the two polarities are the same, we use

$$S(++) = NF\left(\frac{1}{2}\exp\left[\frac{-E+\epsilon-\Delta}{kT}\right] + \frac{1}{2}\exp\left[\frac{-E-\epsilon+\Delta}{kT}\right]\right) \quad (4)$$

or

$$\frac{S(++)}{S(00)} = \cosh\left(\frac{\epsilon-\Delta}{kT}\right) \quad (5)$$

When the two polarities are opposite we use

$$S(-+) = NF\left(\frac{1}{2}\exp\left[\frac{-E+\epsilon+\Delta}{kT}\right] + \frac{1}{2}\exp\left[\frac{-E-\epsilon-\Delta}{kT}\right]\right) \quad (6)$$

or

$$\frac{S(-+)}{S(00)} = \cosh\left(\frac{\epsilon+\Delta}{kT}\right) \quad (7)$$

Δ is a measure of the asymmetry due to the electric field applied at the time of illumination.

EXPERIMENT

An experiment in which the voltage is applied both at the time of illumination and at the time of measurement is illustrated in Figure 2. A type 1538-A Strobotac was used to illuminate the sample of chloroplasts. The light was filtered by 3.5 cm of concentrated $CuSO_4$ to remove the red light. The rate of flashing was just under 100 flashes/min. The delayed light from the sample was passed through a Corning filter 2403 to remove the blue exciting light.

The intensity of the delayed light was measured with an EMI 9558-B photomultiplier and a Tektronics 545A oscilloscope. The sweep was started at the time of each flash. Figure 2, a time

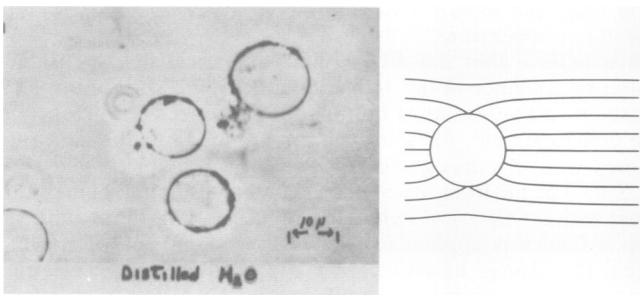

FIG. 1. Left: Photomicrograph of chloroplasts in distilled H_2O showing the "blebs." Right: Drawing of calculated equipotentials around spherical insulator in conducting medium.

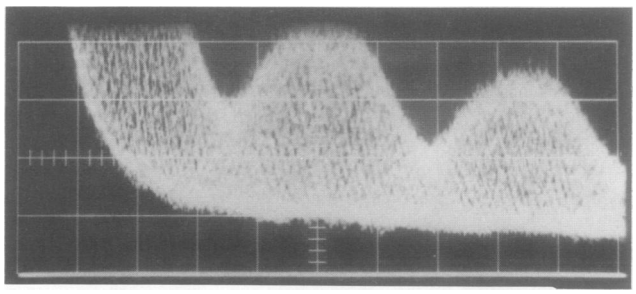

FIG. 2. Time exposure of many traces of oscilloscope. Ordinate: intensity of delayed light. Abscissa: time after flash, 5 msec/division; 100 flashes/min; chloroplasts in distilled H_2O; 250 v rms 60-cycle AC between electrodes.

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exposure photograph of many different traces, each at a different phase, shows the delayed light for the first 50 msec after each flash of exciting light with 250 v rms 60-cycle AC applied to the platinum electrodes. The exact speed of the Strobotac was adjusted so that the phase angle between the flash and the AC was slightly different for each flash from the one before.

The bottom envelope of all of the traces gives the intensity of the delayed light at the instant that the voltage on the sample goes through zero. The top envelope of all of the traces gives the delayed light at the time of peak voltage on the sample.

For 60-cycle AC one period (P) is 16.66 msec. If we measure at ZP, where Z is an integer, we know that the flash occurred at the peak voltage and that it had the same polarity as the voltage at the time of measurement. Therefore, at 16.66 msec, 33.33 msec, and 50 msec, if we divide the distance from the top envelope to the trace at the bottom of the photograph made with the shutter closed (to give zero light) by the distance from the bottom envelope to this zero line, we will have $S(++)/S(00)$.

If we make the measurement at time $(Z + 1/2)P$, then the flash will be at the peak voltage of opposite polarity to that at the time of measurement. The ratio at 25 msec and at 41.66 msec will give us the value of $S(-+)/S(00)$.

These measurements and calculations are presented in Table I.

From the calculations in the table we see that the ratio between ϵ, the change in activation energy, and the calculated voltage across one membrane is much the same as we found in the 1972 experiment in spite of the different conditions. In the 1972 experiment we used long flashes of light, with no voltage applied at the time of illumination. Delayed light was measured 20 sec after the flash with an electrometer to integrate the 120 bright flashes/sec. We used the rms voltage of the AC to calculate the field.

The present experiment was done with short flashes of light on the sample at the peak of the applied voltage. The delayed light was measured at 16.66 to 50 msec after the flash and at the peak voltage. We used the peak voltage of the AC for calculating the fields.

TABLE I

Measurements and Calculations

The measurements are from Figure 2. Equations 5 and 7 are used to calculate ϵ and Δ.

Time after flash (msec)	$\frac{S(++)}{S(00)}$	$\frac{S(-+)}{S(00)}$
16.66	$\frac{28.2}{9.5} = 2.968$	
25.00		$\frac{40.4}{8.7} = 4.644$
33.33	$\frac{20.8}{6.9} = 3.014$	
41.66		$\frac{31.5}{6.5} = 4.846$
50.00	$\frac{17.9}{5.8} = 3.086$	
	Average: 3.023	4.745
	$\frac{\epsilon - \Delta}{kT} = 1.7708$	$\frac{\epsilon + \Delta}{kT} = 2.2389$
	$\epsilon - \Delta = 0.04427$	$\epsilon + \Delta = 0.05597$
	$\epsilon = 0.0501$ ev $\Delta = 0.00585$ ev	
Peak field on suspension	$= \frac{250 \times \sqrt{2}}{1.59}$	$= \frac{222.4 \text{ v}}{\text{cm}}$
Peak voltage on bleb	$= 222 \times 13 \times 1\frac{1}{2} \times 10^{-4}$	$= 0.434$ v
Voltage on one membrane (4 membranes)		$= 0.108$ v
Voltage on one membrane (6 membranes)		$= 0.072$ v
	$\frac{0.0501}{0.108} = 0.464$	$\frac{0.0501}{0.072} = 0.696$

CONCLUSIONS

The results presented in this paper show that an electrical field across a suspension of chloroplasts stimulates the delayed light emission and that, for chloroplasts in distilled H_2O, where we have blebs, the stimulation of delayed light is astonishingly large. The geometry of the bleb concentrates the field across the membranes, and the stimulation which disappears as soon as the field is removed can be explained as a change in the activation energy for delayed light. An electric field applied at the time of illumination makes a change in the activation energy that can be seen for several sec; this "permanent" change in the activation energy is about 12% of the change made by the same field at the time of measurement. This gives a new way to polarize chloroplasts.

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