THE PHOTOCONDUCTIVITY OF CHLOROPLASTS AND THE FAR RED LIGHT EFFECT

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ABSTRACT The photoconductivity of chloroplast films in the dry and the wet state has been investigated. These films were found to behave like typical semiconductors, in agreement with the results of Arnold and co-workers, who have used only dry films. It was found that a steady photocurrent produced in a chloroplast film by blue or red light, can be either stimulated or quenched—depending on the intensity of the illumination—by simultaneous application of far red or near infrared light. This reminds one of the quenching of the photoconductivity of phosphors by infrared light. The interaction of long wave light with light of shorter wavelength in the production of photoconductivity is of interest in relation to the "second Emerson effect," which suggests a similar interaction in photosynthesis.

INTRODUCTION

The studies of Emerson and Arnold (1, 2) and of Gaffron and Wohl (3) indicated that the primary photochemical act in photosynthesis is carried out not by a single chlorophyll molecule, but by cooperation of several hundred such molecules. These results suggested the occurrence in photosynthesis of excitation energy migration over several hundred pigment molecules; in other words, the absorption of light by chlorophyll in a chloroplast seems to lead to the creation of an exciton. Resonance energy transfer accounts also for the experimentally established fact (4-17) that light energy absorbed by accessory pigments is transferred, often with high efficiency, to chlorophyll *a* (or, in purple bacteria, to bacteriochlorophyll B-890).

In 1949, Katz (18) discussed the possibility that chlorophyll molecules in the chloroplasts may form a two-dimensional crystal, possessing conduction bands in which excited electrons or holes could migrate. In 1955, Bassham and Calvin (19) discussed the same idea and pointed out that a semiconducting structure could facilitate spatial separation of the oxidizing and reducing agents—free electrons and holes—and that this may help photosynthesis by preventing back reactions between these agents.

In 1956, the investigations of Commoner, Heise, and Townsend (20) and of Sogo, Pon, and Calvin (21) gave evidence of the formation of unpaired electron spins in illuminated spinach chloroplasts, while Arnold and Sherwood (22-24) found in 1957 that dried chloroplast films exhibit thermoluminescence as well as other typical properties of semiconductors.

In addition, studies by Arnold, Strehler, and co-workers (25-28) have demonstrated the occurrence of a temperature-dependent, long-lived phosphorescence in algae and in chloroplasts, which could be attributed to a slow recombination of trapped electrons and holes.

However, the concept of photosynthesis as a "solid state process" has been opposed, particularly by Rabinowitch (17), mainly for the following reasons.

The chlorophyll monolayers, which appear to be present in chloroplasts, are *not* crystalline (as suggested by Katz). If they were, the absorption band of chlorophyll *in vivo* would lie beyond 730 mμ, and not at 675 to 680 mμ, in a position typical of chlorophyll in the amorphous state. The absence in the spectrum of chlorophyll *in vivo* of an absorption band, which could be attributed to transition into a conductance level, also argues against the assumption that electron excitation into such a level is the primary result of light absorption. Furthermore, in a monomolecular layer, the separation of the primary oxidation product from the primary reduction product does not require electric "conduction" over distances longer than the diameter of a single molecule; therefore, it could be achieved by a mesomeric transformation (electron loss on one end of the chlorophyll molecule and electron acquisition at the other end; the cycle can be closed by a tautomerization).

Rosenberg (29) objected to Rabinowitch's argument by quoting observations of strong photoelectric currents in noncrystalline hydrocarbon melts, and the absence of a separate band leading into a photoconducting level in organic photoconductors.

Rosenberg suggested that the long-lived triplet levels of molecules in a noncrystalline solid can merge into a conductance band, while the short-lived singlet excited levels remain associated with individual molecules; photoconductance may then result from a secondary singlet-triplet transformation and not affect the absorption spectrum.

The alternative: exciton (*i.e.*, electron *and* hole) migration in illuminated chloroplasts *versus* separate migration of electrons and holes, thus remains open. The following experiments are a contribution to the study of this problem. They confirm the observations of Arnold and Sherwood on dry chloroplast films and add some data on wet chloroplast films. The main new finding is the effect of far red light ($\lambda > 700$ mμ) on the photocurrent produced in dry chloroplast films by light of shorter wavelength.

MATERIALS AND METHODS

Spinach chloroplast fragments were prepared by the method of Spikes (30).[1] The chloroplasts, sedimented in the final centrifugation, were rinsed twice with glass-distilled water, resuspended in this medium, frozen rapidly at −78°C, and lyophilized. The material was then stored at −25°C in the dark.

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[1] Obtained from Dr. Bishop.

To prepare the films, chloroplasts were resuspended in a small amount of water, the suspension painted onto a copper plate (2 X 3 cm), and dried in a stream of nitrogen. The chloroplast-painted plates were kept in a desiccator over drierite.

Sandwiches, 3 X 2 cm in size, were made from a copper electrode carrying the chloroplast film (about 0.2 mm thick), and a glass plate with an electrically conducting layer facing the chloroplast film (obtained from Liberty Mirror Division, Libby-Owens-Ford Glass Co.) and serving as the second electrode. The use of a transparent glass electrode permitted the illumination of a large area of the chloroplast film.

Fig. 1 is a schematic diagram of the apparatus used for the investigation of photoconductivity.

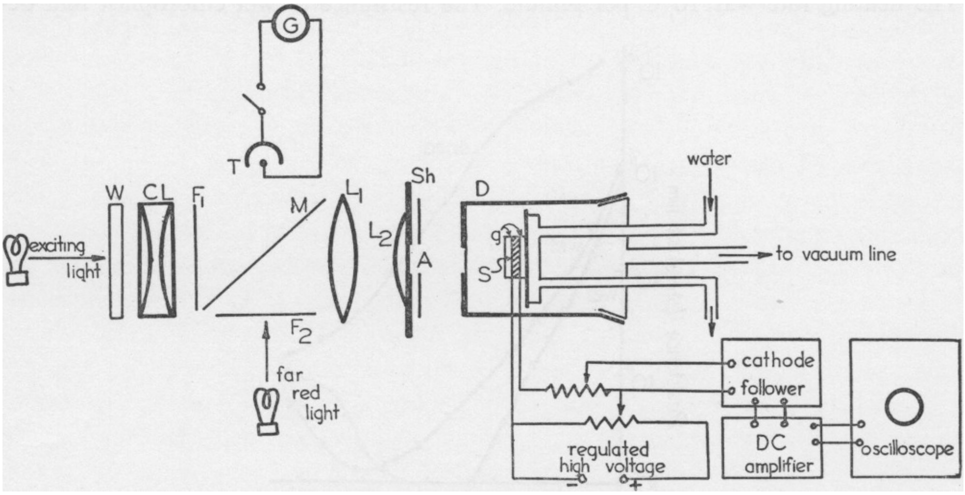

FIGURE 1 Block diagram of apparatus. L_1, L_2, lenses; *W*, 5-cm water cell; *CL*, condenser lens; *T*, thermocouple; F_1, F_2, filters; *M*, 50 per cent transmittance mirror; *G*, galvanometer; *S*, sample; *Sh*, shutter with aperture *A; D*, Dewar vessel; *g*, glass conduction electrode.

The photocurrent generated by light passed through a resistor ranging from 10^8 to 10^{10} ohms, and the voltage developed across the resistor was fed through a cathode follower and a D.C. amplifier (gain = 10^6) into an oscilloscope. In this way, it was possible to follow the decay curve of the photocurrent from about 10 μsec. to about 100 sec. after the light was switched off. The oscilloscope sweep was triggered by the photosignal; thus, it was possible to observe the rise of the photocurrent as well as its decay.

For the measurement of steady state photoconductivity, a 1000-watt incandescent lamp was used. The filament of the lamp was projected on the chloroplast film by a glass condenser lens. A water layer 5 cm thick served to absorb infrared light above 1.4 μ.

Action spectrum measurements were made with the same system by using a Bausch and Lomb grating monochromator.

The chloroplast films were illuminated parallel to the direction of the applied field. A screen with an aperture for the passage of light separated the lamp and the condenser from the glass vessel containing the chloroplast films. In order to protect the films from scattering light, the glass vessel was painted with black ink, with a hole opposite the film layer.

For the measurement of far red light effect on steady state photocurrent excited in chloroplast films by blue or red light, a Corning color filter (No. 7-59) was used in combination with a narrow band pass filter 2412, and Bausch and Lomb 2nd order filter.

RESULTS AND DISCUSSION

1. *Dark Conductivity of Chloroplasts.* The apparatus can easily measure 10^{-15} ampere flowing through the 10^{10}—ohm resistor. This permitted the measurement of the dark current caused in the chloroplast films by a potential of 1000 volts. Fig. 2 shows the resistance of dry and wet films as a function of temperature. The heating rate was 10°C per minute. The resistance of wet chloroplast film decreased in a way typical of semiconductors, but later increased again, probably because of the loss of water by heating (which was carried out in an atmosphere saturated with water vapor at 20°C).

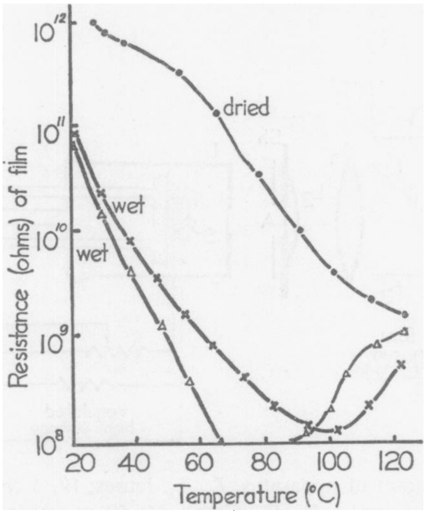

FIGURE 2 Resistance of dried and wet chloroplasts as a function of temperature.

The specific resistance of dried films was never less than 10^{12} ohms at room temperature (20°C); but that of the wet films ranged from 10^{11} to 10^{8} ohms, depending upon the amount of water in the films.

Experiments showed that the conductivity of dry chloroplast films depends not only on temperature but also on the duration of the passage of the dark current, often manifesting hysteresis.

On the whole, the dark conductivity of chloroplast films behaves like that of other organic semiconductors, as already shown by Tollin and Kearns (31).

2. *The Photoconductivity of Chloroplasts.* When dry chloroplast films ($R_d \cong 10^{13}$ ohms) were illuminated *in vacuo* with an incandescent lamp, with a potential of 1000 volts applied to the electrodes, a photocurrent was observed, the

strength of which depended upon the temperature and the intensity of illumination. The direction of the field applied to the chloroplast films did not influence the current, either during the illumination or after it was stopped. No photocurrent was observed when the two electrodes were illuminated without a chloroplast layer between them. Increasing the thickness of the chloroplast film up to 0.5 mm caused the current to rise.

3. *Action Spectrum.* When the chloroplast film was illuminated with monochromatic light obtained from a Bausch and Lomb grating monochromator, the maximum photocurrent was produced by wavelengths corresponding roughly to the visible absorption bands of chlorophyll in the chloroplast. Fig. 3 shows a typical action spectrum of the steady state photoconductivity in a dry chloroplast film at room temperature, at a light intensity of 10^{15} quanta per second per 6 cm^2. In this curve, the peak of the conductivity curve appears at or above 700 mμ, whereas the absorption peak lies at 685 mμ; it remains to be seen whether this shift is significant; if so, it could be taken as evidence that photoconductivity is due to the chlorophyll *a* component absorbing at or beyond 690 mμ—perhaps a polymeric form of the pigment (32).

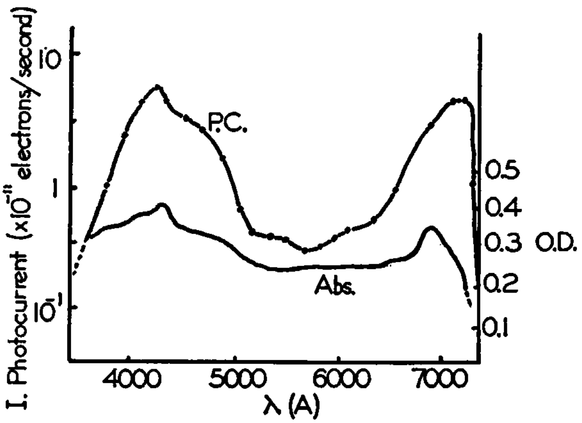

FIGURE 3 Action spectrum of the photoconductivity of chloroplast (at room temperature). Intensity of exciting light: 10^{15} quanta/sec/6 cm^2.

4. *Dependence of the Photocurrent upon Duration of Illumination and Its Decay.* Fig. 4 is a typical curve showing the current as function of time during and after the illumination.

At the beginning of illumination, the conductivity of the dry film *in vacuo* rises approximately linearly. After reaching a maximum, the photocurrent does not become constant, as expected for a pure photoconducting crystal, but begins to decline while the light is still on.

The time required to reach the maximum photocurrent depends upon the thickness of the film, the intensity and spectral composition of light, and the temperature of the film. The rise and decay times were several times longer than those reported for pure chlorophyll (33). When the light was turned off, the current decreased to a steady level somewhat lower than the initial one (Fig. 4); the latter was re-established if the film was left in the dark for several hours.

The maximum photoconductivity of the chloroplast film did not depend upon previous illumination of the layer; but an after effect was manifested by more rapid establishment of maximum conductivity after repeated illumination (Fig. 5). This proves that the internal state of the pigment in the chloroplast film may be different even if photoconductivity is the same during illumination or after it.

The dependence of the decay on temperature has been investigated from 0° to

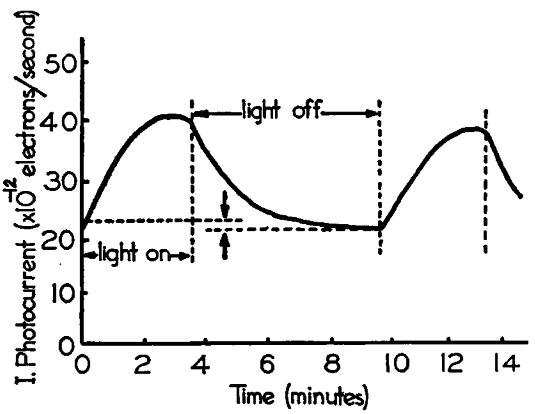

FIGURE 4 Dependence of the photoconducivity upon the duration of illumination and decay of the current after the illumination is stopped (at room temperature). Intensity of light: 10^{15} quanta/sec/6 cm^2.

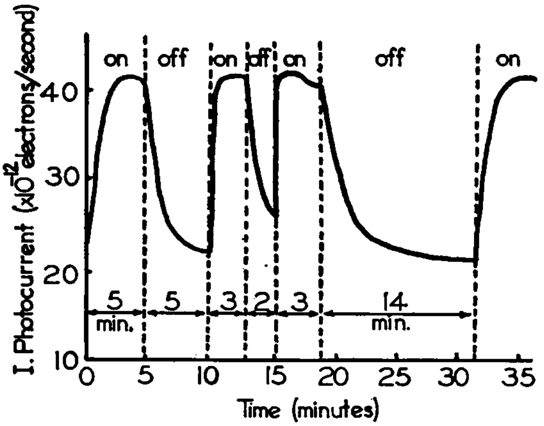

FIGURE 5 Independence of the maximum conductivity of chloroplast films from after effect.

50°C (Fig. 6). (The maximum conductivities at different temperatures were arbitrarily set at 100.) With rising temperature, the decay is accelerated.

5. *Dependence of the Photoconductivity upon Water Content of the Films.* If dried chloroplast films are wetted with a drop of water, the dark current increases, but the photocurrent decreases. Fig. 7 shows that the rise of the photoconductivity in a wet chloroplast film is faster than in a dry film; after the maximum photoconductivity is reached, photocurrent does not decline while light is still on, as it does in a dry film. This suggests that the excitation of conduction electrons may go in dry and in wet films through different excited states, as suggested by Franck (34).

The photoconductivity decreases with increasing water content of the chloroplast

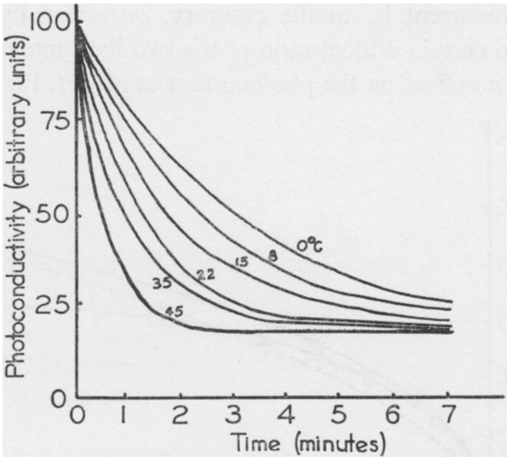

FIGURE 6 Decay of the photoconductivity of chloroplast films at different temperatures.

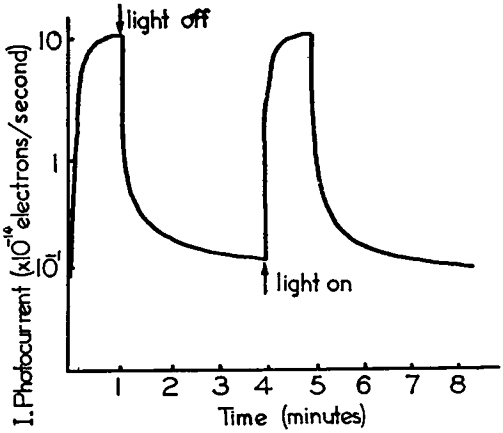

FIGURE 7 Time dependence of the photoconductivity of wet chloroplast of films.

films; at a certain content, no photocurrent is observed at all. It can be surmised that photoconductivity occurs as long as all water is "bound water," but disappears when some of it becomes "free water." Introduction of water molecules into chloroplast films may interrupt the ordered hydrogen bond structure in the protein, leading to the destruction of the conductance band.

6. *Influence of Far Red or Infrared Light on the Photoconductivity of Chloroplasts.* A change in the photocurrent caused by actinic light in a chloroplast film can be produced by simultaneous illumination of the film with far red or near infrared light, which in itself produces no photocurrent. As long as the actinic "short wave" light is comparatively weak, a *stimulation* of photocurrent by far red or near infrared light is observed, but if the intensity of the "short wave" light is increased, the photocurrent is, to the contrary, *quenched* by near infrared light ($\lambda > 850$ mμ). For a certain critical ratio of the two light intensities, the addition of light > 850 mμ has no effect on the photocurrent at all (*cf.* Fig. 8). Far red light of wavelength > 750 mμ produces a stimulation effect even if actinic light is relatively strong. The photoconductivity due to weak visible radiation can be stimulated, by 10 per cent or more, by the addition of far red or near infrared radiation.

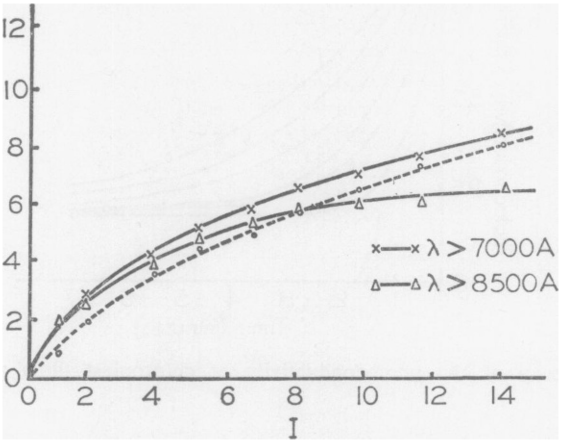

Figure 8 Dependence of photocurrent on intensity: dotted line . . . exciting light only; solid line ——, simultaneous illumation with far red light at $\lambda > 7000$ A; *I*, intensity of exciting light in arbitrary units; *i*, photocurrent in arbitrary units.

In these measurements, the chloroplast films have been made conductive by weak illumination with blue (430 mμ) or red (680 mμ) light obtained by means of an interference filter; after the photocurrent has reached a stationary value, the effect of additional illumination with far red or near infrared light of constant energy has been measured. The energy has been kept constant over the whole investigated region by determining in advance the lamp current needed to produce a given energy flux at the different wavelengths.

Fig. 9 shows the time dependence of the increase and decrease of photoconductivity, produced by visible light, as the >700-mμ light of different intensity (marked in arbitrary units) is applied for 30 sec. and then cut off. With increasing intensity of long-wave radiation the saturation is reached faster. No similar acceleration was observed in quenching.

When quenching occurs, one observes first a rapid rise in photocurrent immediately after the far red light is applied, and later, a slower decay as shown in Fig. 10. The transient stimulation becomes more significant with decreasing temperature, but disappears at temperatures above 35°C. This suggests that the transient stimulation is due to electrons ejected into the conduction band from shallow traps, which are not stable at temperatures higher than 35°C.

CONCLUSION

This paper gives experimental evidence that a purely electronic primary photo-

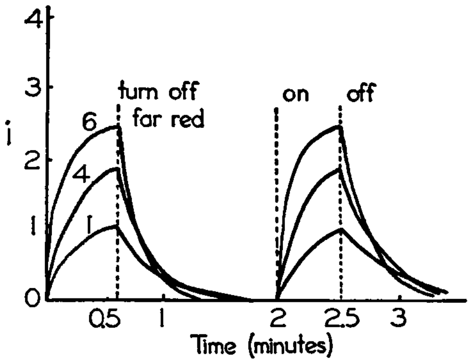

FIGURE 9 Time dependence of the increase and decrease of photoconductivity (i) of chloroplasts by additional illumination with far red light.

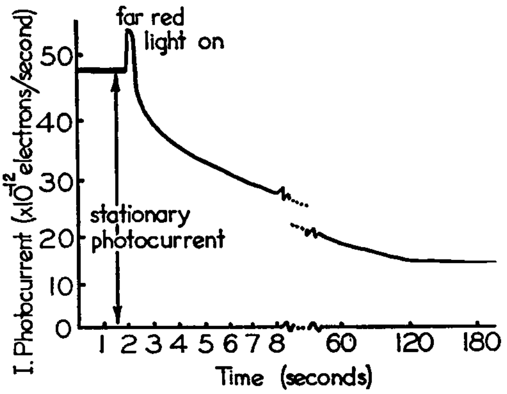

FIGURE 10 Transient change of photoconductivity and its decay resulting from the quenching by far red light.

physical process can occur in chloroplast films. These results are in close agreement with those obtained by Arnold and co-worker.

The main new finding of the present paper is the influence of far red light on photoconductivity of chloroplasts caused by light of shorter wavelength. The photocurrent produced by weak blue or red light can be stimulated when far red or near infrared light ($\lambda > 850$ mμ) is applied simultaneously. The near infrared light can cause quenching instead of stimulation when added to stronger blue or red light. In the case of quenching, a transient increase in photoconductivity immediately after the application of far red light is followed by decay. Many authors have reported quenching of photoconductivity of inorganic phosphors by infrared light, as well as some stimulation phenomena of a transient character (35–39). To explain the rapid transient stimulation, followed by quenching, as observed in CdS, Rose (40) has proposed a model which involves the creation of free holes by infrared radiation.

The dependence of the stimulation or quenching effect on the ratio of the intensities of the exciting light and of the infrared supplementary light, found in the present experiments, could be explained perhaps by using Rose's model. Further study of these phenomena may assist in the understanding of the energy migration in the photosynthetic unit and of the role of electron migration in it.

The interaction of long wave light with light of shorter wavelength in the production of photoconductivity is of interest in its possible relation to the "second Emerson effect" which suggests a similar interaction in photosynthesis (*cf.* Govindjee and Rabinowitch (41)).

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