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PLANT MATERIAL

Power B. Sogo, Ning G. Pon, and Melvin Calvin

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Abstract

Photoinduced electron spin resonance signals have been observed in isolated chloroplasts and other green materials with a growth time not affected by reducing the temperature to -140° . This is interpreted in terms of conduction-band and trapped-electron theory.

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Introduction

In a recent paper,¹ the electron-spin resonance spectra obtained from light-induced paramagnetism in chloroplast suspensions were described and a discussion of the possible role of these unpaired electrons in the processes of photosynthesis was given. We have verified the essential features of the results reported above and have made new measurements in an attempt to more closely establish the origin of the signal. The characteristic features of the resonance absorption are: the g value, the growth and decay times, the steady-state concentration of the absorbing centers, the quantum efficiency for the production of the absorbing centers, and the shape of the spin resonance spectrum. The determination of the identity of the absorbing species, as well as the path of its formation, would seem to require, first: a knowledge of the precise dependence of the above quantities on such variables as temperature, light wavelength and intensity, sample preparation, and sample environment; and second: the correlation of such data with the results of chemical and kinetic studies of photosynthesis. It is hoped that in this way a fundamental insight into the primary processes of energy conversion in green plants will be gained. This paper describes a series of exploratory experiments in which the primary aim was the determination of the relative importance of several variables in affecting the behavior of the signal, in particular temperature, light intensity, and sample preparation.

*The work described in this paper was sponsored by the United States Atomic Energy Commission.

Methods

In these experiments a total weight of 250 g of spinach leaves was required for the preparation of whole chloroplasts and smaller fragments. The leaves were washed with distilled water and the excess water was shaken off. All subsequent operations were carried out at, or near, 0°C.

In a typical experiment, 125 g of fresh spinach leaves was sliced into thin strips and ground for 30 seconds in a blender containing 250 ml of 0.5 M sucrose that is at pH 6.8 and is 0.03 M with respect to potassium phosphate. The homogenate was filtered through six layers of cheese cloth. The filtrate was centrifuged at 200 x g for 5 minutes to remove whole cells and cell debris. The supernatant was carefully decanted and centrifuged at 600 x g for 12 minutes. The supernatant was decanted and saved for the preparation of the small chloroplast fragments, while the precipitate was resuspended in about 200 ml of fresh sucrose solution. A rubber policeman attached to one end of a glass rod was used to stir the mixture so as to obtain an even suspension. The suspension was centrifuged again at 600 x g for 15 minutes. The supernatant was discarded and the precipitate was collected by resuspension in a small volume of fresh sucrose solution and centrifugation at 20,000 x g for 5 minutes. The supernatant was quickly decanted and the packed precipitate so obtained was designated as "whole chloroplasts."

One half of the whole chloroplasts was suspended in about 10 ml. of pH 6.8, 0.05 M phosphate buffer and the suspension was allowed to stand overnight at 0°C. The mixture was centrifuged at 10,000 x g for 10 minutes. The supernatant was discarded while the precipitate was washed once again with phosphate buffer and collected by centrifugation at 10,000 x g for 10 minutes. The supernatant was discarded and the tightly packed precipitate was designated as "large chloroplast fragments."

For the preparation of the small chloroplast fragments, the 600 x g supernatant was centrifuged for 1 minute at 20,000 x g to remove any remaining whole chloroplasts. The light green supernatant was decanted and centrifuged at 20,000 x g for 15 minutes. The supernatant was quickly decanted. The loosely packed green precipitate was designated as "small chloroplast fragments." The latter probably contain, in addition to small fragments of chloroplasts, mitochondrial particles. A flow diagram of the preparations is given in Fig. 1.

All the above preparations were kept at 0°C until ready for spin resonance studies. The spin resonance spectra were obtained with a transmission-cavity spectrometer operating at a frequency of about 9.3 kMc/sec with a bolometer for the detector.² Magnetic field modulation at a frequency of 165 cpm and a conventional lock-in amplifier are employed for the display of the derivative of the absorption on the chart recorder. Frequency stabilization of the Varian V-58 klystron on the peak of maximum cavity transmission is effected with a frequency-modulation feedback system.

The variable-temperature probe is shown in Fig. 2. The samples were painted on the surface of a silver-plated copper rod, 5/32-in. o. d., that was inserted into a clear quartz dewar. The completed assembly was then placed in the cavity resonator. This change in cavity geometry converts the TE₀₁₁ mode right cylinder into a TE₀₁₁ coaxial cylinder with a small decrease in cavity Q. However, the resulting favorable sample shape, with a large ratio of surface area to volume, together with the small value of the electric field throughout the sample volume, more than compensates for the disadvantage of the drop in Q. The sample temperature was changed by immersing the bottom end of the rod in a suitable bath, and the resulting temperature was monitored by a thermocouple attached to the rod in the vicinity of the sample.

The light source consisted of a 200-watt tungsten-filament projection lamp. A Corning No. 3480 sharp-cut filter in conjunction with a special water-cooled Corning infrared filter served to restrict the band width of the incident light to the wavelengths lying between 5800 Å and 8000 Å. A pair of condensing lenses was used to collect and focus the light on the 1-cm hole at the end of the waveguide tee. After traversing the length of the tee, the light entered the cavity through a 6-mm iris.

Results

A summary of the results is given in Table I. Eucalyptus leaves showed a faint light dependent signal when they were air-dried to a proper state of moisture content. This proper state is quite critical and the results were poorly reproducible. The dried whole chloroplasts were prepared by allowing a wet suspension painted on the sample rod to dry at room temperature for two days. The signal growth and decay times for the wet, large chloroplast fragments were obtained by setting the magnetic field on a peak of maximum

Table I

Electron spin resonance observations on various samples from photosynthetic material. At room temperature, the width between points of maximum slope is approximately 10 oersted; at -140° , the width between points of maximum slope is approximately 15 oersted. Light quality: $5800 < \lambda < 8000 \text{ \AA}$. Resonance g value is 2.00.

Substance	Light Intensity	Temperature	Signal Growth Time	Signal Decay Time
Dried leaves	low ^a	25°	minutes	hours
Dried whole chloroplasts	low ^a	25° 60 ^d	minutes seconds	hours seconds
Wet whole chloroplasts	low ^a	25° -140°	seconds seconds	~1 minute hours
Wet small chloroplast fragments	low ^a	25°	seconds	minutes
Wet large chloroplast fragments	low ^a high ^b high ^b	25° 25° -140°	~30 seconds ~6 seconds ~10 seconds	~30 seconds ~30 seconds hours ^c

^a Low light intensity: approximately 10^{15} quanta/sec into cavity; the number of free electrons at equilibrium was approximately 10^{16} .

^b High light intensity: approximately 10^{16} quanta/sec into cavity; the number of free electrons at equilibrium was approximately 10^{16} .

^c The signal began to disappear rapidly when the temperature was raised to about $>50^{\circ}$.

^d This seems to correspond to the temperature at which there is a peak in the thermoluminescence curve. Observed by G. Tollin in this laboratory in an experiment similar to that described by W. Arnold and H. K. Sherwood.³

slope and recording the change in this peak height as a function of time. Representative traces are shown in Fig. 3. The instrumental time constant was set at 2 sec in these measurements. The growth and decay times for the other samples were obtained by repeated scanning of the resonance absorption, and their order of magnitude is given.

The filtered light intensity passing through the cavity iris was estimated for two optical configurations with a General Electric type DW-60 radiation meter, and the steady-state number of unpaired electrons was obtained from a comparison of the product of (signal height) \times (signal width)² to the corresponding product from a standard sample of DPPH. The results indicate that the fastest growth times obtained are still limited by the intensity of the incident light. Thus, the time constant of the signal-producing process is almost certainly shorter than 6 seconds. The quantum efficiency for the photoproduction of the absorption centers appears to be quite high; although the absolute measurements are crude, probably 1 to 10 quanta are required for each absorption center.

Typical resonance curves of a sample of wet large chloroplast fragments are shown in Fig. 4. A change in the shape of the light-induced signal as a function of the sample temperature is evident. The room-temperature resonance has some of the characteristics of exchange-narrowed lines, while the low-temperature resonance is considerably broader and more asymmetric. This behavior may be the effect of anisotropic crystalline or molecular fields that interact weakly via spin-orbit coupling with the unpaired electron in the frozen solid and are partially averaged out by thermal motions at room temperature. The residual signal in the dark differs in shape from the light-induced signal, and it probably arises from a different species of unpaired electrons. It is interesting to note that the relative intensity of the residual signal may be considerably reduced by heating at 40° for a few minutes. No apparent destruction of the samples is caused by the rapid cooling to -140°. When such frozen samples are allowed to warm up to room temperature, their behavior was the same as that of samples kept continuously at room temperature.

Discussion

Some years ago, a proposal was made concerning the nature of the early processes in the transformation of electromagnetic energy into chemical potential.⁴ This proposal involved an ordered array, on a microscopic scale,

of chlorophyll and collateral molecules. The sequence of events was to be an absorption of light leading to the first excited singlet state of chlorophyll, which then was converted into a triplet excitation, followed by an ionization process that would lead to a trapped electron and a hole, these two entities being the reducing and the oxidizing components that must be simultaneously generated. A part of the process involved the extremely rapid neutralization of the hole by the capture of an electron from water, or some product formed from it. (A very similar concept of the semiconductor qualities of the chloroplast was proposed on the basis of fluorescence studies quite independently by Katz.⁵) The resultant material would then ultimately appear as a molecular oxygen. The electron, on the other hand, would pass through a series of carriers (hydrogen carriers) such as are well known in biochemical processes (thioctic acid, pyridine nucleotide, flavin, etc.), ultimately leading to the reduction of carbon dioxide.

According to this scheme, one should expect at least three seats of electronic paramagnetism to occur, namely, the triplet state of chlorophyll, the trapped unpaired electrons, and the radicals of the semiquinone type among the hydrogen carriers on the path between the trapped electron and carbon dioxide. Within experimental error, the signal growth time for frozen wet large chloroplast fragments at -140° appears to be the same as the growth time for the corresponding fluid sample at 25° . This fact seems sufficient for ruling out the ordinary enzymatic reactions as intermediates in the signal-producing process. Thus, according to the above scheme, we are left with the chlorophyll triplet, the trapped electron, and, additionally, some species of free radical resulting from the direct dissociation of a chemical bond in the absorption act. Although it is possible to suppose that the cooling would enhance the lifetime of the chlorophyll triplet to the extent of hours, it does not seem likely. We are thus left with the trapped electron and the possibility of a dissociated bond.

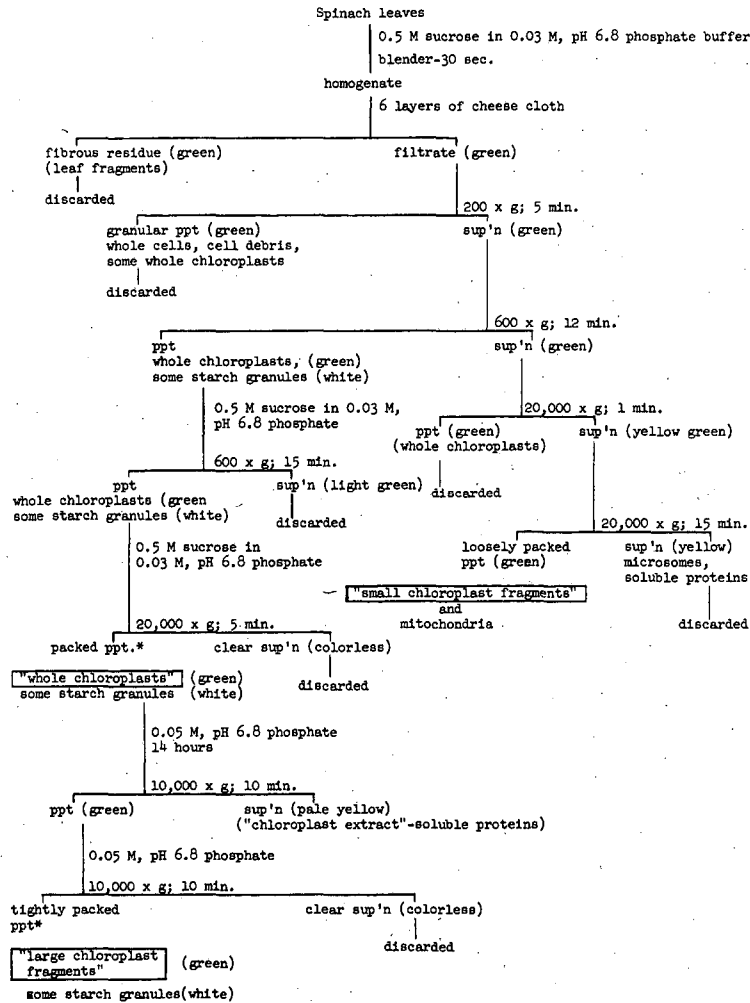
It is perhaps worth noting that whatever the nature of the unpaired electron producing this signal, its coupling with the lattice around it must be rather poor in order to produce a signal as narrow as the one we see, suggesting its location in a rather delocalized pi-type of orbital. It is to be expected that improvements in technique will lead to a more precise identification of the variety of unpaired electrons which almost certainly result from the illumination of the photochemical apparatus in plants.

References

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5. E. Katz, Photosynthesis in Plants, edited by W. E. Loomis and J. Franck (Iowa State College Press, Ames, Iowa, 1949), Chapter 15, p. 291.

Figures

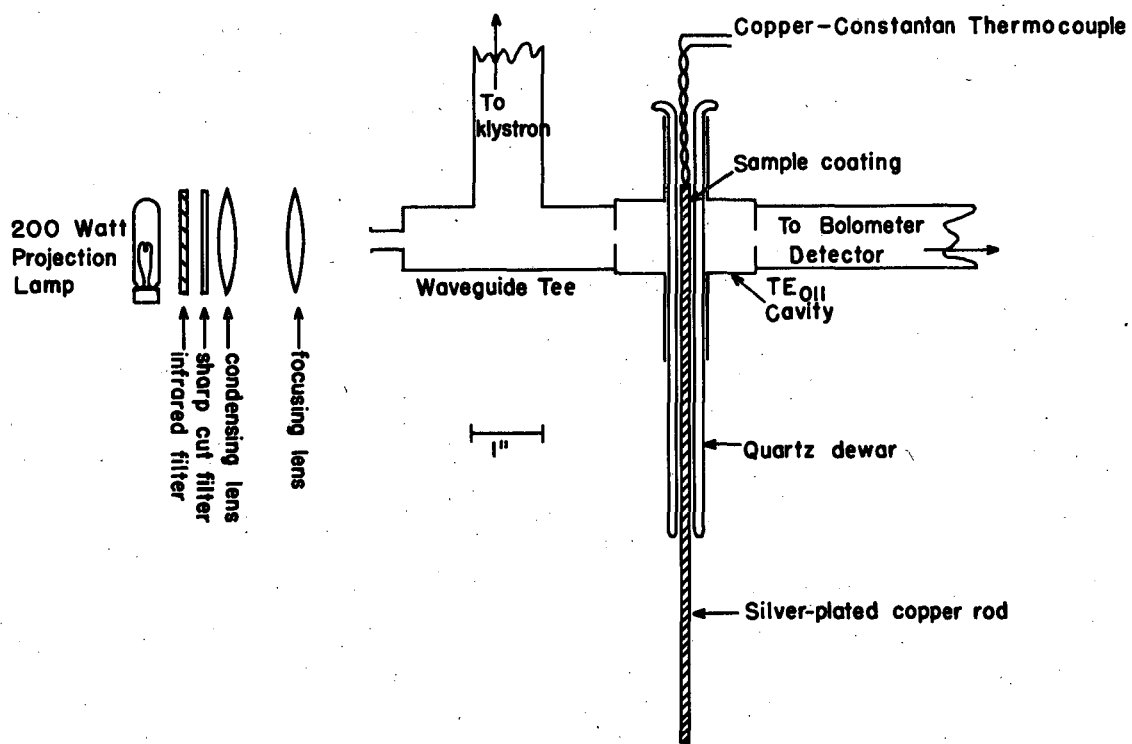
- Fig. 1. Flow diagram for the preparation of chloroplasts and fragments.
All operations are carried out at, or near, 0°C.
- Fig. 2. Variable-temperature irradiation apparatus.
- Fig. 3. Signal growth and decay time curves.
- Fig. 4. Spin resonance spectra from wet large chloroplast fragments.



* Samples were taken by skimming material from the top, white starch material was at the very bottom of the centrifuge tube.

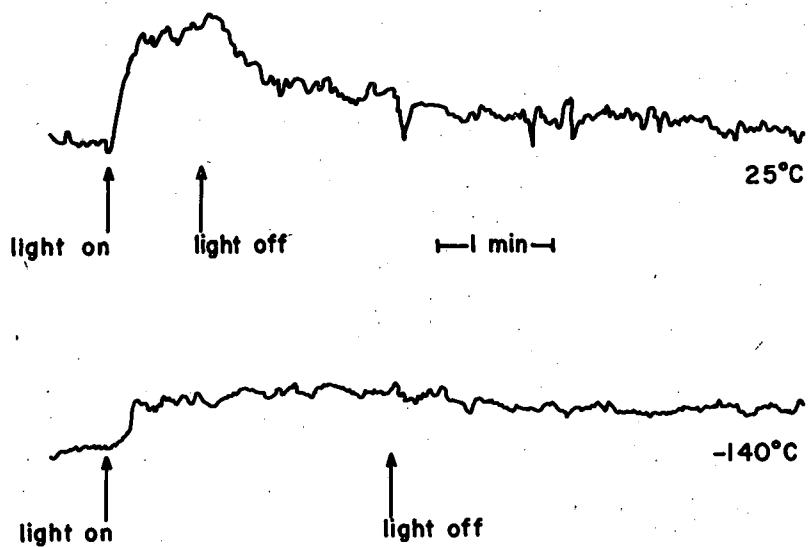
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Figure 1



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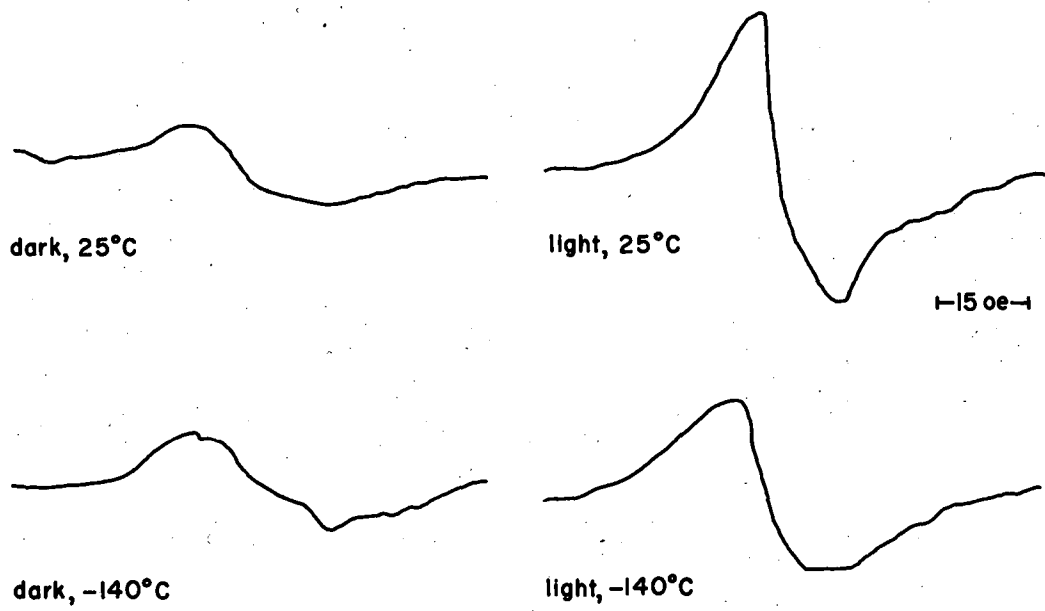
Figure 2



Signal Growth and Decay Time Curves

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Figure 3



Spin Resonance Spectra from Wet, Large Chlorophyll Samples

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Figure 4