LIGHT SATURATION STUDIES ON THE ELECTRON SPIN RESONANCE SIGNALS OBSERVED WITH CHLORELLA PYRENOIDOSA

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The light-induced electron spin resonance signal observed with Chlorella pyrenoidosa cells has been shown by Commoner et al. (1957) to exhibit fine structure indicating the presence of more than one type of unpaired electrons upon illumination. Others have confirmed this finding, and report that there are two signals superimposed--one a sharp, fast decay signal (signal I) of about 11 gauss width and g = 2.002 and a second broad, slow decay signal (signal II) of about 19 gauss width and g = 2.005. Allen et al. (1961) postulate that these signals are dependent upon chlorophyll b and a respectively. This paper reports light saturation studies made with white light on these two signals with whole and sonicated Chlorella pyrenoidosa cells.

Fig. 1 relates the amplitude of each component of the light-induced ESR signal to light intensity. Signal II approaches saturation at about 1500 foot-candles light intensity, and actually decreases at higher intensity. Signal I, however, is small in this range, shows a marked response to light intensity after signal II saturates, but does not saturate at the highest light intensity used (17,000 foot-candles). The normal intensity range for growing Chlorella is below 1500 foot-candles, where the broad signal is predominant. Much higher light intensities are required to produce corresponding amplitudes of the sharper signal.

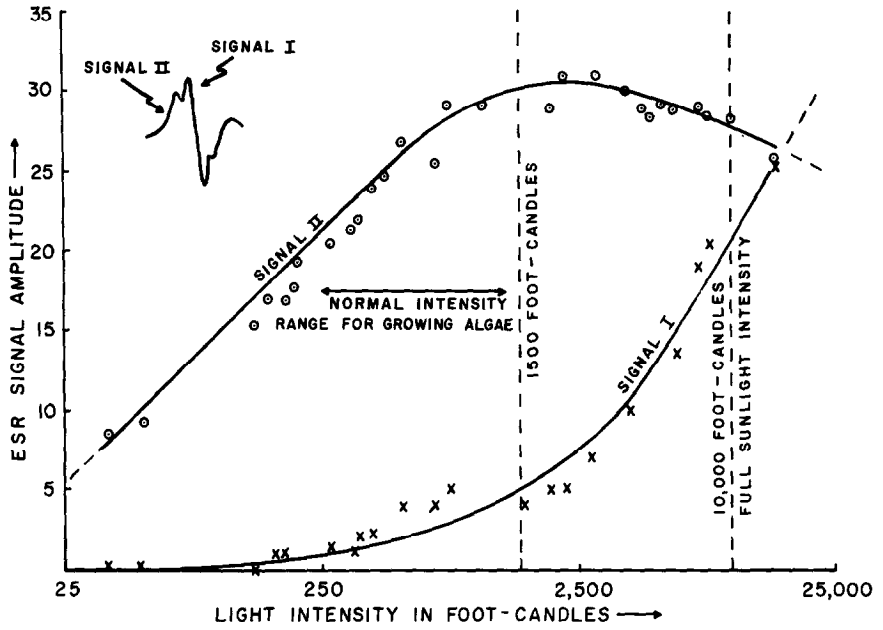

Figure 1. Light-induced ESR Signal Amplitudes as a Function of Light Intensity for Whole Cells of Chlorella pyrenoidosa.

Light intensity is plotted on a logarithmic scale. Signal II is about 19 gauss wide and has a decay time of several hours. Signal I is about 11 gauss wide and decays within seconds.

Signal II is completely missing in chloroplast fragments obtained by sonicating Chlorella cells for 20 minutes (phosphate buffer pH 7 with . 4 M sucrose) with a 10 KC Raytheon sonic oscillator. Particles which sediment between 25,000 x G. and 140,000 x G. produce only a sharp, fast decay signal very similar in line width, g value, response time and relaxation time to signal I, but somewhat larger in magnitude. It appears, therefore, that only signal I is associated with the primary electron transfer processes which occur in chloroplast fragments.

The light absorption spectrum of the sonicated chloroplast fragments is very similar to the spectrum of intact, whole cells. Both exhibit chlorophyll a and b peaks. Such particles are also capable of performing quinone and ferricyanide Hill reactions. It seems unlikely, therefore, that the two

signals observed with whole cells represent different photochemical reactions of chlorophyll a and chlorophyll b as postulated by Allen et al. (1961).

The response to light intensity of the light-induced, sharp signal observed with chloroplast fragments is shown in Fig. 2. It is evident that this signal is much greater at lower light intensities than the signal I observed with whole cells. The implication is that by blocking the production of signal II, one enhances the production of signal I, especially in the low to normal light intensity range.

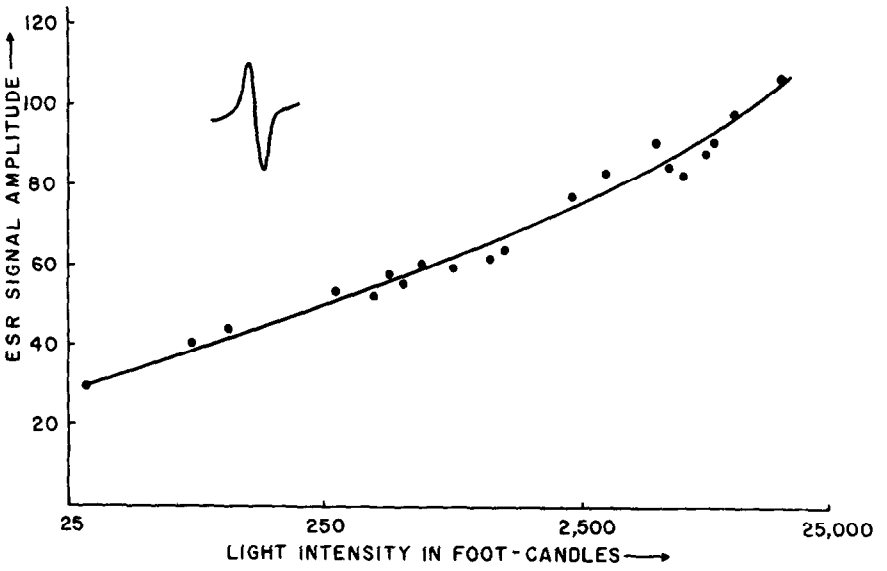

Figure 2. Light-induced ESR Signal Amplitude as a Function of Light Intensity for Chloroplast Fragments from Chlorella pyrenoidosa Cells.

Chloroplast fragments produce only one signal at g = 2.0. The signal is about 11 gauss wide and decays rapidly in the dark. It appears very similar to the signal I observed in whole cells.

Since signal I has fast rise and decay times, saturates only at very high light intensities, is strongly evident at temperatures down to -160° C, and appears to be the same signal as observed in Chlorella chloroplast

fragments, it is most likely due to a photophysical type response. Signal II, on the other hand, appears to be related to physiological processes since it apparently requires the intact cell, shows slow decay in the dark, saturates at normal light intensity for cell growth and is less evident (but does not disappear) at below freezing temperatures. The light intensity responses of the two signals in the whole cell and in chloroplast fragments indicate that the unpaired electrons responsible for signal I may lead to production of the free radicals responsible for signal II, thus yielding a low intensity of signal I unless the free radicals producing signal II are saturating (Fig. 1) or are incapable of being formed (Fig. 2).

The light saturation studies were made with a Varian Model 4500 EPR spectrometer equipped with aqueous sample cell and slotted cavity for illumination. The light source was a 1000 watt GE No. DRS-T20 tungsten lamp projected through 4 cm of water with 2.5% $CuCl_2$ as a heat filter and focused on the cavity window. Light intensity measurements were made with a Weston Model No. 2445 foot-candle meter. Appropriate corrections for light intensity were made to take into account the effective area of sample illuminated in the cavity. The sample temperature rise was less than 2° C. Wire mesh screens were used as neutral density filters to adjust the light level. This minimized spectral changes in the light source such as would occur if voltage on the lamp were changed to adjust intensity.

Chlorella cells (4 days growth) were concentrated by centrifugation and packed in a flat 1 cm x 5 cm quartz sample holder. The chlorophyll content of the .05 ml sample volume was calculated to be 0.575 milligrams. The experiment was repeated with much thinner suspensions and similar responses to light intensity were obtained. The experimental procedure

entailed starting at the lowest light intensity and advancing to higher light intensities to compensate for the slow decay characteristics of signal II.

REFERENCES

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