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ELECTRON-SPIN-RESONANCE STUDIES ON PHOTOSYNTHETIC MATERIALS

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ABSTRACT

A number of organisms have been examined for their ability to produce electron-spin-resonance signals at low temperatures in response to illumination. The efficiency of the response is of the order of not less than 5%, and the wavelength for maximum response is generally slightly on the longer side of the wavelength of maximum absorption, with a minimum appearing at the wavelength of maximum absorption.

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I. INTRODUCTION

During the past four years, observations of photoinduced electron-spin-resonance (ESR) signals have been reported by Commoner et al.,[1,2] Calvin and Sogo,[3] and Sogo et al.[4,5] in a variety of plant materials. These measurements have been carried out primarily by two groups -- one at the University of California at Berkeley under the direction of M. Calvin, and the other at Washington University in St. Louis under the direction of B. Commoner. The impetus for these studies stems from an interest in the basic mechanism by which plants absorb, transfer, and convert light energy emitted by the sun into the chemical potential ultimately required for the

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evolution of molecular oxygen and the synthesis of carbohydrates from carbon dioxide and water. It has yet to be established that the unpaired electrons responsible for the photoinduced signals play a primary role in this quantum conversion process of photosynthesis. This paper presents a brief review of these studies followed by a discussion of our recent measurements on quantum yield.

II. REVIEW OF KINETIC STUDIES

Plant materials in which photoinduced ESR signals have been observed include whole algae (e.g., Chlorella, Scenedesmus, Romaria, Nostoc, and Anacystis), whole purple photosynthetic bacteria (e.g., Rhodospirillum rubrum, Rhodopseudomonas spheroides, and Chromatium), chromatophores derived from Chromatium, and chloroplasts derived from spinach and tobacco leaves. The samples consisted of thick, wet, centrifugally packed pastes of material with a minimum amount of water. Sensitive X-band microwave spectrometers with restricted integrating time constants were used, and illumination systems consisted mainly of tungsten-filament lamps with associated lenses and filters. A camera shutter was used to provide a pulse of light containing a known number of light quanta for certain experiments.

The intrinsic rise time of the ESR signal with high light intensity such that the rate at which light quanta are absorbed is not a limiting factor is not known. The rise time of the signal in Rhodospirillum rubrum at room temperature is at least of the order of 0.02 sec, but the deterioration of the signal-to-noise ratio as the spectrometer band width is increased has made it impossible to establish both upper and lower limits for the rise time. The

decay of the signal when the light is turned off has a characteristic time of the order of 1 to 10 sec at room temperature. As the temperature is lowered from room temperature to about -170° C, the rise time is not observed to change within the limitations imposed by the spectrometers used, but the decay time is clearly seen to be a function of temperature. For the chlorophyll-containing substances such as spinach chloroplasts, one observes a lengthening of decay time and a decrease of signal amplitude as the temperature is lowered. For the bacterio-chlorophyll-containing substances such as Rhodospirillum rubrum, the signal decay time lengthens until a temperature of about -50° C is reached and then becomes shorter so that the decay time is less than 1 sec below about -100° C. The signal intensity reaches a peak value at about -15° C. The shape of the decay curves show that the "half-life," or the time required for the signal to decrease to one-half of its original amplitude, depends on the initial signal amplitude. A high initial spin concentration gives a short half-life, while a low spin concentration gives a long half-life. This may indicate that a recombination or "bi molecular" process is responsible for the disappearance of the signal.

The steady-state concentration of photoinduced spins is a function of light intensity. At very low light intensities, the signal intensity varies linearly with the rate of absorption of light quanta. At high light intensities, a saturation spin density is achieved so that a further increase in light intensity has no effect on the size of the signal. Crude estimates indicate that the order of magnitude of the spin density is about one unpaired spin per 1000 chlorophyll molecules present in the sample.

III. QUANTUM YIELD OF PHOTOINDUCED SPINS

We have recently measured the quantum efficiency for the production of the unpaired electrons that give rise to the ESR absorption in spinach chloroplasts. A value of $0.03 \pm .02$ has been obtained for the ratio of the number of unpaired electrons produced to the number of incident light quanta. It is assumed that all light quanta hitting the sample are absorbed.

The experimental arrangement has been described in a previous paper by Sogo et al.[5] The method that was used for these experiments follows. The wave length of the incident light was restricted to a band about 400 Å wide centered at about 7350 Å by a combination of band-pass interference filters and a Corning infrared filter. The incident light intensity was measured with a bolometer which had been calibrated by a standard lamp. The 300-watt tungsten-filament projection lamp, filters, and condensing optics that were used gave a mean intensity of 1.1×10^{16} quanta per sec into the microwave cavity. The fraction of this light in the cavity that reaches the sample was estimated to be 70% by the actinometer method involving the photolysis of uranyl oxalate. An ultraviolet source was substituted for the tungsten lamp for this calibration.

The ESR spectrometer was set to monitor the height of the signal, and the change in height was recorded when the light was admitted. A known number of light quanta were injected by a calibrated camera shutter set for 1/5 sec. From the known shape of the light-induced signal curve in the steady state and a subtraction of the dark signal, we were able to obtain a curve corresponding to the light signal produced by the flash of light. This signal was compared to the signal arising from a sample containing eight 10^{-3}M Mn^{++} ions in aqueous solution. The Mn^{++} sample was taped to the

silver-plated sample rod and the chloroplasts painted on the remainder of the exposed rod so that the two spectra could be obtained simultaneously.

The wave length of 7250 Å was found to be the most favorable for obtaining a reproducibly high quantum efficiency under the conditions of this experiment. For example, 6800 Å gave a quantum efficiency on the order of one-fifth to one-tenth that at 7250 Å.

IV. DISCUSSION

The interpretation of the results described above is made difficult by the nature of the samples that have been used. The centrifugally-packed material is optically very dense, and thick samples (of the order of 0.2 mm or so) have been required for the observation of large signals. The light intensity varies exponentially from a maximum value at the surface to zero in the interior, and the photoinduced spin concentration is also a function of depth. Thus, measurements of the shapes of the rise and decay curves and the wave length dependence of quantum efficiency have, at best, only qualitative significance with these opaque samples.

The ideal sample is one in which the light intensity can be treated as a constant throughout the volume. At the same time, the sample must contain sufficient material to produce a respectable ESR signal. A dilute aqueous suspension of material with a volume of about 1 cm^3 should meet these requirements. The primary problem in the use of such samples is the attendant loss in spectrometer sensitivity due to the dielectric loss of water at the microwave frequencies that have been used. A working high-sensitivity ESR spectrometer capable of accommodating cubic-centimeter amounts of water would provide an instrumental breakthrough for this as well as other studies of free radicals in biological systems.

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