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INTERMEDIATE ELECTRON TRANSPORT IN PORPHYRIDIUM: EPR STUDIES

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EPR (electron paramagnetic resonance) is a form of spectroscopy in which the measuring beam has a wavelength of 3 cm., and thus has no effect on living matter. Photosynthetic organisms, or their chloroplasts or chromatophores, become paramagnetic when illuminated with visible light. What this means is diagrammed in Figure 1; paramagnetism is due to unpaired electrons. It is one of the first detectable events after the absorption of a photon. If enough electrons are unpaired (or there are enough free radicals of a given species), one sees an absorption peak at a given ratio of magnetic field and microwave frequency which is expressed as a so-called g-value and is analogous to wavelength in light spectroscopy.

$$g = h\nu / \beta H_o,$$

Where h = Planck's constant, ν is the frequency in hz, β is the Bohr magneton, and H_o is the d.c. magnetic field in gauss. Most free radicals occur at a g value of 2.00. (*)

All plants have a light-induced EPR signal I (1) with similar characteristics in a steady state. The presence of this signal is

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*Ref. (1) provides the background information on light-induced EPR signals in photosynthetic organisms. Ref. (2) describes experimental techniques.

dependent on a functioning photosystem I reaction center, since it is absent in the Scenedesmus mutant lacking a photosystem I reaction center (3). The cross section for signal I formation is approximately 115 chlorophyll molecules in a photosystem 1 sub chloroplast particle derived from spinach, and in this preparation the quantum requirement for spin production is one (4). These facts, plus a body of evidence from the laboratories of Vernon and others (5, 6) make it highly probable that the signal can be equated with oxidized P_{700}. It should be noted that bulk chlorophyll is not observed; only the reaction center undergoing oxidation-reduction is detected.

Our interest in these signals lies in what they can tell us about photosynthetic electron transport. By observing the oxidation state of P_{700}, under the influence of system 1 and/or system 2 light, we can infer what is happening between the two photosystems. The results are to some extent complementary to those derived from fluorescence yield measurements, being on the system 1 side of the chain rather than the system 2 side.

The results reported herein were obtained with intact cells of Porphyridium cruentum. Light of wavelength I (λ = 680 to 703 nm) or wavelength II (λ = 550 nm) could be alternated or superimposed on the cuvette.

A signal is readily produced (Fig. 2) with less than saturating red light; green light about twice as intense produces less signal. The signal resulting when the two beams are combined is considerably smaller than that with light I alone, indicating a reduction in the steady-state number of oxidized reaction centers by the electrons

being transferred from photosystem II by green light. If DCMU (3-3', 4' dichlorophenyl 1, 1 dimethyl urea) is added, then the combined beams increase the signal. When the signal is light saturated, and with the addition of DCMU, the concentration of spins is approximately 1 per 200 chlorophyll molecules.

In Figure 3, the amplitude of the signals as a function of intensity of light I is shown in the upper curve, and with the addition of a low intensity of green light, in the lower. At moderate intensities, the reduction effected by light II is relatively constant.

The amplitude of the signal produced by a less than saturating red light is reduced by increasing amounts of green light, as illustrated in Figure 4. A weak green light ($3 \cdot 10^2$ ergs $\cdot$ cm$^{-1} \cdot$ sec^{-1}) can reduce the signal some 30%, although by itself it has no detectable EPR effect.

Let us now look at the kinetics of signal decay in the dark after a steady state has been reached (Fig. 5). The time course is apparent first order with a $t_{\frac{1}{2}}$ of 0.3 sec. If light II is substituted for light I, the decay is speeded to an extent dependent on the intensity of light II; the time required for the signal to drop to half its original amplitude is 0.1 to 0.05 sec.

Rise kinetics are more complex, since the net rate of signal formation represents a balance between oxidation and reduction. Electrons return to the reaction center along a cyclic pathway as well as being funneled in from photosystem II (7). The size of the reducing pool between the photosystems profoundly affects the rate of accumulation of $P_{700}^{ox.}$. The rate of rise is strongly influenced by the length of the

preceding dark time (Fig. 6), by the intensity and wavelength of the exciting light (cf. ref. 8). It can also be slowed by preillumination with system II light.

The kinetics of signal amplitude following the superposition of light II upon light I (as was illustrated for steady-state signals in Fig. 1) can be informative (Fig. 7). A signal is produced by illumination with light I; the addition of light II produced first a rapid drop, and then a slower one, resulting after some 15 sec. in a signal one-third the amplitude of the original one. The fast drop is an immediate reduction, the slow one perhaps the filling of an intermediate pool and/or some conformational change which facilitates electron transport. The two phases may be indicative of the two pools, A_1 and A_2, postulated by Kok, Joliot and McGloin (9). If the intensity of light II is increased (Fig. 7b), there is a transient drop after light II is switched off. This drop could indicate that some of the green photons were going into system I oxidation. In addition, the signal resulting after exposure to light II reaches a higher steady state than that after dark, which indicates to us that perhaps light II has induced the state II described by Duysens and Talens (10) in their fluorescence studies (see also Fig. 7c). Probably light II is doing three things: reducing P_{700}, also contributing to its oxidation, and third, bringing about a change of state. A thirty-second dark period restores the original conditions. If light II is switched on and off while light I remains on, a repeating set of transients is obtained as illustrated in Fig. 7c. Note that the amplitude of the signal increases with repeated exposures to light II.

The rate of passage of electrons between the two photosystems has a substantial temperature coefficient. If the same sort of experiment is performed at 4°C, green light is far more effective in producing signal I than it is when the temperature is 25°C (Figure 8), meaning that it is less effective in generating a reductant. If light II is superimposed upon light I at the lower temperature, the initial drop is as fast and as large, but it is followed by a rise in signal rather than a continued drop. Continued transfer of electrons is not taking place rapidly enough to keep P_{700} reduced.

We are confident that valid estimations of pool sizes and temperature coefficients of the rate limiting steps will soon be at hand.

FIGURE CAPTIONS

Fig. 1 A schematic representation of the transition between the diamagnetic state of material and the paramagnetic state. The arrows represent electrons.

Fig. 2 Signals produced by 680 nm light (1.5×10^4 ergs·cm^{-2}·sec-1), 550 nm light (3×10^4 ergs·cm^{-2}·sec^{-1}), and the two beams combined.

Fig. 3 Amplitude of signal I as a function of the intensity of light I, (680 nm), with and without the addition of light II (550 nm). The arrow indicates where the two light beams are of equal intensity.

Fig. 4 Amplitude of signal I, generated by light I (680 nm, 1.5×10^4 ergs·cm^{-2}·sec^{-1}) with increasing intensity of light II (550 nm) added.

Fig. 5 Amplitude of signal I as a function of time. An arrow ↑ indicates a given light is turned on, and ↓ indicates it is turned off. Decay of the signal is faster when light II is on than in the dark.

Fig. 6 Rate of rise of signal I as a function of the length of preceding dark period.

Fig. 7 Amplitude of signal I as a function of time. In (7a) lights I and II are of equal intensity. In (7b) the intensity of light II has been doubled. In (7c) light I is kept on and light II is switched on and off at ten second intervals.

Fig. 8 Amplitude of signal I as a function of time. The only difference in the upper and lower traces is temperature. See text.

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