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CIDEP OBSERVATIONS IN PHOTOSYSTEM I OF GREEN PLANT AND ALGAL PHOTOSYNTHESIS*

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Summary: Flash photolysis experiments with electron paramagnetic resonance detection were carried out between 10 K and 300 K on samples of green plant and algal species. Chemically induced dynamic electron polarization was evident for the signals observed in the g = 2.0 region for 100 KHz modulated detection and also for a system with no magnetic field modulation. The light reversible signals decaying in about 1 ms at low temperatures are interpreted as arising from photosystem I of the green plant and algal samples. Evidence is presented which indicates that the origin of the electron spin polarization is the well established radical-pair mechanism.

There have been several reports (1-6) of CIDEP in photosynthetic systems where the normal photochemistry of charge separation was shown to be operative under the experimental conditions. Blankenship and coworkers (1) first reported observing strongly polarized EPR transients from spinach chloroplasts at room temperature. They reported observing only microwave emission signals; these results were interpreted in terms of a triplet precursor mechanism operating in PS I of green plants. Recently, Dismukes and coworkers (4-6) have revised this interpretation in terms of a radical-pair mechanism giving rise to strongly polarized EPR transients.

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ABBREVIATIONS: CIDEP: chemically induced dynamic electron polarization; EPR: electron paramagnetic resonance; PS I: photosystem I; PS II: photosystem II; mT: 10 Gauss.

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In a previous report (2), we presented some experimental evidence for what we believed was triplet-state involvement in PS II of intact algae at low temperatures. In this report, we demonstrate that the origin of the transient signals is due to a radical-pair mechanism in PS I. In these respects, our results are in agreement with those of Dismukes and coworkers. However, an intriguing new result is that our observations at temperatures below 300 K reveal the presence of EPR signals which cannot be attributed to a chlorophyll species alone. These signals do indicate the involvement of some other organic free radical.

MATERIALS AND METHODS

A Varian E-12 EPR spectrometer with 100 KHz field modulated detection was modified so as to achieve an instrument limited rise time of 30 μs** for magnetic-field-modulated experiments (7). We also obtained direct-absorption EPR measurements where the absorption signal was taken directly from the Varian E-101 microwave bridge which constitutes part of the E-12 spectrometer system. This absorption signal was then amplified by a home-built 30 db gain wide-band amplifier with a noise figure of 7 db. The measured rise time of this direct EPR absorption system was 0.4 μs. For flash-photolysis experiments with modulated detection, the light source was a Photochemical Research Associates Model 610 flash system with a flash half life of 25 μs usually filtered with a Corning CS-262 filter. For direct-absorption measurements, the light source was a Phase-R 2100 dye laser operating at (620 ± 10) nm with a flash half life of 200 ns. The light flash of either system was not of a saturating light intensity. The EPR signals were digitized by a Nicolet 2090 Explorer III digital oscilloscope which was interfaced with a Nicolet 1180 instrument computer for signal averaging and data analysis. In turn, the instrument computer was interfaced with the Varian E-12 EPR spectrometer so that the stepping of the magnetic field was controlled by the computer. In each experiment, kinetic traces were measured at approximately 30 to 50 different magnetic field positions. For each kinetic trace, a photodiode was used to detect the rising edge of the flash, and this rising edge was placed at a common origin by means of the "cursor" mode of the transient recorder. The kinetic traces were then signal averaged and stored in the computer's memory for subsequent data presentation.

Particles enriched in photosystem I were prepared from spinach according to the method of Vernon et al. (8) with the detergent Triton X-100. Such particles were also prepared from the algae Anacystis nidulans and Scenedesmus obliquus using a combination of the French press and detergent treatment methods (9). All photosynthetic samples including intact algae were buffered close to pH = 8, and there were no exogenous redox agents.

**The time to reach (1-1/e) of the final value.

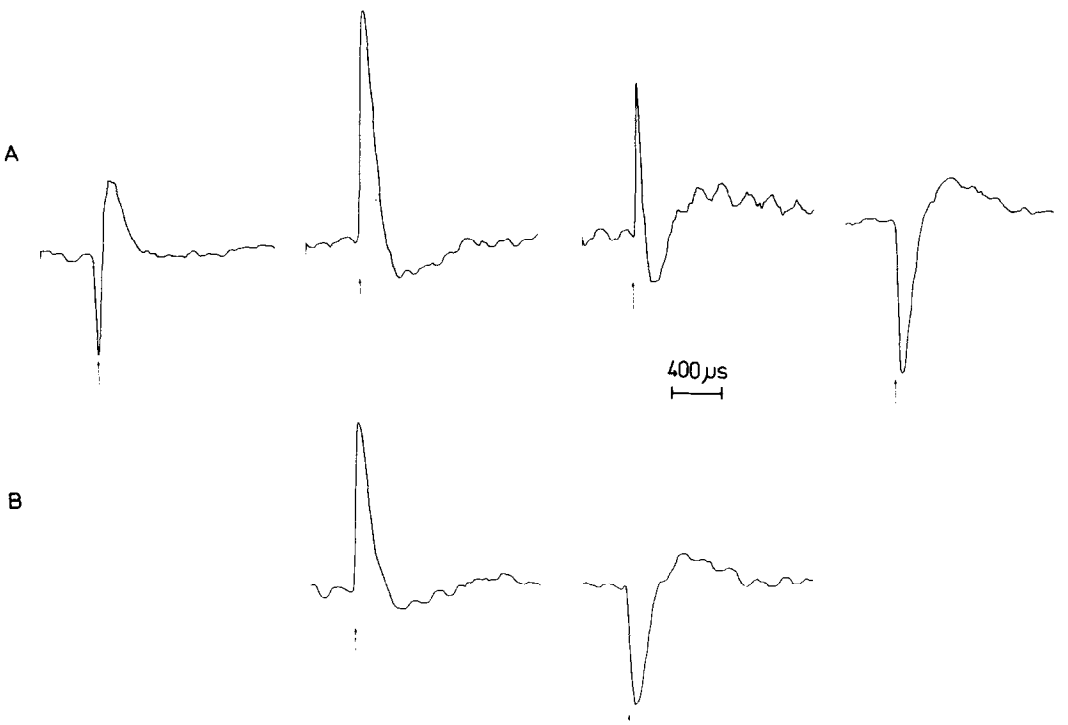

Figure 1: Some typical profiles for flash photolysis with 100 KHz detection of EPR signals observed from whole cells of algae at 100 K. The flash position is marked by an arrow. The modulation amplitude is 0.2 mT and the microwave power is 1 mW at 9.2 GHz for all traces. (A) Some flash induced time profiles observed from the protonated alga Anacystis nidulans. (B) Some flash induced time profiles observed from the deuterated alga Scenedesmus obliquus.

RESULTS

We have monitored the rapid light-reversible transients (2) in the g = 2.0 region in flash photolysis experiments with modulated EPR detection from 10 K to 300 K for green plant and algal samples. The 1 ms transients of Fig. 1 show a crossing of the zero baseline in a time of $\sim$ 200 μs which is normally characteristic of spin-polarized emission signals.

Typical time-resolved EPR spectra of these transients are presented in Fig. 2 for spinach PS I particles and for algae.

At about 200 K and higher temperatures, a more slowly decaying ($t_{1/2} \sim 100$ ms) $P700^+$ signal was also observed. The $P700^+$ first derivative peak-to-peak line width from normal Scenedesmus obliquus was 0.73 mT, and the line width from 97% deuterated Scenedesmus obliquus was 0.34 mT.

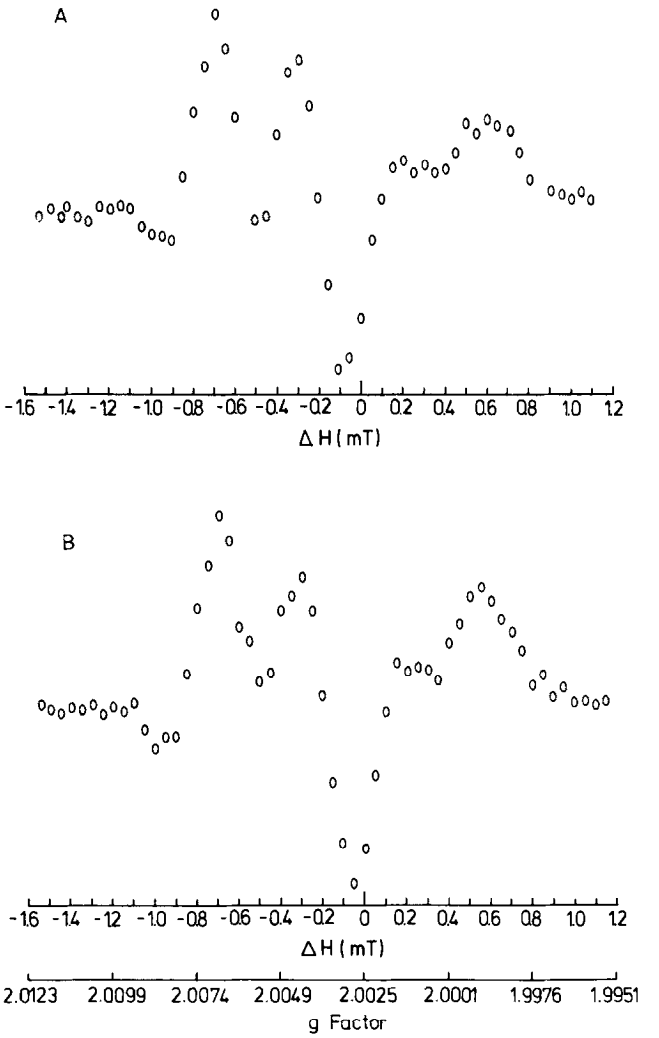

Figure 2: Time-resolved EPR spectra observed from flash photolysis experiments with 100 KHz EPR modulated detection. The EPR instrumental conditions were T = 100 K, modulation amplitude = 0.2 mT, and microwave power = 1 mW at ∿ 9.2 GHz. The individual signal amplitudes were selected at 30 μs after flash excitation. The number of flashes is 256 at each magnetic field position. (A) The spectrum observed from a sample of whole cells of the alga Anacystis nidulans. (B) The spectrum observed from a sample of photosystem I enriched subchloroplast particles prepared from spinach with the Triton X-100 detergent fractionation method. The concentration of chlorophyll was ∿ 1 mg/ml.

Fig. 2 shows that there are kinetic responses at magnetic-field positions down to -0.8 mT from the $P700^+$ g factor of 2.0025. $P700^+$ only has a significant absorption down to about -0.6 mT. Similar field profiles were measured at

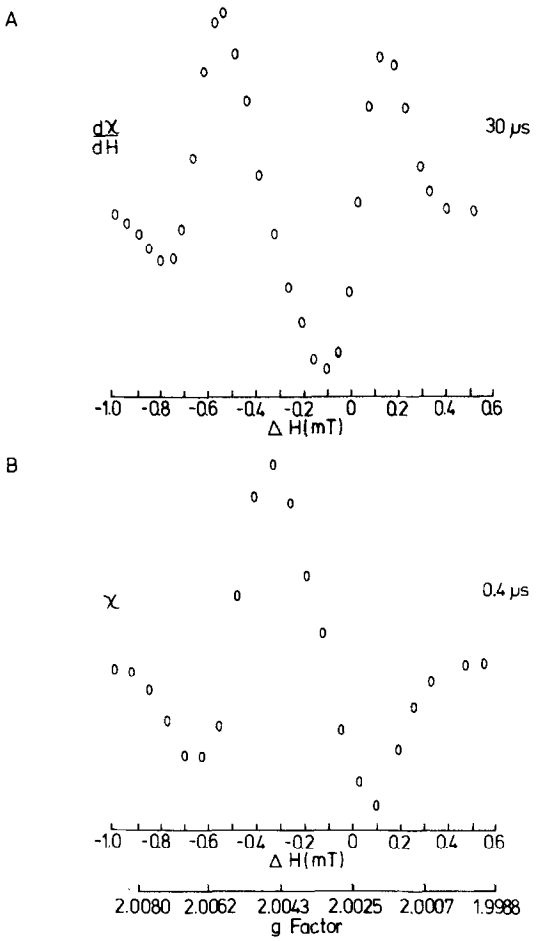

Figure 3: Time resolved EPR spectra observed from flash photolysis experiments with two kinds of EPR detection. In both A and B, the sample was whole cells of the 97% deuterated alga Scenedesmus obliquus observed at 100 K. The EPR microwave power was 1 mW at 9.2 GHz. The number of flashes is 64 at each magnetic field position. (A) The spectrum observed at 30 μs after flash excitation with 100 KHz modulated detection. The modulation amplitude is 0.2 mT. (B) The spectrum observed at 0.4 μs after flash excitation with the direct absorption detection system. In the figure, the upward direction is that of microwave absorption and downward is microwave emission.

temperatures above 200 K up to 300 K, and the signals at lower magnetic fields could still be observed at room temperature and were particularly strong for the 97% deuterated algae.

Because the EPR line widths were reduced in the deuterated algae samples, we obtained better resolution and better signal-to-noise ratios. Flash photo-

lysis field profiles were measured at 100 K for the deuterated algae as shown in Fig. 3 where both 100 KHz modulated detection and direct absorption detection results are presented.

DISCUSSION

It is evident from Fig. 2b that the PS I enriched preparation from spinach exhibited 1 ms transients very similar to those of the algae (Fig. 2a) with regard to the respective field profiles. It was also observed that PS I enriched preparations from the protonated and deuterated algae revealed the same magnetic-field profiles from 1 ms transients as for the samples of the parent intact algae. Thus, the membrane fractionation experiments point to the assignment of the 1 ms transients to PS I activity. Further support for this PS I assignment came from a redox titration with ferricyanide. As the $\sim$ 1 ms transients exhibited a mid-point potential of (500 $\pm$ 30) mV when observed at 100 K, we assume that this observation gives further support (4) for the PS I assignment of the 1 ms transients.

This revision of our earlier assignment is apparently in agreement with other reports (10,11) of rapidly reversible components in PS I photochemistry at low temperatures and at room temperature. The PS I assignment does represent a departure from the commonly held view that nearly all of the observable photoinduced signals in PS I are irreversible at low temperatures. In earlier work (12), no exogenous redox agents were added to PS I subchloroplast particles, and only a few percent of the total P700 appeared to be photoreversible with regard to $\sim$ 100 ms transients. It was later shown that the light reversibility of the $P700^+$ and X^- components was much enhanced (13-15) when the secondary membrane-bound ferredoxin components (16,17) were chemically reduced.

We will now consider the important new information which is contained in the results of the direct-absorption measurements on deuterated algae as presented in Fig. 3b. The key observation in this figure is that both enhanced microwave absorption and emission are observed in the field profile. This ob-

servation is strong evidence for a radical-pair mechanism (18) being the origin of the spin polarization. A pure triplet precursor mechanism (18) should give rise to doublet radicals exhibiting the same sign of polarization, either enhanced absorption or emission, but not both.

Furthermore, modulated detection gave kinetic traces which appeared to have a remarkably consistent time profile for deuterated algae with a crossing of the zero baseline in time always present as shown in Fig. 1. This phase inversion may not in fact be indicative of enhanced emission signals, but may be caused by rapid-passage effects (19) which distort the kinetic profiles. The results of direct-absorption detection probably give more credible kinetic information because of the long spin-lattice relaxation time of chlorophyll species at these low temperatures.

Recently, Dismukes and coworkers (4-6) have presented an interpretation of their results obtained by 1 MHz modulated detection of the flash photolysis of chloroplasts and algae at room temperature. They found evidence for the acceptor (20-23) species X^- with its principal g factors of 2.08, 1.88 and 1.78. They also suggested that a chlorophyll molecule acted as an electron acceptor.

It is clear that both our direct absorption and field modulated results present evidence for kinetic responses from PS I in the g factor region between 2.0025 and about 2.0055. This is true for all samples examined including normal and deuterated algae and spinach chloroplasts. Thus, we propose that these observations may represent evidence for the presence of at least one electron acceptor having a g factor probably between 2.0040 and 2.0055. Clearly, such a g factor cannot be a normal chlorophyll (24) species. Thus, there is some evidence for an organic free radical other than chlorophyll in the PS I reaction center.

Some preliminary redox measurements were performed on the reducing side of PS I. It was found that for redox potentials more negative than -600 mV (about the potential of the more negative membrane bound ferredoxin center)

that the field profile of the CIDEP signals changed in the g = 2.0 region. It is clear that these CIDEP signals are not directly associated with the more slowly decaying ($\sim$ 100 ms) signals (12-15) observed for $P700^+$ and for the acceptor X at very low temperatures. It is suggested that these measurements constitute some evidence for two kinds of electron acceptors in PS I and hence two kinds of PS I reaction centers.

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