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A STUDY OF CHEMICALLY INDUCED DYNAMIC ELECTRON POLARIZATION (CIDEP) IN PHOTOSYSTEM I OF WHOLE ALGAL CELLS AT AMBIENT TEMPERATURES *

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Time-resolved electron paramagnetic resonance (EPR) studies were carried out at room temperature and at 273 K on whole-cell samples of the photosynthetic algae: *Anacystis nidulans* and *Scenedesmus obliquus*, the latter being 97% deuterated from the growing medium. These photosynthetic organisms show greatly enhanced EPR signals which result from the generation of nonequilibrium spin populations, a phenomenon known as chemically induced dynamic electron polarization (CIDEP). We report magnetic-field profiles of the early transient signals of Photosystem I which are very similar to those observed at low temperatures. The results suggest that one or more early reduced electron acceptors in Photosystem I are being observed at ambient physiological temperatures.

Introduction

Recent studies of the photochemistry of the primary electron-transfer reaction of Photosystem I of green plants and algae have led to an increasingly complex model for the reaction-center complex [1–3]. A recent proposal by Bonnerjea and Evans [4] and independently by Gast and coworkers [5] suggests the following linear electron-transport scheme: P-700-A_0-A_1-X-$Fd_{B,A}$, where $Fd_{B,A}$ designates the complex of ferredoxin A and B [6–8], X is the acceptor species sometimes referred to as A_2 [9–11], A_1 is an acceptor species with an EPR signal at $g = 2.0051$ [4] or $g = 2.0054$ [5], A_0 is an acceptor having an EPR signal with $g = 2.0024$ [4] and P-700 is the primary electron donor of Photosystem I. The photo-oxidation of P-700 is accompanied by the observation of spin-polarized EPR transients which exhibit the CIDEP phenomenon [12]. Previous EPR studies of these spin-polarized transients in Photosystem I have addressed the question of the chemical identity of the various electron acceptors in the reaction-center complex. EPR spectra and g factors have been measured at ambient temperatures [13–18] and at low temperatures [19–25]. Species X has been tentatively assigned as an iron-sulfur center of unknown geometry, partly on the basis of optical measurements [3,11]. At this early stage it is difficult to speculate on the identity of the A_1 species, except to note that its g-factor is too high for a chlorophyll or pheophytin-based center. Several groups have proposed that the earliest electron-acceptor species A_0 is a chlorophyll-based species, some claiming a monomeric structure [26–30] while others postulate a dimeric structure [24,31–33]. McCracken and Sauer [25] have reported that A_0^-

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Abbreviations: CIDEP, chemically induced dynamic electron polarization; $Fd_{B,A}$, complex of ferredoxin A and B, ESE, electron spin echo.

may have a slightly anisotropic g tensor with g values ranging from 2.0026 to 2.0031, which is consistent with its being a chlorophyll or pheophytin entity.

The EPR technique has played a very significant role in the detection and assignment of the intermediate electron acceptors A_0, A_1 and X. It is also evident that the CIDEP phenomenon has provided additional information about interactions between P-700 and the intermediate acceptors as well as structural information [16,17,24,5]. However, there is an apparent discrepancy in the literature concerning the magnetic-field profiles observed for the CIDEP transients, when one compares the field profiles measured at low temperatures (less than 150 K) with those measured at ambient temperatures. The range of g factors or the magnetic-field span reported for the CIDEP transients at room temperature [14,15,17,18] is narrower than that seen at low temperatures [19–25]. On the other hand, measurements with the electron spin echo (ESE) technique [34–36] and with EPR at K-band (24 GHz) [37], all at room temperature, yield a field profile which is more in agreement with those results obtained with conventional EPR (X-band or approx. 9 GHz) at low temperatures.

In this paper we report a study of CIDEP signals from whole algae at room temperature obtained on a conventional EPR apparatus but employed in a direct-detection mode which allows for a time resolution of approx. 400 ns. Our results indicate these CIDEP signals are very similar to those previously measured at low temperatures [19–25].

Material and Methods

Whole cells of the algae *Anacystis nidulans* and *Scenedesmus obliquus* were investigated, the cultures were maintained in our laboratory and originated from the Culture Collection of Algae at Indiana University. *S. obliquus* was also grown in a deuterated growing medium from an original culture generously donated by Dr. J.J. Katz of Argonne National Laboratory; the resulting cells were approx. 97% deuterated. The $P\text{-}700^+$ EPR signal from the 97% deuterated algae was narrowed considerably (0.24 mT, as compared to 0.73 mT for the corresponding protonated algae). The intact algal cells were isolated from the growth medium and packed in a solution of 50 mM tricine at pH 7.8 with 10 mM NaCl/1 mM $MgSO_4$/0.01 mM EDTA. The cells were introduced into a large aqueous EPR flat cell for a Varian Model E-238 microwave cavity operating in the TM_{110} mode. The samples were static or non-flowing and the typical total chlorophyll concentration was approx. 5 mg/ml in the whole-cell samples. In some experiments at approx. 273 K, the algal cells were introduced into a small aqueous EPR flat cell which was then placed into a quartz insert dewar in the same EPR cavity to provide temperature control [21].

EPR transients were recorded as before [21] in the direct-detection mode (no magnetic-field modulation). The time response of the system was limited by the excitation light pulse which was provided by a Photochemical Research Associates Model 610 C pulsed light source with a xenon flashlamp [full width at half maximum (FWHM) of approx. 1 μs]. While the xenon flash exhibits a small approx. 1% amplitude long tail (approx. 10 μs), the modulation-free EPR detection system generally does not have the sensitivity required to resolve the corresponding tail in the EPR transient decay profiles even with extensive signal averaging. Thus, deconvolution of the flash profile is not worthwhile under these noisy signal-averaged conditions. As before [21], all EPR signals were digitized by a Nicolet 2090-III Digital Oscilloscope which relayed its transient waveforms along an interface to a Nicolet Model 1180 Computer for signal averaging and data analysis. The xenon flash was filtered by a Corning CS2-62 filter transmitting light with wavelengths longer than 590 nm. For normal protonated algae samples at 300 K, approx. 32000 flashes were needed at each magnetic-field position to obtain adequate signal-to-noise ratios. The energy per pulse incident on the sample (after filtering) was approx. 100 μJ on an active area of approx. 1 cm^2. By splitting the data accumulation into several segments, we determined that the photoresponse of the algae samples did not vary during the course of an experiment (approx. 4–10 h) at ambient temperatures where approx. 40–50 magnetic-field positions were measured separately. Great care was taken to en-

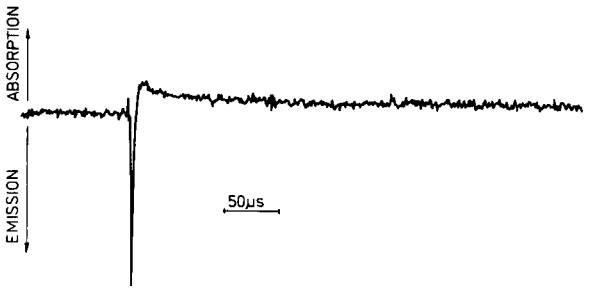


Fig. 1. Typical time profile of the EPR signal using direct detection as observed from flash photolysis of whole cells of *S. obliquus* (more than 97% deuterated). The conditions were: microwave power, 1 mW; microwave frequency, 9.0 GHz; $T = 273$ K; $\Delta H = +0.1$ mT, where $g = 2.0025$ corresponds to the reference magnetic field (see Fig. 3).

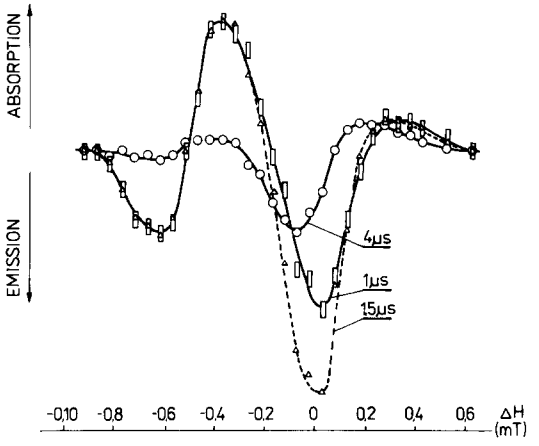


Fig. 2. Direct-detection, time-resolved EPR magnetic-field profiles observed from flash photolysis of whole *S. obliquus* (more than 97% deuterated) algae. Conditions: microwave power 20 mW; microwave frequency 9.4 GHz, $T = 293$ K. Time equals zero refers to the beginning of the flash; the maximum flash-induced intensity occurs at approx. 1 μs. $g = 2.0025$ corresponds to the reference magnetic field which is assigned $\Delta H = 0$ at approx. 0.336 T with the sample in an aqueous-solution quartz flat cell.

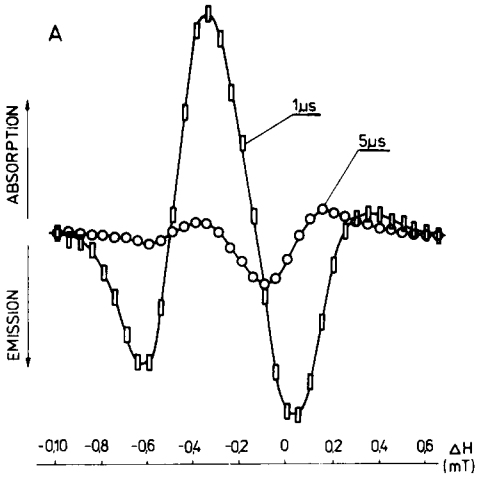
sure that virtually no flash artifacts, originating from the flashlamp and auxiliary circuits, were present in the observed kinetic profiles. Nearly all flash artifacts in the EPR transients were removed [21] by subtracting an off-resonance (approx. +10 mT) kinetic profile from each profile obtained at a given magnetic-field position. An example of a corrected kinetic trace is presented in Fig. 1.

Results and Discussion

We report here the EPR magnetic-field profiles in the early time regime (approx. 1 μs) of the CIDEP transients for whole algal cells at physiological temperatures between 273 and 300 K. Most of the experiments were performed on the deuterated *S. obliquus* cells because the lines were narrower and hence signal-to-noise ratios were much better than for protonated algae. Time-resolved EPR field profiles at 1, 1.5 and 4 μs are shown in Fig. 2 (where time equals 1 μs refers to the peak of the flash-induced instrument response). These profiles are very similar to those we have observed in frozen matrices at low temperatures [19–22], but the decay rate of the transients is much faster at room temperature than at low temperature. Our findings are in contrast to earlier work where narrower signals were obtained [14,15,17,18], but we are in agreement with results using the time-resolved ESE method [34–36] and the time-resolved EPR results at K-band [37].

A comparison of the field profiles in Fig. 2, measured at 1.0 and 1.5 μs after the flash, reveals a rise of microwave emission centered at $g = 2.0025$, although the low- and high-field parts of the signal are unchanged within experimental error. In the 4 μs trace, the only significant component left is the decaying emission signal at $g = 2.0025$, presumably due to $P\text{-}700^+$; the low-field components have almost completely decayed. We believe that these marked changes in line shape are due to the presence of at least two spectral components, each of which decays in polarization at a different rate.

By decreasing the temperature of deuterated *S. obliquus* cells to 273 K, the polarization decay rates are slower; it was thus possible to capture the more intense stages of the polarization decay within the time resolution available. Hence, we were able to obtain good signal-to-noise ratios with only 1 mW microwave power and 8192 repetitions. In Fig. 3 four field profiles are presented at 1.0, 5.0, 15 and 300 μs after the flash. The last field profile is very similar, in terms of line-shape, g factor and linewidth, to EPR absorption spectra obtained under steady-state conditions. Presumably by this time, the polarization has decayed almost com-



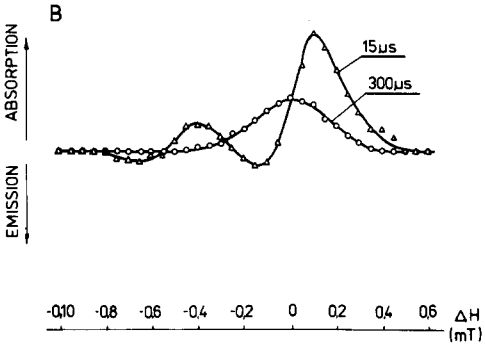


Fig. 3. Direct-detection, time-resolved EPR magnetic-field profiles from flash photolysis of whole *S. obliquus* (more than 97% deuterated) algae. Conditions: microwave power, 1 mW; microwave frequency 9.0 GHz; $T = 273$ K. $g = 2.0025$ corresponds to the reference magnetic field which is assigned $\Delta H = 0$ at approx. 0.320 T with the sample contained in a quartz dewar insert. (a) Field profiles at 1 and 5 μs; (b) field profiles at 15 μs and 300 μs; these profiles are shown at 5× amplitude in comparison with those in (a).

pletely, leaving a normal EPR absorption signal of $P\text{-}700^+$ arising from a Boltzmann distribution of spins. Again, the marked changes in line shape are indicative of at least two decaying components.

We present the EPR CIDEP field profile at 1 and 4 μs for protonated *A. nidulans* algae at 273 K in Fig. 4. (We have established that protonated *S. obliquus* algae give identical results within experimental error to those from *A. nidulans*. We chose to use the latter algae as they were more convenient to grow and gave the stronger signals shown in Fig. 4.) The profile at 1 μs, which was obtained at

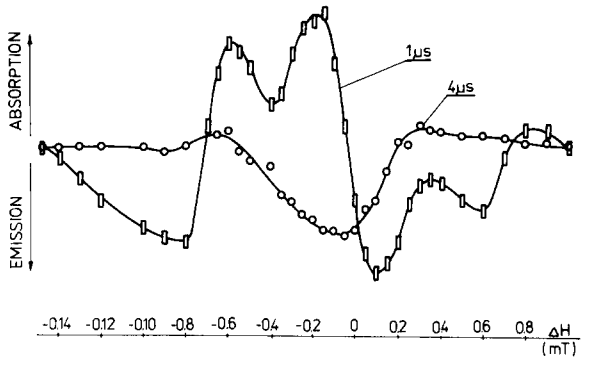


Fig. 4. Direct-detection, time-resolved EPR magnetic field profile observed from flash photolysis of protonated *A. nidulans* algae. Conditions were the same as in Fig. 3.

the peak of the light pulse, was again very similar, in line shape and g factors, to field profiles measured [19–21] for protonated algae at 100 K; although as for the deuterated algae, the polarization decay rates are faster. Fig. 5 presents analogous field profiles to those in Fig. 4 but at 293 K. The 1 μs profile maintains some resemblance to that obtained at 100 K; however, the low-field part is less pronounced, and clearly the decay rate is on the edge of the time resolution of the apparatus. The field profile 2 μs after the start of the flash in Fig. 5 is mainly in emission. This is probably the signal which has been observed previously in modulated detection experiments under non-flowing conditions at room temperature [14]. This stage of the CIDEP signal is observed at all

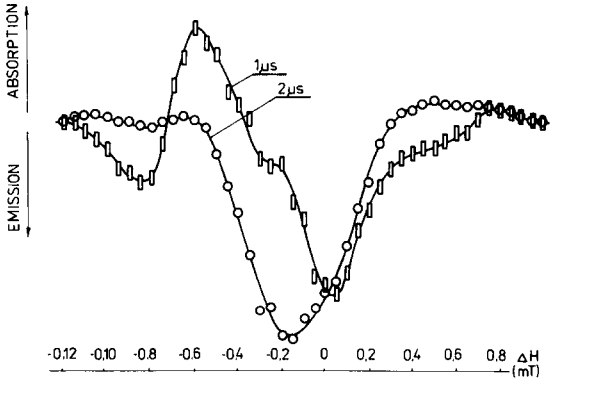


Fig. 5. Direct-detection, time-resolved EPR magnetic-field profiles observed from flash photolysis of protonated *A. nidulans* algae. Conditions were the same as for Fig. 2.

temperatures studied, but at lower temperatures it develops at a later time after the flash.

Almost all of the changes in field profile with time are due to the decay of the spin polarization. This decay is controlled by two factors [38]: the inherent spin-lattice relaxation time T_1 of each chemical species and the microwave power level. An estimate of T_1 can be made only if one extrapolates decay rates as a function of microwave power to zero power [39]. At 100 K we have been able to separate these effects (our unpublished results), and, in addition, we have shown that the CIDEP field profile remains undistorted for all microwave power levels less than 1 mW [21]. Unfortunately, at room temperature or 293 K in Figs. 2 and 5, we have not been able to obtain reasonable signal-to-noise ratios for power levels less than 20 mW. Because the relaxation times are shorter at the higher temperature, we believe that the field profiles are still undistorted by any saturation effects; however, we are unable to draw any quantitative conclusions about spin-lattice relaxation times.

Conclusions

We have established that the CIDEP EPR field profiles from Photosystem I of whole algae and chloroplasts are independent of temperature between approx. 10 and 300 K. Previously observed deviations are probably caused by limited time resolution of the EPR spectrometers used. As expected, the decay of polarization is much faster at room temperature than at lower temperatures.

Deuteration of the algae has two effects: the CIDEP line shapes are narrower and the decay of polarization is slower than for corresponding protonated algae. This latter effect permits us to study the time dependence of the CIDEP field profiles in more detail. The cause of the slower spin-lattice relaxation in the deuterated species may be the much-reduced deuterium hyperfine fields, relative to the proton hyperfine fields, resulting in a poorer coupling to the phonon energy manifold of the lattice.

A striking feature of the CIDEP field profiles at an early time is that they are completely different from the steady-state P-700$^+$ profile, and yet after some microseconds the field profile decays to one which is very similar to that of P-700$^+$. The CIDEP field profiles at early times are of a type which was predicted by Pedersen [40]. Pedersen's theory, which is an adaptation of the standard radical pair theory, states that these lineshapes must contain contributions from two different radical species, and the theory has been used in two recent studies [5,25] to explain the low temperature CIDEP profiles due to Photosystem I. We interpret the results of Figs. 2–5 to indicate that at early times we are seeing the superposition of profiles from at least two distinct radical centers. One is almost certainly P-700$^+$, while the other is probably one or more of the acceptor species. This assignment is in agreement with attempts [4,5,25] to explain both steady-state and time-resolved EPR results, particularly the latter because a CIDEP multiplet effect [40] on a polarized P-700$^+$ alone could not account for the observed orientation dependence [25,41] of the CIDEP field profile. In view of the fact that the low-field parts of the field profiles in Figs. 2–5 decay more rapidly than the high-field parts, we feel that the acceptor species is contributing primarily in the low-field region with a faster polarization decay rate than that of P-700$^+$. Hence, the acceptor species probably has a g factor higher than that of P-700$^+$ and could arise from the A_1 species which has a g factor of 2.0051 [4] or 2.0054 [5,25]. In previous analyses [5,25] of the low temperature CIDEP data, the low field portion of the EPR spectrum has been attributed to the dynamically polarized A_1^- species created by electron transfer from spin polarized A_0^-. Thus, it is probable that A_0^- is not being observed directly in this work or in previous reports [5,25] of time-resolved CIDEP field profiles; however, A_0^- was observed in deconvoluted steady-state EPR spectra [4] of reduced Photosystem I particles.

After completion of this work, a paper by Furrer and Thurnauer [37] appeared which reports time-resolved EPR measurements at the X-band and at the K-band on deuterated *Synechoccus lividus* algae at room temperature. Their X-band results are in agreement with ours and, most gratifyingly, their K-band results show clear evidence for at least two species with one at $g = 2.0056$ and the other (presumably P-700$^+$) at 2.0026.

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