TIME-RESOLVED ELECTRON SPIN ECHO SPECTROSCOPY APPLIED TO THE STUDY OF PHOTOSYNTHESIS

M. C. THURNAUER, M. K. BOWMAN and J. R. NORRIS

Chemistry Division, Argonne National Laboratory, Argonne, IL 60439, USA

1. Introduction

A recent development in the research of photosynthesis has been the application of fast time-resolved electron paramagnetic resonance (EPR) [1–4]. The EPR method is particularly promising for systems which cannot readily be studied by optical techniques. The radicals which have been observed in photosynthetic systems by the magnetic resonance technique exhibit chemically-induced dynamic electron polarization (CIDEP) [5]. The mechanism which produces this spin polarization (a non-Boltzmann distribution of spin population) reflects the earlier fate of the radicals.

The reported studies of photosynthetic systems by time resolved magnetic resonance have utilized what are now fairly standard EPR techniques [6]. They have been limited by lack of adequate time resolution, possible distortions or artifacts due to the use of magnetic field modulation [3,7], and the problem of positively identifying the observed radical species. We are applying time-resolved electron spin echo (ESE) spectroscopy, a pulsed form of EPR, to the study of photosynthetic systems. This new technique not only avoids many of the problems described above but also provides several new advantages. We report here on the first observation of spin-polarized signals in the blue–green alga *Synechococcus lividus* using time-resolved ESE, and illustrate advantages of the technique.

2. Methods

The time-resolved ESE experiment has been described [8,9]. We employ the same experimental

set-up which can give ~20 ns time resolution. (We routinely operate with 30–40 ns time resolution.) The excitation at 579 nm is from a Molectron DL 14 dye laser pumped by a Molectron UV24 nitrogen laser. Laser pulse duration is ~10 ns. The echo intensity as a function of magnetic field taken at a fixed time with respect to the laser pulse is recorded (EPR spectrum), or the echo intensity as a function of time with respect to the laser pulse taken at a fixed magnetic field value is recorded (kinetic trace). A schematic description of the experiment is given in fig.1.
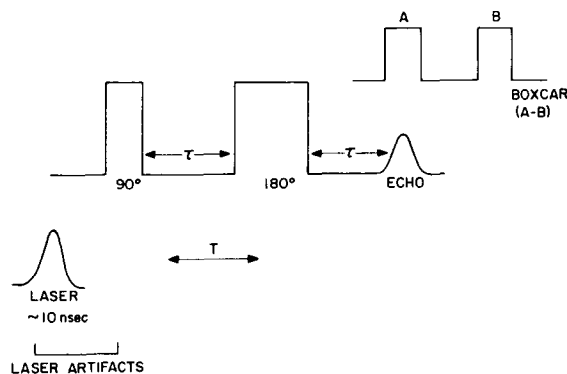

Fig.1. The basic ESE experiment consists of a $90°-\tau-180°$ microwave pulse sequence. In the time resolved ESE experiment the microwave pulse sequence is carried out at some time T relative to the laser pulse. There are two modes of operation. (a) T is fixed. The echo intensity as a function of the magnetic field is recorded. This gives a non-derivative absorption EPR spectrum. By varying T, one can conveniently record the change in the entire spectrum with time. (b) The magnetic field is fixed at a value where the sample is in resonance. The entire ESE pulse sequence is started before the laser pulse and stopped in time until a predetermined interval after the laser pulse. Thus, the echo intensity as a function of T is recorded.

Techniques for culturing *S. lividus* and fully deuterated *S. lividus* have been described [10,11]. Fresh whole cells recirculated at room temperature through a flat cell in the spectrometer cavity were used.

3. Results

Figure 2 shows the EPR spectrum (echo intensity as a function of the magnetic field) observed in fully deuterated *S. lividus* at 2 different times with respect to the laser pulse. At least 2 distinct signals appear
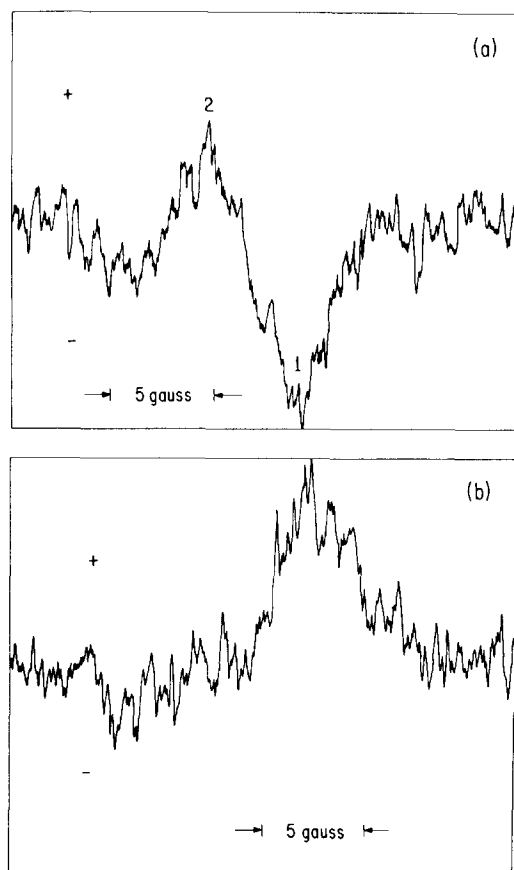
in the spectra for ~300 ns following laser excitation. The high field signal at $g = 2.002$ appears in emission at early times. The low field responses show low field emission and high field, enhanced absorption. The spectrum in fig.2b shows that in $\ll 1$ μs the high field signal appears in absorption, and the low field responses are no longer visible.

The properties of the spectra in fig.2 are conveniently illustrated by the kinetic traces of the signals observed in fully deuterated *S. lividus* shown in fig.3. The high field signal (fig.3a) appears in emission with a ~40 ns rise time and is instrument limited. There is a fast decay into absorption. With a pulse duration of 20 ns we are able to resolve this fast decay rate. The decay time of the final absorption
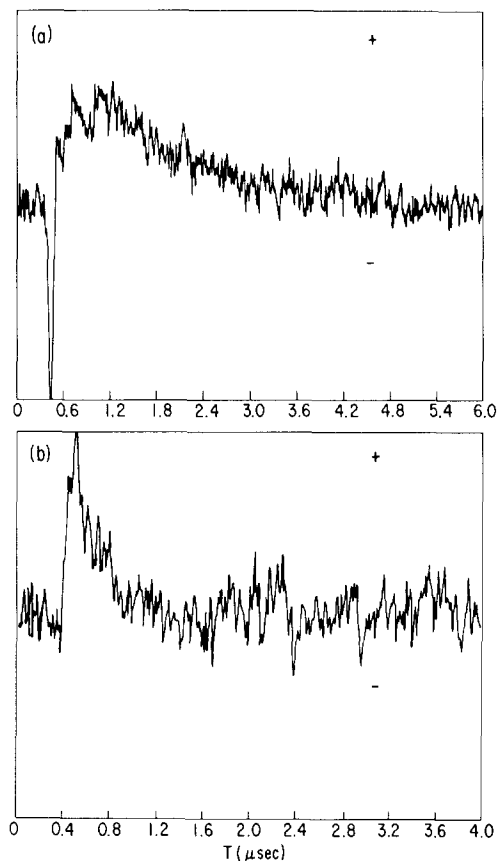

Fig.2. Echo intensity as a function of magnetic field (EPR spectrum) observed in fully deuterated *S. lividus* at 2 times with respect to the laser pulse. (a) Spectrum recorded with first 90° microwave pulse at approximately the same time as laser pulse. (b) Spectrum recorded with first 90° pulse ~610 ns after laser pulse.

Fig.3. Echo intensity as a function of T (kinetic trace) recorded at 2 fixed magnetic field values. (a) Magnetic field fixed at point 1 in fig.2a. (b) Magnetic field fixed at point 2 in fig.2a.
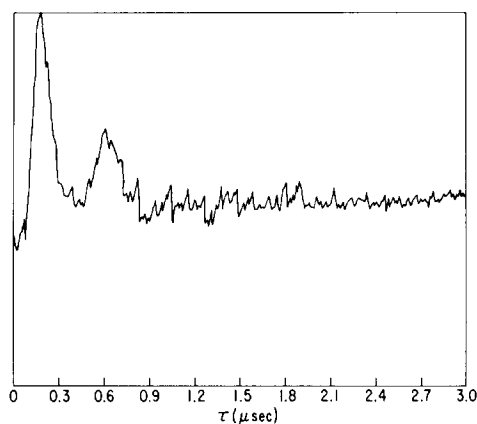
Fig.4. Nuclear modulation observed in fully deuterated *S. lividus*. The magnetic field fixed at point 1 in fig.2a. T fixed. The primary echo decay envelope is obtained by observing echo intensity as a function of τ. This trace was obtained using the N_2 laser for excitation.

component of the signal is ~1.5 μs. Figure 3b shows the kinetic trace of the absorption part of the lower field signals which illustrates an instrument-limited rise time and a decay time of ~300 ns.

We have also measured the primary echo decay (echo intensity as a function of τ (fig.1) at a fixed magnetic field and a fixed time with respect to the laser pulse) for some of the signals described above. Nuclear modulation [9] is seen superimposed on the echo decay envelope (fig.4).

4. Discussion

Advantages of time-resolved ESE spectroscopy over conventional EPR techniques have been summarized [8]. We emphasize here those which are most relevant to the time-resolved EPR studies in photosynthesis.

First of all our ESE spectrometer has achieved 20 ns time resolution while maintaining excellent sensitivity. This is important for the study of photosynthetic systems where the primary reactions are very fast. We discuss below 2 factors which determine time resolution.

In the time-resolved EPR experiment, data cannot be acquired either during or close in time after the

laser pulse since the laser excitation produces microwave cavity and sample perturbations. There is always a problem of distinguishing between the kinetic response of a true photo-induced EPR signal and the response of the EPR spectrometer to a laser or light pulse (laser artifacts). In order to do so magnetic field modulation is employed.* This not only limits time resolution but passage effects from the modulation may produce distortions of the transient EPR signals [3,7]. In the ESE experiment, the time between the first microwave pulse and the observation of the echo (2τ) can be used to temporally separate the signal from laser artifacts. Thus, we are able to probe by ESE what was essentially the magnetization of the sample either before, during or after the laser pulse (see fig.1) without interference from laser artifacts.

In the ESE technique the echo represents a rapid, direct measurement of the z component of the magnetization, M_z. However, it is M_y that is detected in both ESE and EPR experiments. In the conventional time resolved EPR experiment at typical microwave powers the time necessary for M_z to be converted into M_y is related to T_1 (the electron spin lattice relaxation time). So, in fact, the time resolution in the EPR experiment is ultimately limited by the T_1 of the spin. In contrast, in ESE the ultimate time resolution is determined by the duration of the first microwave pulse, and thus is not sample limited.

In photosynthetic systems, decay by chemical reaction (electron transfer) can compete effectively with decay of the electron spin polarization (T_1). ESE spectroscopy can be used to independently determine T_1 in species such as a static, chemically produced P-700$^+$ in order to separate various contributions to the decay kinetics. After the initial rise, the kinetic trace in fig.3a shows a 2 component change in the magnetization. Considering the τ of 400 ns used in these experiments the fast component cannot be a first order decay of a radical formed directly by the laser pulse, since this would imply an impossible degree of electron spin polarization,

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* Typically 1 or 2 MHz field modulation has been used in time-resolved ERP experiments. In direct detection EPR experiments it is often necessary to acquire a kinetic trace both on and off resonance and subtract the two. This is in effect low frequency field modulation

i.e., a normalized spin population > 1. This fast changing component must be due to the superposition of the kinetics of > 1 radical. Thus, this observation is direct evidence that the CIDEP we observe at the $g = 2.002$ resonance represents the kinetic consequences of > 1 paramagnetic species in the first 40 ns after laser excitation. This result would have been difficult to establish by EPR.

In the photosynthetic systems studied to date by time-resolved EPR techniques positive identification of the radical species observed has been difficult. The problem of identification is not unexpected since the radicals which have been observed in photosynthetic systems by steady state EPR methods generally have structureless spectra which are not readily distinguishable. In the time-resolved EPR experiments there are the additional problems of the possible appearance of new radical species and of possible signal distortions as discussed above. The observation of nuclear modulation on the primary echo decay envelope provides a powerful tool to positively identify the observed radicals (fig.4). In particular, we can conveniently 'fingerprint' a chlorophyll-like cation radical by the characteristic modulation pattern due to the nitrogen nuclei [12], and we are able to distinguish chlorophyll cation radicals in systems enriched with ^{25}Mg from pheophytin cation radicals [13].

5. Conclusions

Using the time-resolved ESE technique we have observed within 40 ns of laser excitation at least two spin-polarized EPR signals in fully deuterated *S. lividus*. ESE spectroscopy enjoys the advantages of magnetic resonance while avoiding some of the problems encountered in time-resolved EPR. Moreover ESE provides several new advantages particularly for the study of photosynthesis. The unique properties of ESE allow us to directly observe more than one of the very early reactants in photosynthesis.

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