# Resolution of signals attributed to photosystem I primary reactants by time-resolved EPR at K band

Reinhard Furrer and Marion C. Thurnauer*

*Freie Universität Berlin, Fachbereich Physik (FB20/WEIA), D-1000 Berlin 33, Germany* and **Chemistry Division, Argonne National Laboratory, Argonne, IL 60439, USA*

Received 24 January 1983

Abstract and keywords not received

## 1. INTRODUCTION

There are now several reports of the application of time-resolved EPR methods to study the early events of the photosynthetic light reactions. Most of these studies have been directed toward green plant photosystems [1–9]. Transient signals (and electron spin polarization) have also been observed in reaction center preparations and membrane fractions of purple photosynthetic bacteria in which magnetic interactions between the quinone acceptor and the non-heme iron have been disrupted [9,10]. Paramagnetic transients have been observed in green photosynthetic bacteria [11].

In the green plant photosystems the studies have been done on chloroplasts, whole cells of several photosynthetic algae as well as on relatively enriched photosystem I (PSI) particles. Although the experimental conditions have varied considerably, it is now generally believed that the transient EPR responses (at least the ones around $g = 2.0023$) arise from PSI reactions and that the observed chemically-induced dynamic electron polarization (CIDEP) is due to the so-called radical pair mechanism. However, to date it has been difficult to understand the details of all the observations both in terms of the current models of PSI reactions (review [12]) as well as the CIDEP mechanisms [13,14].

One problem in interpreting the EPR results is the distinct possibility that in photosynthetic systems several of the early intermediates might interact magnetically with each other on the experimental time scale. In cases of spectrally overlapping EPR lines the resulting transient electron-spin polarization can produce spectral shapes which are not readily interpreted and possibly give distorted kinetic traces at certain magnetic field values. For instance, a type of transient signal has been reported in several of these studies under different experimental conditions and in different photosynthetic systems [1e,2,3,8,9]. The signal type is distinguished by its overall shape, or polarization pattern, showing low-field emission, middle-enhanced absorption, and high-field emission. Such a signal was observed in low-temperature experiments on chloroplasts and Triton PSI particles which have the PSI iron–sulfur centers A and B reduced [1e,8]. It was simulated, and thereby interpreted, in two different ways. In one case [1e] the data were simulated to fit the current views of PSI primary reactions, where it is suggested that the initial sequence of events involves two acceptors $A_1$ and $A_2(X)$ functioning prior to the iron–sulfur centers A and B. ($A_1$ is believed to be a chlorophyll species and $A_2(X)$ an EPR characterized species is possibly an iron–sulfur center [12].) With this interpretation [1e] the transient signal itself is attributed to one radical: $P700^+$ (the oxidized primary donor of PSI). Another simulation [8,9] required the existence of two acceptors functioning prior to $A_2(X)$. (The identities of these acceptors were not specified.) In this case [8], the overall transient signal is attributed to two radical species, a donor and acceptor.

To uniquely interpret the transient signals and

*Published by Elsevier Biomedical Press*
00145793/83/0000–0000/$3.00 

realize the information contained in the CIDEP [14] it is essential:

(i) To distinguish the nature of the interactions which give rise to the observed spectral splitting; e.g., whether it is hyperfine, exchange or due to $g$ factor difference.

(ii) To determine the spin polarization of a given radical; i.e., whether it is all in emission, absorption, or a combination of emission and absorption.

A convenient and non-invasive way to resolve these questions is to carry out the experiment at different and preferentially higher microwave frequencies. Here, we report on our initial application of time resolved EPR at K band microwave frequency (~24 GHz) to study the early reactions in the photosynthetic alga *Synechoccus lividus*. The results are discussed in terms of reported experiments at X band (~9.5 GHz) [1–3,8,9].

## 2. MATERIALS AND METHODS

The K band spectrometer and a few basic experimental procedures have been described [15]. Typical microwave frequencies in this experiment were around 23.8 GHz. In addition, we have modified our K-band bridge to incorporate a balanced mixer (Honeywell CK-1), so that our instrumental time resolution is as short as 1 ns. For magnetic field scans taken at times delayed with respect to the laser pulse we have used a dual channel PAR boxcar integrator (Model 162/165) in the (A–B) mode with one gate set at a time before the laser pulse and one at various time delays following the laser pulse. The boxcar gatewidth used was 200 ns. For the time resolved spectra (kinetic traces) the boxcar integrator was continuously scanning its 200 ns window with respect to the laser. $g$ factor determinations were made by obtaining the microwave frequency with a counter (HP 5342A) and the static magnetic field value, $B_o$, outside the microwave cavity by use of proton NMR resonances. The field difference between outside the microwave cavity and inside was taken into account by calibrating with a DPPH probe. Freeze-dried deuterated (99.7%) cells of *Synechoccus lividus* [16] were resuspended in $D_2O$ and circulated at room temperature through a quartz flat cell in the spectrometer cavity. For these experiments we have employed deuterated algal cells in order to make good comparisons with the X band spectra where EPR line narrowing due to deuteration gave better resolution and better signal-to-noise ratios (3,2). The samples were excited with an AVCO-Everett, 100 kW $N_2$ laser with pulse duration 5 ns and repetition rate of typically 70 Hz.

## 3. RESULTS

Fig.1 shows the transient EPR spectra observed in *S. lividus* (99.7% deuterated). These spectra (fig.1(a–f)) were obtained as a function of the delay time, $t_D$, with respect to the laser pulse (0.1–3 $\mu$s). To emphasize changes in overall spectral profile with time the individual spectra (a–f) are plotted with about equal intensity. Thus, for later delay times, $t_D$, the data accumulation time was considerably longer than it was for earlier $t_D$-values. All the spectra exhibit both emissive and absorptive components. The assignments, emission (E) and absorption (A), have been made on the basis of comparison with previous reports of similar experiments at X band (see below, fig.3) but have not yet been determined absolutely at K band.

At $t_D = 0.1$ $\mu$s there are three spectral features at $g = 2.0057(2)$,* $g = 2.0048(2)$, and $g = 2.0026(2)$. These three remain until ~1.0 $\mu$s when a new resonance appears at $g = 2.0033(3)$. Finally at $t_D = 3.0$ $\mu$s this latter is the only remaining signal since the others have decayed into the noise. The apparent line broadening at $t_D = 0.1$ $\mu$s relative to later delay times is probably due to so-called uncertainty broadening [17,18]. The shoulders appearing in some of the spectra (for instance high field side in fig.1b) will require more examination to determine whether they are true transient signals or artifacts due to some possible frequency instability.

A kinetic trace of the signal at $g = 2.0057(2)$ is shown in fig.2. The rise is determined by the 200 ns boxcar gatewidth, and the 1/e decay time $\tau = 1.0(1)$ $\mu$s. This trace provides the possibility of setting into perspective the temporal behavior of the

* The errors refer to the last assigned digits of the $g$-factors; they represent standard deviations as derived from the comparison of the $g$-factors from all the experiments taken at different times

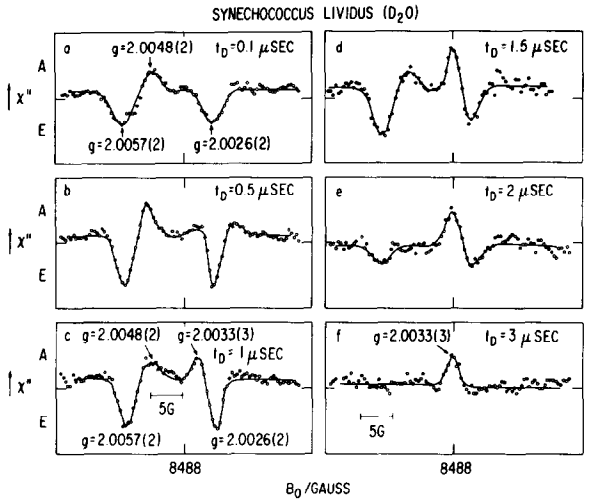


Fig.1. Magnetic field scans taken at different fixed delay times $t_D$ following the laser pulse in whole cells of *S. lividus* (99.7% deuterated). $t_D$ refers to the time from the center of the 5 ns laser pulse to the center of the 200 ns boxcar gate. The lines drawn through the points are simply 'eyeball' fits. Since no field modulation is employed, these are non-derivative absorption mode spectra. The sense, E(emission), A(absorption) is by comparison to previous work on the same system [3,7]

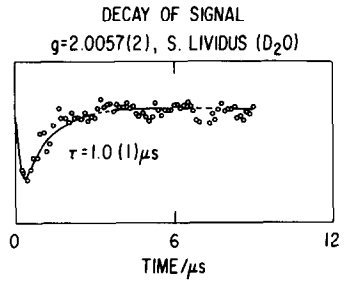


Fig.2. Kinetic trace of signal at $g = 2.0057(2)$ (see fig.1).

responses at $g = 2.0048(2)$ and $g = 2.0026(2)$. From such an analysis it appears that the decay times of the lines at $g = 2.0057(2)$ and $g = 2.0026(2)$ are similar ($\sim 1\ \mu s$) with the signal at $g = 2.0048(2)$ decaying slightly faster, possibly matching the rise at $g = 2.0033(3)$.

## 4. DISCUSSION

In fig.3 we plot the spectrum from fig.1b ($t_D$ = 0.5 μs) together with those reported taken at X band on the same system under similar experimen-

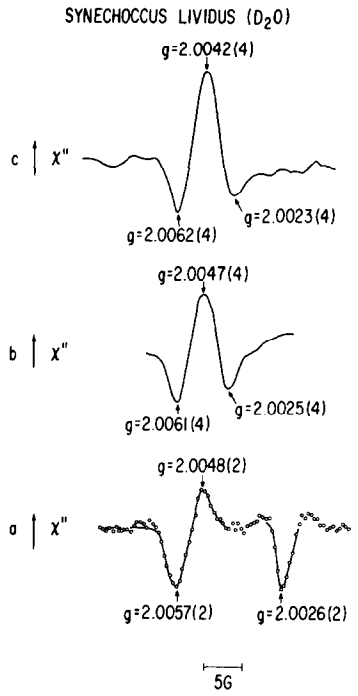


Fig.3. Field scans at early delay times taken in *S. lividus* ($D_2O$) at both K and X band lined up at common low field *g*-factor and plotted on same magnetic field scale. Absorption (A) is up and emission (E) is down. The magnetic field ($B_o$) increases to the right. The order of magnitude difference in $t_D$ (c *vs* a and b) reflects experimental time resolutions: (a) K band, $t_D = 0.5\ \mu s$ (see fig.1b); (b) X band, continuous microwaves, $t_D \cong 0.6\ \mu s$; (c) X band, electron spin echo field scan, $t_D \cong 0.05\ \mu s$. (In the pulsed EPR experiments it is the so-called standard signal (see [3]) which is comparable with the cw EPR signal.)

tal conditions using both pulsed [3] and continuous wave (cw) [7] time-resolved EPR techniques. The 3 spectra are lined up (within experimental error) at the common *g* factor 2.0057(2)* and plotted on the same magnetic field scale. This presentation clearly illustrates the important new information obtained here. Since the magnitudes of the splittings between the high-field resonance and those at low-field are frequency dependent, the overall spec-

* The X band *g*-factors 2.0062 and 2.0061 in fig.3 are taken to agree with the K band value at 2.0057. The larger error in *g*-factors in the X band spectra is consistent with the conclusions of this paper that at X band we are dealing with overlapping lines

trum must be assigned to at least 2 different radicals. The absence of a frequency dependence for the splitting between the two low field resonances suggests that they belong to one single species showing low field emission and high field absorption with $g$-factor between 2.0057 and 2.0048.

Many reports of transient EPR experiments on photosynthetic systems have in common the same type of signal as shown in fig.3; that is, one showing low-field emission, middle-enhanced absorption, high-field emission [1e,2,3,8,9]. This includes experiments on bacterial photosystems [9]. The comparison in fig.3 clearly illustrates the general problems in interpreting this type of signal. When there is insufficient spectral resolution, as in the X band spectra (fig.3b,3c), one must guess not only at the number of radicals giving rise to the signal but also at the polarization of a given radical; i.e., whether it is all in emission, absorption, or a combination. Yet this is the information necessary to interpret this type of signal in terms of primary reactions [14].

Before further discussion of our results we point out that there have been some difficulties in establishing a relationship between the several reports of transient EPR signals seen in similar green plant photosystems but under different experimental conditions. These include factors such as instrumental approach (field modulation *vs* no field modulation, continuous *vs* pulsed microwaves) microwave power, temperature, and initial redox conditions. In addressing this problem we have identified the signals displayed in fig.3 with the one which has been observed at low temperature in other protonated and deuterated photosynthetic systems [2b–d] and which was demonstrated to occur when centers A and B are initially reduced [1e,8]. Our correlation is based first on the similarity in overall shape and $g$ factors of maxima and minima* and second on the conclusion of experiments describing the effects of ambient redox potential at room temperature [3c].

Therefore a crucial point we wish to emphasize here is that our results are in sharp contrast with the interpretation which attributes the overall signal to only one radical, $P700^+$ [1e]. Moreover, in considering the presence of at least two radical species it is necessary to explain the relative signal phases (e.g., emission *vs* enhanced absorption) for a unique interpretation in terms of possible PSI reactions [8].

Another observation is the appearance at longer $t_D$-values (fig.1) of the signal at $g = 2.0033(3)$. This signal had not been reported in the X band experiments. It is possible at longer $t_D$-values that the observations become dependent on both the type of photosynthetic sample and the experimental conditions. Future experiments are designed to check this.

* Under the same experimental conditions of the X band spectra shown in fig.3 but in normal protonated *S. lividus* we observe signals with similar overall shape but broadened to cover a field range of 20–25 G

## ACKNOWLEDGEMENTS

We wish to acknowledge H. Crespi and H. DaBoll for supplying the *S. lividus*. We thank R. Lawler and A. Trifunac for encouragement and M.C.T. acknowledges many discussions with J. Norris. We thank A.J. Hoff for forwarding a preprint of his work. The work was performed at Argonne National Laboratory under the auspices of the Office of Basic Energy Sciences, Division of Chemical Sciences, US-DOE under contract number W-31-109-ENG-38. The work performed at Freie Universität Berlin was supported by the Deutsche Forschungsgemeinschaft (Sfb 161).

## REFERENCES

[1] a. Blankenship, R., McGuire, A. and Sauer, K. (1975) Proc. Natl. Acad. Sci. USA 72, 4943–4947.
b. Dismukes, G.C., McGuire, A., Blankenship, R. and Sauer, K. (1978) Biophys. J. 21, 239–256.
c. Friesner, R., Dismukes, G.C. and Sauer, K. (1979) Biophys. J. 25, 277–294.
d. Dismukes, G.C., McGuire, A., Friesner, R. and Sauer, K. (1979) Rev. Chem. Int. 3, 197–230.
e. McCracken, J.L., Franck, H.A. and Sauer, K. (1982) Biochim. Biophys. Acta 679, 156–168.

[2] a. McIntosh, A.R. and Bolton, J.R. (1976) Nature 263, 443–445.
b. McIntosh, A.R., Manikowski, H., Wong, S.K., Taylor, C.P.S. and Bolton, J.R. (1979) Biochem. Biophys. Res. Commun. 87, 605–612.
c. McIntosh, A.R., Manikowski, H. and Bolton, J.R. (1979) J. Phys. Chem. 83, 3309–3313.
d. McIntosh, A.R. and Bolton, J.R. (1979) J. Rev. Chem. Int. 3, 121–129.

e. McIntosh, A.R., Manikowski, H. and Bolton, J.R. (1981) in: Proc. 5th Int. Congr. Photosynthesis. Sept. 7–13, 1980, Halkidiki (Akoyunoglou, G. ed) vol.2, pp.687–695, Balaban Int. Sci. Services, Philadelphia PA.

[3] a. Thurnauer, M.C., Bowman, M.K. and Norris, J.R. (1979) FEBS Lett. 100, 309–312.
b. Thurnauer, M.C. and Norris, J.R. (1980) Chem. Phys. Lett. 76, 557–561.
c. Thurnauer, M.C., Rutherford, A.W. and Norris, J.R. (1982) Biochim. Biophys. Acta 682, 332–338.

[4] Warden, J.T. and Adrianowycz, O.L. (1981) in: Proc. 5th Int. Congr. Photosynthesis, Sept. 7–13, 1980, Halkidiki (Akoyunoglou, G. ed) vol.1, pp.83–191, Balaban Int. Sci. Services, Philadelphia PA.

[5] Dismukes, G.C. and Tycko, R. (1981) in: Proc. 5th Int. Congr. Photosynthesis, Sept. 7–13, 1980, Halkidiki (Akoyunoglou, G. ed) vol.3, pp.785–803, Balaban Int. Sci. Services, Philadelphia PA.

[6] Heathcote, P. and Evans, M.C.W. (1981) in: Proc. 5th Int. Congr. Photosynthesis, Sept.7–13, 1980, Halkidiki (Akoyunoglou, G. ed) vol.2, pp.665–675, Balaban Int. Sci. Services, Philadelphia PA.

[7] Thurnauer, M.C., Furrer, R., Clark, C.O. and Norris, J.R. (1981) Proc. Schultagung Mod. Meth. der Hoch Frequenze Spektroskopie, Nov. 2–11, Schloss Reinhardsbrunn.

[8] Gast, P., Swarthoff, T., Ebskamp, F.C.R. and Hoff, A.J. (1983) Biochim. Biophys. Acta 722, 163–175.

[9] Gast, P. (1982) PhD Thesis, State University of Leiden.

[10] a. Hoff, A.J., Gast, P. and Romijn, J.C. (1977) FEBS Lett. 73, 185–190.
b. Gast, P. and Hoff, A.J. (1979) Biochim. Biophys. Acta 548, 520–535.
c. Hoff, A.J. and Gast, P. (1979) J. Phys. Chem. 83, 3355–3358.

[11] Heathcote, P. and Warden, J.T. (1982) FEBS Lett. 140, 277–281.

[12] Malkin, R. (1982) Annu. Rev. Plant Physiol. 33, 455–479.

[13] Muus, L.T., Atkins, P.W., McLauchlan, K.A. and Pedersen, J.B. eds (1977) Chemically Induced Magnetic Polarization, Reidel, Boston MA

[14] Pedersen, J. and Boiden D. (1979) FEBS Lett. 97, 305–310.

[15] Furrer, R., Fujara, F., Lange, C., Stehlik, D., Vieth, H.M. and Vollman, W. (1980) Chem. Phys. Lett. 75, 332–339.

[16] a. DaBoll, H.F., Crespi, H.L. and Katz, J.J. (1962) Biotech. Bioeng. 4, 281–297.
b. Taecker, R.G., Crespi, H.L., DaBoll, H.F. and Katz, J.J. (1971) Biotech. Bioenerg. 13, 779–793.

[17] Trifunac, A.D. and Thurnauer, M.C. (1979) in: Time Domain Electron Spin Resonance (Kevan, L. and Schwartz, R.N. eds) ch.4, pp.107–152, Wiley, New York.

[18] Varma, N.C. and Fessenden, R.W. (1973) J. Chem. Phys. 58, 2501–2506.