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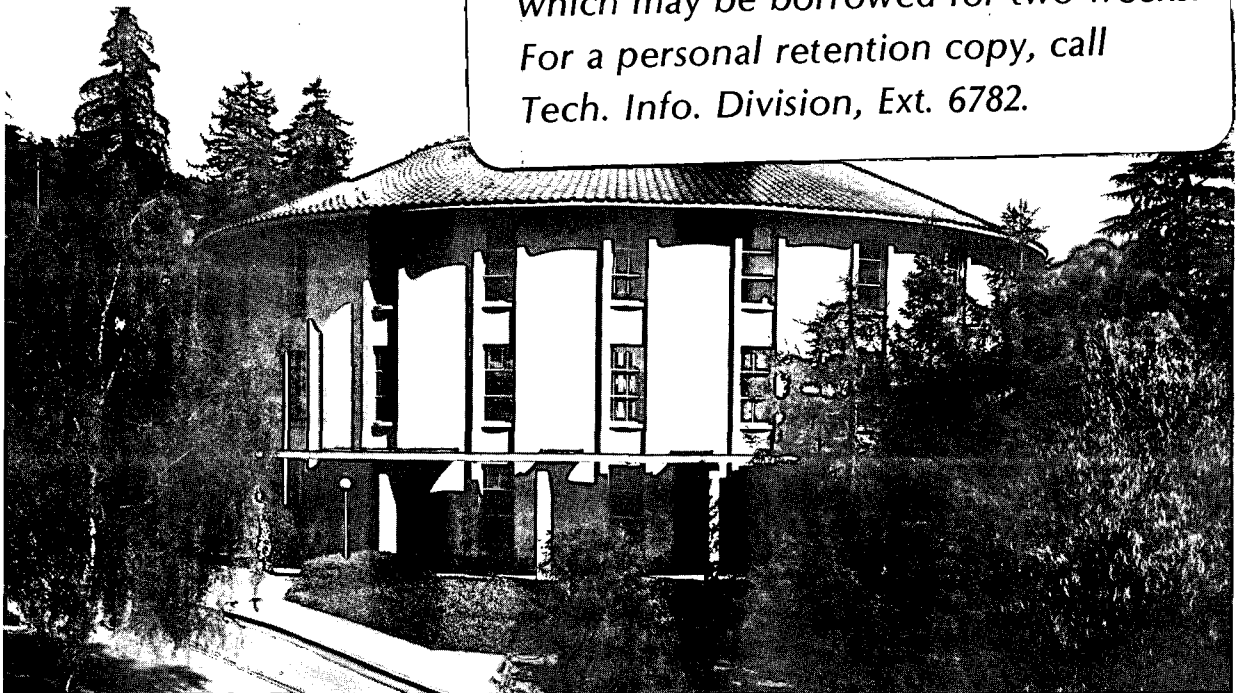
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March 1981

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RADICAL PAIR INTERACTIONS IN SPINACH CHLOROPLASTS

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Key Words: Photosystem I, time resolved EPR

SUMMARY

Time resolved Electron Paramagnetic Resonance (EPR) studies were done on broken spinach chloroplasts under reducing conditions at low temperature (10 K). A dramatic dependence of signal dynamics and lineshape in the $g=2.00$ region on the reduction state of Photosystem I is demonstrated. Computer simulations of the spin polarized lineshapes obtained in this work lead to the conclusion that the primary electron acceptor in Photosystem I, a species known as A_1 , is a chlorophyll a dimer.

INTRODUCTION

Light induced electron spin polarization is an important consequence of charge separation in the primary photoreactions of photosynthesis. Spin polarization has been studied in a wide variety of green plant [1-4], algal [3-6] and bacterial [7] preparations using electron paramagnetic resonance (EPR) techniques at room temperature and at low temperature. All of these studies make use of pulsed light excitation and fast time-resolved EPR to probe the initial charge transfer events of the photosynthetic process. The information contained in these measurements is of four types (a) structural--the arrangement of the primary photoreactants with respect to each other and with respect to the membranes in which they reside is contained in the spectra from flow oriented [2] or magnetic field oriented [4] photosynthetic systems; (b) the chemical identities of the donors and acceptors involved in the primary photochemistry of photosynthesis is obtained from the g factor and hyperfine coupling constants required to simulate the observed spectral lineshapes [7]; (c) the observed kinetic profiles can be resolved into chemical and spin lattice relaxation dynamics by studying the kinetics of the system as a function of microwave power and temperature [8]; (d) interactions between the photoreactants can be analyzed [9].

We have investigated the low potential redox dependence of the fast transient EPR signals which are observed in whole chloroplasts and in photosystem I preparations from spinach. Our choice of sample conditions for this study was based on the current model of the photosystem I reaction center organization, which may be represented as $P_{700} A_1 X Fd_B Fd_A$ [10]. P_{700} is the primary electron donor. A_1 is

thought to be the primary electron acceptor [10]. X is an EPR-characterized species having g values of 2.07, 1.86 and 1.75 [11]. Fd_B and Fd_A are iron sulfur centers which can be reduced chemically by sodium dithionite in the dark [12,13]. X and A_1 , which have very low reduction potentials, can be reduced by sodium dithionite plus illumination while freezing to 77 K [14].

For samples poised in the redox state $P_{700} A_1 X Fd_B^- Fd_A^-$ we have essentially duplicated for chloroplasts the results reported by McIntosh et al [3,4] for algae and subchloroplast particles. However, if the photosystem I reaction center is poised in the redox state $P_{700} A_1 X^- Fd_B^- Fd_A^-$ prior to the low temperature pulsed-light EPR measurement, a substantial change in both dynamics and spectral lineshape of the observed transient species is observed. It is possible to interpret these new findings to distinguish between two candidates for the primary acceptor A_1 , which has been attributed to a chlorophyll a monomer [15,16] or a chlorophyll a dimer [17]. We also raise questions concerning the validity of g-value assignments that ignore interactions between the primary photoreactants, and we describe the use of a quantum mechanical model that permits a quantitative analysis of the experimental lineshapes.

MATERIALS AND METHODS

Time resolved EPR measurements were made at X-band using a Varian E-109 spectrometer employing 1 MHz magnetic field modulation and equipped with an Air Products Helitran cryostat. The measured response time of the instrument was 2 μ sec. The Varian TE₁₀₂ cavity (E-231) used in these experiments was fitted with an optical flange made from K-band

waveguide to allow 100% transmission of the exciting light. The output of the 1 MHz lock-in amplifier was fed directly into a Nicolet Explorer IIIA Oscilloscope with a Model 204 plug-in. The recorder traces were then transmitted to a NIC-80 minicomputer for signal averaging and analysis.

The light source for these experiments was a Phase-R DL-1400 dye laser operating at 590 nm (Rhodamine 6G in methanol) with a 500 nsec pulse width. Typical pulse energies of 100 mJ were utilized, and care was taken to insure that the light was always saturating. Pulse repetition rate was 1 Hz. Temperature measurements were made with a gold/chromel thermocouple.

The possibility of rapid passage distortion of the EPR signals was tested using direct detection of resonance. In these experiments, the magnetic field was not modulated, and the output of the microwave mixer was fed into a wideband amplifier with a bandwidth of 300 Hz - 10 MHz and a gain of 30-80 dB. After amplification, the signals were recorded as above. Transient signals which arise from heating of the sample and cavity by the laser flash were eliminated by recording signals on and off resonance and subsequent subtraction after signal averaging. It should be noted that these artifacts arise only accompanying direct detection and are small at the low microwave power levels used in our experiments.

Broken spinach chloroplasts were prepared as described [2] in 0.4 M sucrose, 0.05 M HEPES buffer, 0.01 M NaCl, pH 7.5. After isolation, the chloroplasts were resuspended in 0.2 M glycine buffer, pH 10.1 and treated with 0.2mM EDTA. These resuspensions were then treated in two different ways:

Treatment with dithionite: Chloroplast suspensions were degassed and mixed with sodium dithionite under N_2 atmosphere so that the concentration of the reducing agent added was 50 mM. 10 μ M methyl viologen served as a redox mediator.

Treatment with ferricyanide: Chloroplast suspensions were mixed with potassium ferricyanide such that the concentration of the oxidant added was 1 mM.

In some experiments digitonin particles were used instead of broken spinach chloroplasts. These particles were prepared as previously described [19]. In each case, the treated suspensions were combined with an equal amount of ethylene glycol, sealed in EPR tubes, and stored at 77°K. Some samples treated with dithionite were poised at a lower redox potential by illumination with a 400 watt tungsten lamp during freezing.

Decay constants were obtained from digitized kinetic traces by fitting them with a non-linear least squares computer program (ZXSSQ of the IMSL library).

RESULTS

Spinach chloroplasts which were frozen dark in the presence of dithionite give rise to a fast-decaying EPR signal (60 μ sec at 50 μ W of microwave power at 10°K, see Fig. 1) after a light pulse from a laser. A plot of the amplitude of the EPR transient versus magnetic field strength is shown in Fig. 2. The spectrum contains both emissive and absorptive components which are observed over a 30 G range near $g = 2.00$. This EPR transient is also observed without using field modulation (Fig. 3), and its spectrum exhibits a lineshape that

corresponds to the first derivative seen using field modulation (Fig 2). Because there is qualitative agreement between the lineshapes in Figs. 2 and 3, we conclude that rapid passage effects owing to field modulation detection of the transient amplitudes are small. Furthermore, these rapid transient signals do not decay directly to zero. When examined at longer (msec) times after the laser pulse, the signals cross the baseline and decay subsequently to zero only after approximately 1 msec (Fig. 4). Both of these kinetic components show a dependence on microwave power and temperature. A plot of the dynamics of the faster component versus microwave power is given in Fig. 5.

Chloroplasts or digitonin photosystem I particles prepared in the state $P_{700} A_1 X^- Fd_B^- Fd_A^-$ by freezing under illumination in the presence of dithionite gave rise to a much faster-decaying transient (6 μ sec at 50 μ W of microwave power at 10 K; Fig. 6) after a light pulse from a laser. The relaxation time of this transient signal decreases to 2 μ sec (our instrumental time resolution) as the microwave power is raised to 100 μ W. A plot of the amplitude of the transient signal (observed using field modulation) versus magnetic field strength (Fig. 7) reveals a mixed emissive and absorptive pattern which is significantly different from the spectrum shown in Fig. 2. The transient EPR spectrum of chloroplasts prepared in this condition was also observed using direct detection (Fig. 8). No longer-lived (msec) component is observed at longer sweep times in these samples, however.

An orientation effect of the transient spectra was observed on magnetically aligned chloroplasts prepared only in the redox state, $P_{700} A_1 X Fd_B^- Fd_A^-$ [21]. Transient EPR signals identical to those shown in Figs. 1 and 6 were observed in photosystem I-enriched preparations. No

transient EPR signals were observed in the $g = 2.00$ region of spinach chloroplasts which were treated with $K_3Fe(CN)_6$, thereby oxidizing P700 to $P700^+$.

DISCUSSION

Theoretical model

The Hamiltonian for two interacting spins can be written

$$H = H_{Zee} + H_{hf} + H_{ex} \quad (1)$$

where

$$H_{Zee} = \beta [H_0 \cdot g_1 \cdot S_1 + H_0 \cdot g_2^t \cdot S_2] \quad (2)$$

$$H_{hf} = \sum_i S_1 \cdot A_1^i \cdot I_1^i + \sum_j S_2 \cdot A_2^j \cdot I_2^j \quad (3)$$

$$H_{ex} = S_1 \cdot J \cdot S_2 \quad (4)$$

The first term is the Zeeman interaction where β is the Bohr magneton, H_0

is the magnetic field vector, g_1 is a diagonal g-tensor for the spin of radical 1, and g_2^t is the g tensor for the spin of radical 2 transformed into the g_1 coordinate system by an Euler angle rotation matrix. S_1 and S_2 are the total spin operators for radical 1 and radical 2, respectively. The second term represents the hyperfine interaction summed over the i and j possible hyperfine states having the coupling constant tensors A_1^i and A_2^j and nuclear spin operators I_1 and I_2 for radicals 1 and 2, respectively. The third term is the isotropic electron spin exchange interaction.

We generate the Hamiltonian matrix by assuming the high-field basis set:

$$\begin{aligned}
 |S\rangle &= 1/\sqrt{2} (\alpha\beta - \beta\alpha) \\
 |T_{+1}\rangle &= \alpha\alpha \\
 |T_0\rangle &= 1/\sqrt{2} (\alpha\beta + \beta\alpha) \\
 |T_{-1}\rangle &= \beta\beta
 \end{aligned}
 \tag{5}$$

The special case of this Hamiltonian matrix which is pertinent to our problem is given by eq. (6).

This matrix assumes isotropic g_1 , g_2 , A and J and may be solved numerically by inserting appropriate values of the parameters g_1 , g_2 , J , A_1^i , A_2^j and diagonalizing the matrix. Iteration over the hyperfine states is necessary.

The transition field positions, E_{ij} , are calculated from differences between the eigenvalues. The oscillator strengths, P_n , for the transitions are determined from the relation

	$ T_{+1}\rangle$	$ S\rangle$	$ T_0\rangle$	$ T_{-1}\rangle$
$\langle T_{+1} $	$\frac{1}{2}BH_0(g_1 + g_2) - J/4$ $+\frac{1}{2}(A_1^i M_1^i + A_2^j M_2^j)$	0	0	0
$\langle S $	0	$\frac{3J}{4}$	$\frac{1}{2}[BH_0(g_1 - g_2) +$ $A_1^i M_1^i - A_2^j M_2^j]$	0
$\langle T_0 $	0	$\frac{1}{2}[BH_0(g_1 - g_2) +$ $A_1^i M_1^i - A_2^j M_2^j]$	$-\frac{J}{4}$	0
$\langle T_{-1} $	0	0	0	$-\frac{1}{2}BH_0(g_1 + g_2) - J/4$ $-\frac{1}{2}(A_1^i M_1^i + A_2^j M_2^j)$

[6]

$$P_n \propto |\langle \psi_a | g_1 S_{1x} + g_2 S_{2x} | \psi_b \rangle|^2 \quad (7)$$

where ψ_a and ψ_b are the wave functions of the levels which are coupled by the microwave field. The probabilities are then convoluted using a gaussian lineshape function having a fixed width, Δ , across the field profile, i.e.

$$I(H_0) = \sum_n P_n \times \exp[-(E_{ab} - H_0)^2 / \Delta^2] \quad (8)$$

In some cases an anisotropic g_2 tensor was needed. In these instances the second Zeeman term was given as $\beta \underline{g}_2 \cdot \underline{H}_0$ and the "powder pattern" spectrum was generated by integrating over the angle variables which project the \underline{H}_0 field onto the \underline{g}_2 axes.

Because our experimental results show significant spin polarization it is necessary to incorporate into this analysis a provision for allowing a non-boltzmann population of spin states to weight the oscillator strengths. Assuming that the charge transfer is coherent and occurs from a singlet precursor one weights the oscillator strengths determined by equation (7) by the calculated singlet coefficient given in the interacting system basis set.

The oscillator strengths for the four radical pair transitions derived from equation (7) are

$$\begin{aligned} p_1^{ij} &= 1 - J/\omega_{ij} \\ p_2^{ij} &= 1 + J/\omega_{ij} \\ p_3^{ij} &= 1 + J/\omega_{ij} \\ p_4^{ij} &= 1 - J/\omega_{ij} \end{aligned} \quad (9)$$

where

$$\omega_{ij} = \{ [g_1 - g_2] \beta H_0 + (A_1^i M_1^i - A_2^j M_2^j)^2 + J^2 \}^{\frac{1}{2}} \quad (10)$$

In the high field limit $S - T_{+1}$ and $S - T_{-1}$ mixing are normally small (18) and so $S - T_0$ mixing dominates. We therefore ask what percent of singlet character ends up in the $|+\rangle = C_1|S\rangle + C_2|T_0\rangle$ level and the $|-\rangle = C_3|S\rangle - C_4|T_0\rangle$ levels. The coefficients C_1 and C_3 are obtained from the diagonalization of the matrix equation [1] and are given as

$$c_1 = (1 + J/\omega_{ij})^{\frac{1}{2}} \quad ; \quad c_3 = (1 - J/\omega_{ij})^{\frac{1}{2}} \quad (11)$$

The corrections to the oscillator strengths from spin polarization are given by the square of these coefficients, and are $1 + J/\omega_{ij}$ for P_1^{ij} and P_4^{ij} because these involve transitions to the $|+\rangle$ level and $1 - J/\omega_{ij}$ for P_2^{ij} and P_3^{ij} because these involve transitions to the $|-\rangle$ level. The net effect is that all oscillator strengths are equal in magnitude but positive for absorptions and negative for emission.

Dynamics of the Transient Signals

An interesting feature of the transient EPR signals of Figs. 1 and 6 is the fact that the relaxation is dependent on redox potential. Upon prior reduction of X the half life of the observed decay decreases by an order of magnitude. This is what one might expect to observe from a radical in close proximity to a fast relaxing paramagnetic species, (e.g. X^-), or if the dynamics reflects the back reaction of the radical pair.

The decay of electron spin polarization when monitored by continuous wave EPR techniques is a convolution of three processes [7,8]. The

dominant process at high microwave powers can be stimulated emission or absorption of microwave radiation [7]. The other processes are spin-lattice relaxation and chemical decay of the radical pair. Thus, the observed decay rate is a convolution of rates of differing magnitude. The relative contributions of these processes can be sorted out as described by Atkins et al., [8], by studying the microwave power dependence of the decay dynamics. In the absence of chemical relaxation and distortions due to magnetic field modulation [22], the transient solutions to the Bloch equations predict a simple exponential decay with decay constant given by

$$k_{\text{eff}} = 1/T_{\text{eff}} = 1/T_1 + (\gamma H_1)^2 T_2 / (1 + \delta\omega^2 T_2^2) \quad (12)$$

where T_1 and T_2 are the spin lattice and spin spin relaxation times, γ is the gyromagnetic ratio, H_1 is the magnitude of the microwave field and $\delta\omega$ is the frequency offset from resonance. The assumptions made in the derivation of equation (12) are $T_1 \gg T_2$ and $\delta\omega \neq 0$. Equation (12) predicts that a plot of the observed decay constant versus microwave power (which is proportional to H_1^2) will yield a straight line. Extrapolation to zero power will give a value for T_1 . The linearity of a plot of the effective decay constant at low microwave power provides evidence for the validity of the assumption that chemical decay was not important.

The spin polarized signals from spinach chloroplasts with only Fd_B and Fd_A reduced (Fig. 1) are slow enough at low temperature so that they can be time resolved with our instrument. The power dependence of the effective rate of this decay is shown in Fig. 5. It is nearly linear, indicating that the chemical back reaction rate is not important, and

extrapolation to zero power gives a T_1 of 60 μ sec.

Preliminary studies on the slower kinetic component (Fig. 4) arising in spinach chloroplasts prepared in this same redox state show a non-linear dependence on microwave power suggesting that chemical decay is contributing at longer times. Future studies will focus on extracting the relative contributions of each of the dynamical processes from the effective decays of the spin polarized transients as a function of redox potential, temperature and microwave power.

Application of the model to the results obtained from chloroplasts prepared in the redox state $P700 A_1 X^- Fd_B^- Fd_A^-$

Illumination while freezing the chloroplasts in the presence of sodium dithionite not only reduces X to X^- but is reported to reduce A_1 to A_1^- [15]. However, because of the low potential expected for the A_1/A_1^- couple and because we observe a transient EPR signal following a pulse of light in photosystem I-enriched preparations, it appears that A_1 is not fully reduced under our conditions. We conclude that the observed spectra are representative of the $P700^+ A_1^-$ radical pair. This conclusion is consistent with previous studies which detected radical pair recombination triplet states in photosystem I preparations and spinach chloroplasts prepared under the same conditions [19]. An alternative explanation is that A_1 is fully reduced under these conditions and there exists another acceptor preceding A_1 . Because there is no supporting evidence for this possibility we favor the first hypothesis.

The spectrum shown in Fig. 7 displays no detectable orientation dependence in magnetically aligned chloroplasts, indicating that contributions from anisotropic g tensors or other anisotropic

interactions are negligible. The absence of an anisotropic g-tensor at this stage of photosynthetic electron transport is reasonably consistent with the view that the primary donor and acceptor of photosystem I are chlorophyll species.

To test the hypothesis that A_1 is a chlorophyll dimer or monomer, computer simulations of the possible radical pair spectra were done using the analysis presented above. Two sets of simulations were done. In the first set the radical pair consisted of a Chl a dimer cation species and a Chl a monomer anion, while in the second set of simulations the radical pair was taken to be a Chl a dimer cation and a Chl a dimer anion. The g-values and hyperfine coupling constants used in our simulations were taken from the work of Fajer, *et al.* [20]. The results of these simulations for various values of the exchange integral, J, are shown in Fig. 9.

The criteria used for choosing the best fit to the spectrum of Fig. 7 are the splittings between the peaks and their relative amplitudes. We found that for a J value of 1.5 G the splitting between the peaks was nicely predicted in both cases. We also found that the amount of asymmetry in the spectrum was very sensitive to the differences in the hyperfine fields of the two radicals if they are weakly coupled (low J) and the g-value difference between them is small. This effect can be seen clearly in Fig. 9. As J is increased above 10 G, the asymmetry in the predicted radical pair spectra vanishes. Further, these simulations are in much closer agreement with the experimental results (Fig. 7) under the assumption that A_1 is a dimeric chlorophyll species.

Application of the model to the results obtained from chloroplasts prepared in the redox state $P_{700}^+ X Fd_B^- Fd_A^-$.

At first glance the spectrum shown in Fig. 2 appears to be fit by a model where J is zero. The spectrum qualitatively resembles a convolution of two gaussians with nearly identical g values, but one broad in emission and the other narrow in absorption. However, the spectrum cannot be fit within the limits of the experimental error by such a model. A major inadequacy of this model is that it does not explain the orientation dependence of the spectrum [4,20]. Isotropic spins would not produce such a result unless the interaction between the species is orientation dependent (e.g., dipolar). The prospect of dipolar coupling contributing to the spectra was ruled out when attempts at simulating the lineshapes in Fig. 2 through equation (6) proved unsuccessful. Most likely, the anisotropy arises from the g tensor anisotropy of X .

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REFERENCES

1. Blankenship, R., McGuire, A. and Sauer, K. (1975) Proc. Natl. Acad. Sci. U.S.A 72, 4943-4947.
2. Dismukes, G.C., McGuire, A., Blankenship, R. and Sauer, K. (1978) Biophys. J. 21, 239-256.
3. McIntosh, A.R., Manikowski, H., Wong, S.K., Taylor, C.P.S. and Bolton, J.R. (1979) Biochem. Biophys. Res. Comm. 87, 605-612.
4. McIntosh, A.R., Manikowski, H. and Bolton, J.R. (1979) J. Phys. Chem. 83, 3309-3313.
5. McIntosh, A.R. and Bolton, J.R. (1976) Nature 263, 443-445.
6. Thurnauer, M.C., Bowman, M.K. and Norris, J.R. (1979) FEBS Letts. 100, 309-312.
7. Hoff, A.J., Gast, P. and Romijn, J.C. (1977) FEBS Letts. 73, 185-190.
8. Atkins, P.W., McLauchlan, K.A. and Percival, P.W. (1973) Mol. Phys. 25, 281-296.
9. Friesner, R., Dismukes, G.C. and Sauer, K. (1979) Biophys. J. 25, 277-294.
10. Sauer, K., Mathis, P., Acker, S. and Van Best, J.A. (1978) Biochim. Biophys. Acta 503, 120-134.
11. McIntosh, A.R. and Bolton, J.R. (1976) Biochim. Biophys. Acta 430, 555-559.
12. Ke, B. (1973) Biochim. Biophys. Acta. 301, 1-33.
13. Malkin, R. and Bearden, A.J. (1971) proc. Natl. Acad. Sci. U.S.A. 63, 16-19.
14. Heathcote, P., Timofeev, K.N., Evans, M.C.W. (1979) FEB Letts. 101, 105-109.

15. Heathcote, P. and Evans, M.C.W. (1980) FEBS Letts. 111, 381-385.
16. Baltimore, B. and Malkin, R. (1980) Photochem. Photobiol. 31, 485-490.
17. Shuvalov, V.A., Dolan, E. and Ke, B. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 770-773.
18. Adrian, F. (1971) J. Chem. Phys. 54, 3918-3923.
19. Frank, H.A., McLean, M.B. and Sauer, K. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 5124-5128.
20. Fujita, I., Davis, M.S. and Fajer, J. (1978) J. Amer. Chem. Soc. 100, 6280-6282.
21. Frank, H.A., McCracken, J.L. and Sauer, K. (unpublished results).
22. Friesner, R., McCracken, J.L. and Sauer, K. (1981) J. Magn. Reson., in press.

FIGURE CAPTIONS

Figure 1. EPR transient signal from broken spinach chloroplast suspended under N_2 gas in 0.2M glycine buffer, pH = 10.1, treated with 50mM sodium dithionite, 10 μ M methyl viologen, and ethylene glycol before freezing in the dark. The spectrometer settings were: microwave power, 50 μ W; temperature, 10 K; field modulation frequency, 1 MHz; modulation amplitude, 4G. This trace is the average of 400 events.

Figure 2. A plot of transient signal amplitude versus magnetic field strength for broken spinach chloroplasts prepared as in Fig. 1. The conditions for the measurement were identical to those given in Fig. 1.

Figure 3. A plot of transient signal amplitude versus magnetic field strength for broken spinach chloroplasts prepared as in Fig. 1. The conditions for the measurement were: microwave power, 50 μ W; temperature, 10 K, and direct detection of the EPR transient was utilized.

Figure 4. EPR transient signal identical to that of Fig. 1 except that the sweep time of the transient recorder has been lengthened to 10 msec.

Figure 5. Plot of the decay constant versus microwave power for the rapid transient signal displayed in Fig. 1. The decay constant was obtained by fitting the digitized data to a single exponential using a non-linear least squares computer program.

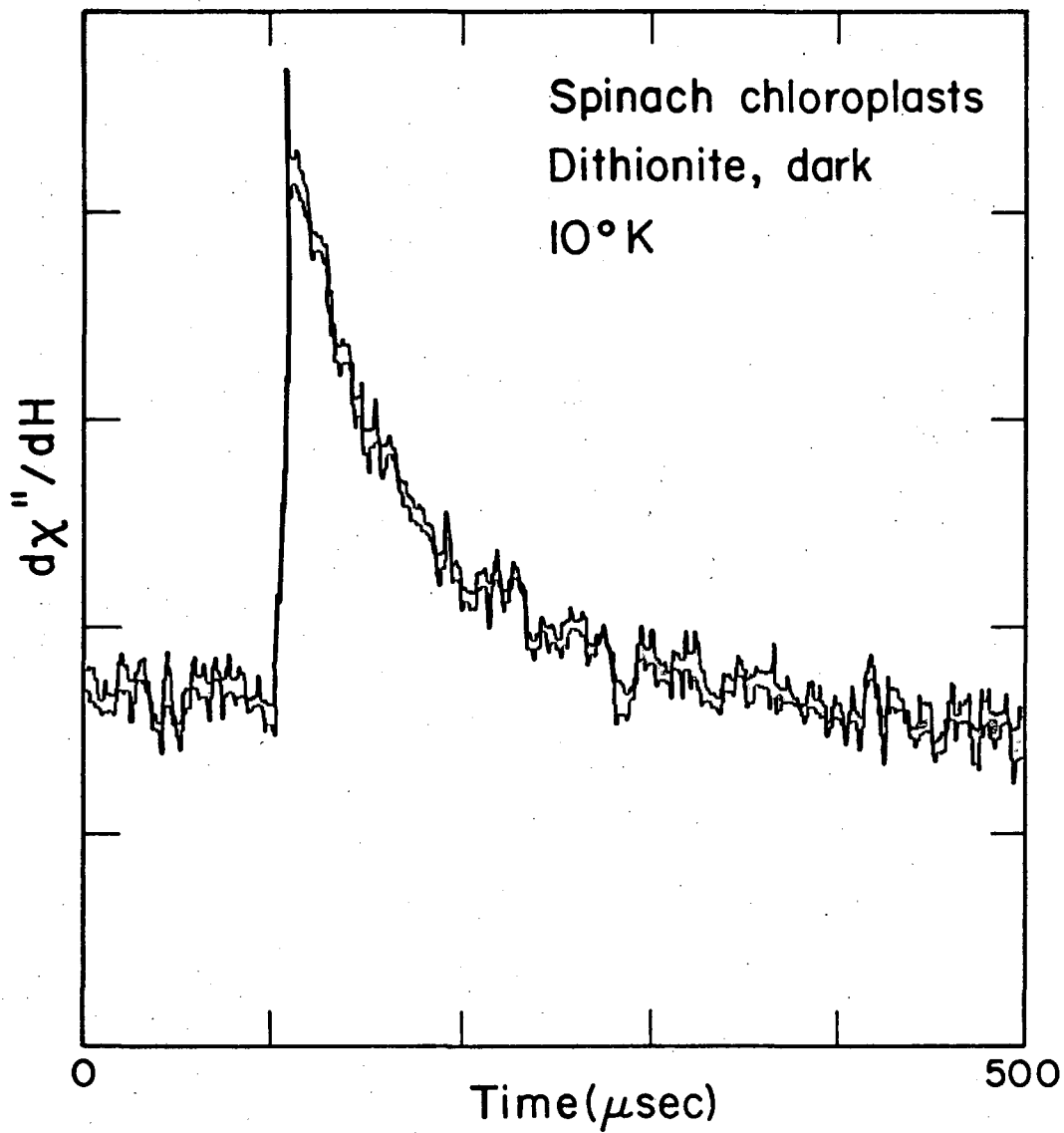
Figure 6. EPR transient signal from broken spinach chloroplasts suspended under N_2 gas in 0.2M glycine buffer, pH = 10.1 treated with 50mM sodium

dithionite, 10 μ M methy viologen and ethylene glycol. The sample was then frozen during illumination with a 400W tungsten lamp. The spectrometer settings for this measurement were: microwave power, 50 μ W; temperature, 10 K; field modulation frequency, 1 MHz; field modulation amplitude, 4G. This trace was the average of 400 events.

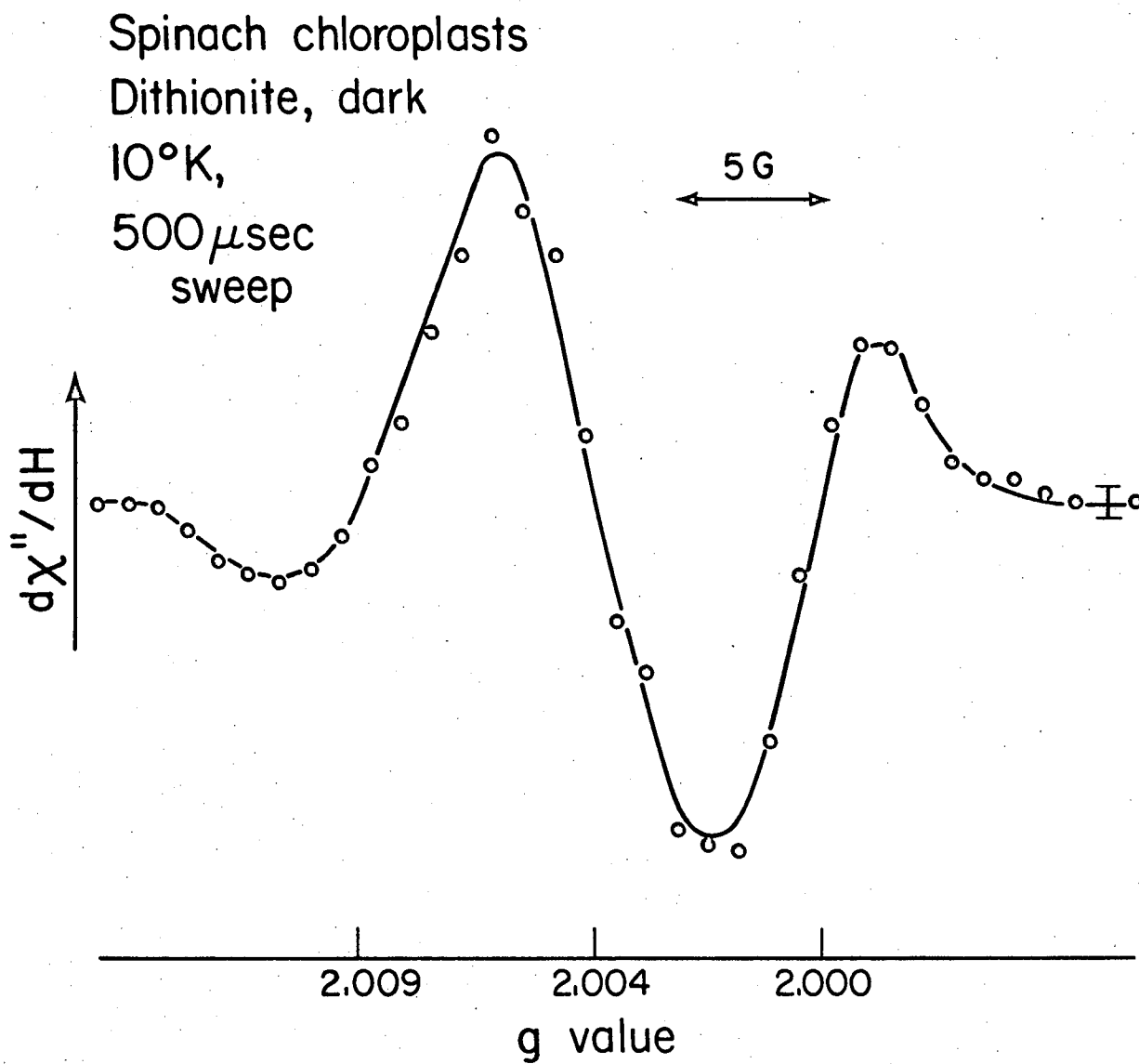
Figure 7. A plot of transient signal amplitude versus magnetic field strength for broken spinach chloroplasts prepared as in Fig. 6. The spectrometer settings were identical to those of Fig. 6.

Figure 8. A plot of transient signal amplitude versus magnetic field strength for broken spinach chloroplasts prepared as in Fig. 6. The spectrometer settings were: microwave power, 50 μ W; temperature, 10 K; direct detection; and gain - 80dB. The kinetic traces from which the points were obtained were the average of 400 events.

Figure 9. Computer simulations of the transient EPR spectrum obtained from spinach chloroplasts treated with dithionite and frozen under illumination. The simulations on the left were done assuming A_1 to be a chlorophyll monomer anion species while those on the right assume A_1 to be a chlorophyll dimer anion species. The magnitude of J is given in gauss for each simulation. The g-values and hyperfine coupling constants used are: for Chla dimer cation, $g = 2.0025$, hf coupling constants - 2.1G (4 protons), 1.3G (6 protons); for Chla monomer anion, $g = 2.0029$, hf coupling constants - 4.05G (3 protons), 1.95G (3 protons); for Chla dimer anion, $g = 2.0029$, hf coupling constants = 2.02G (6 protons), .98G (6 protons).



XBL 7912-5128



XBL 7912-5126

Frank, etal.

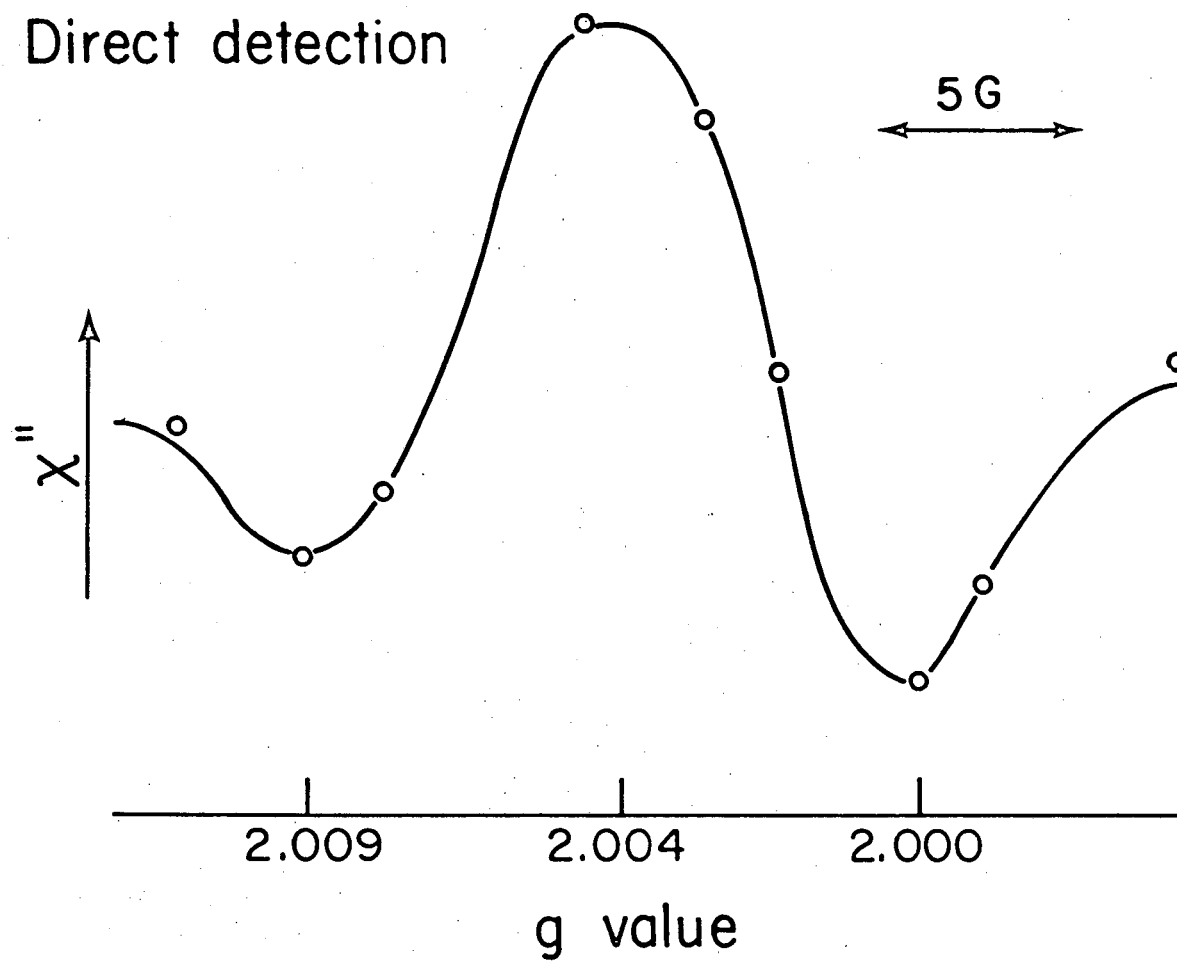
Fig. 2

Spinach chloroplasts

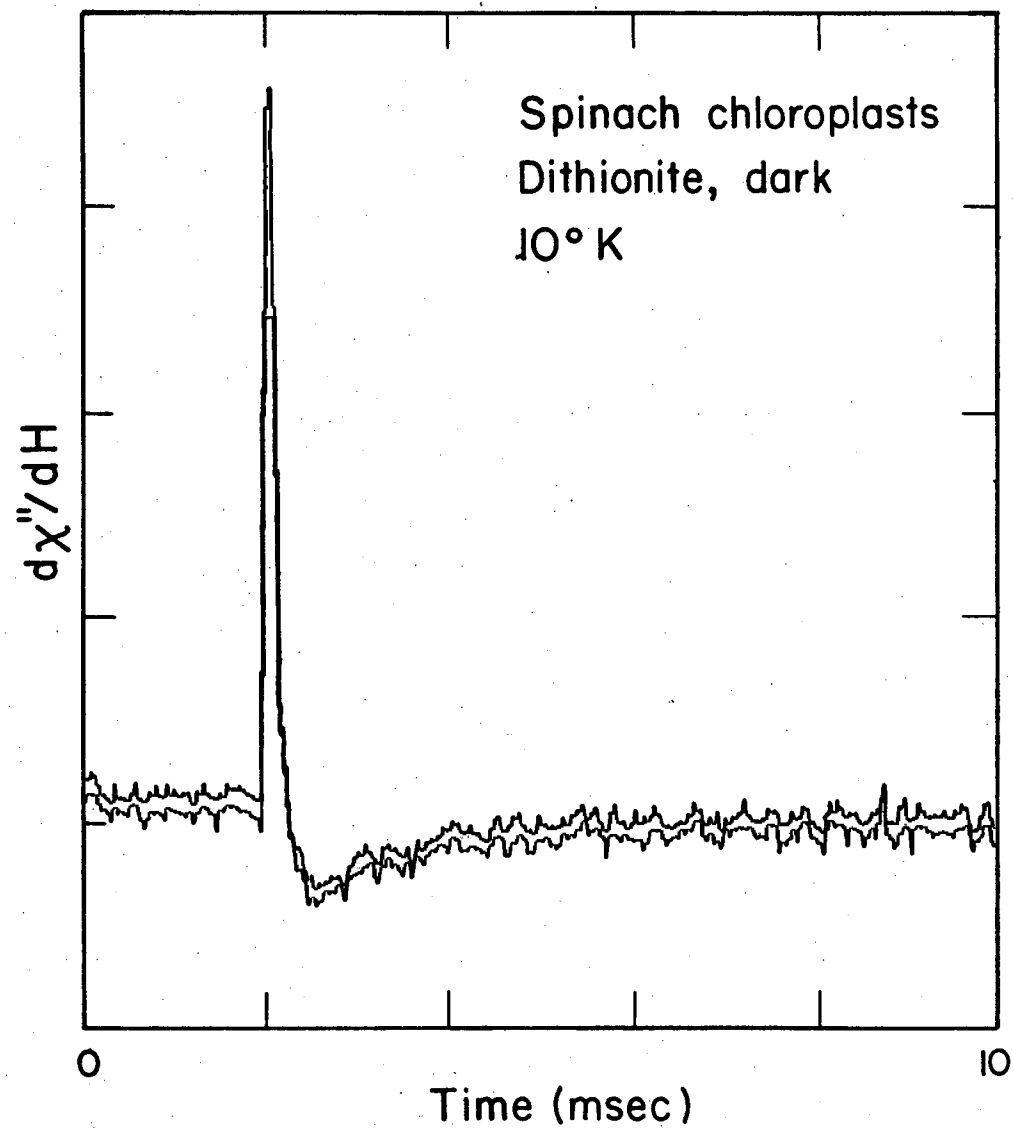
Dithionite, dark

10°K, 500 μ sec sweep

Direct detection

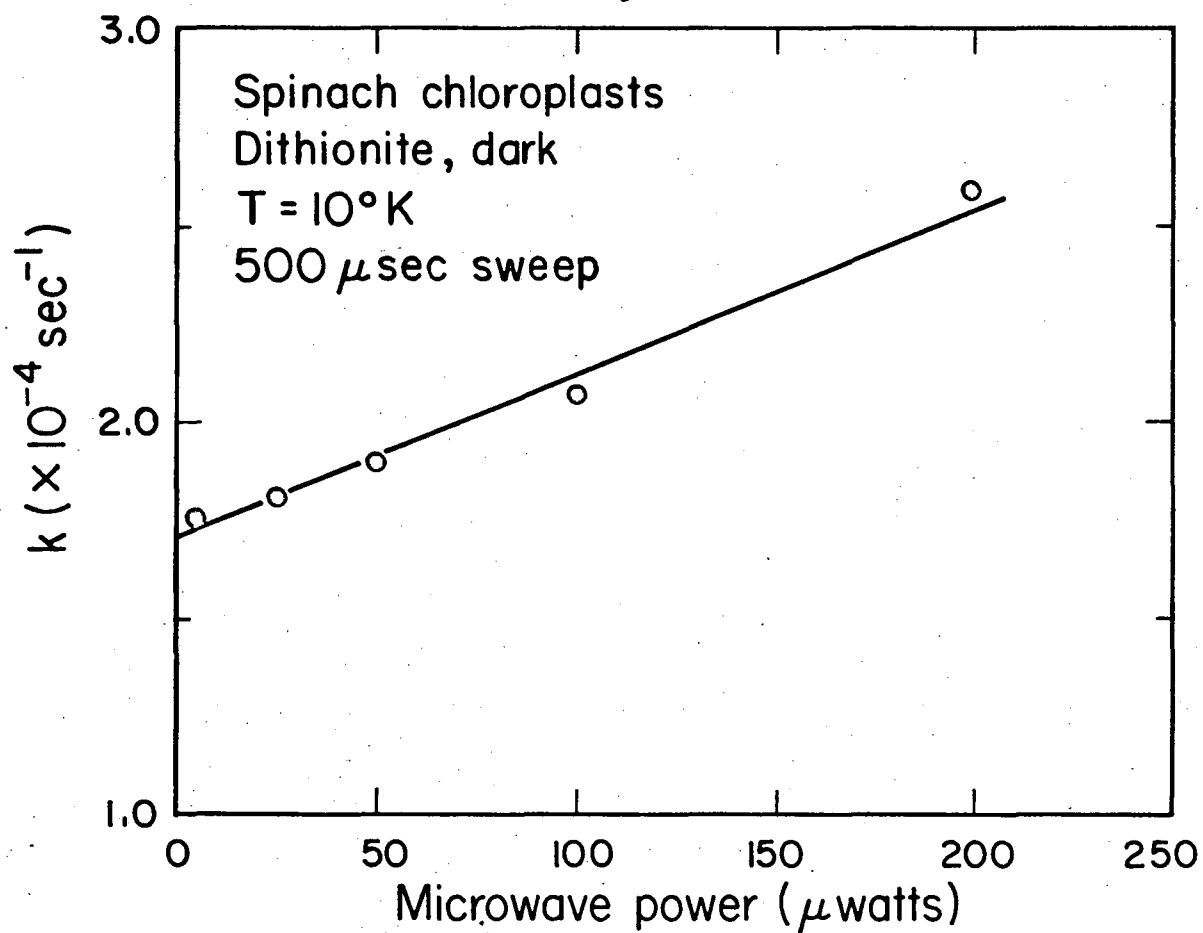


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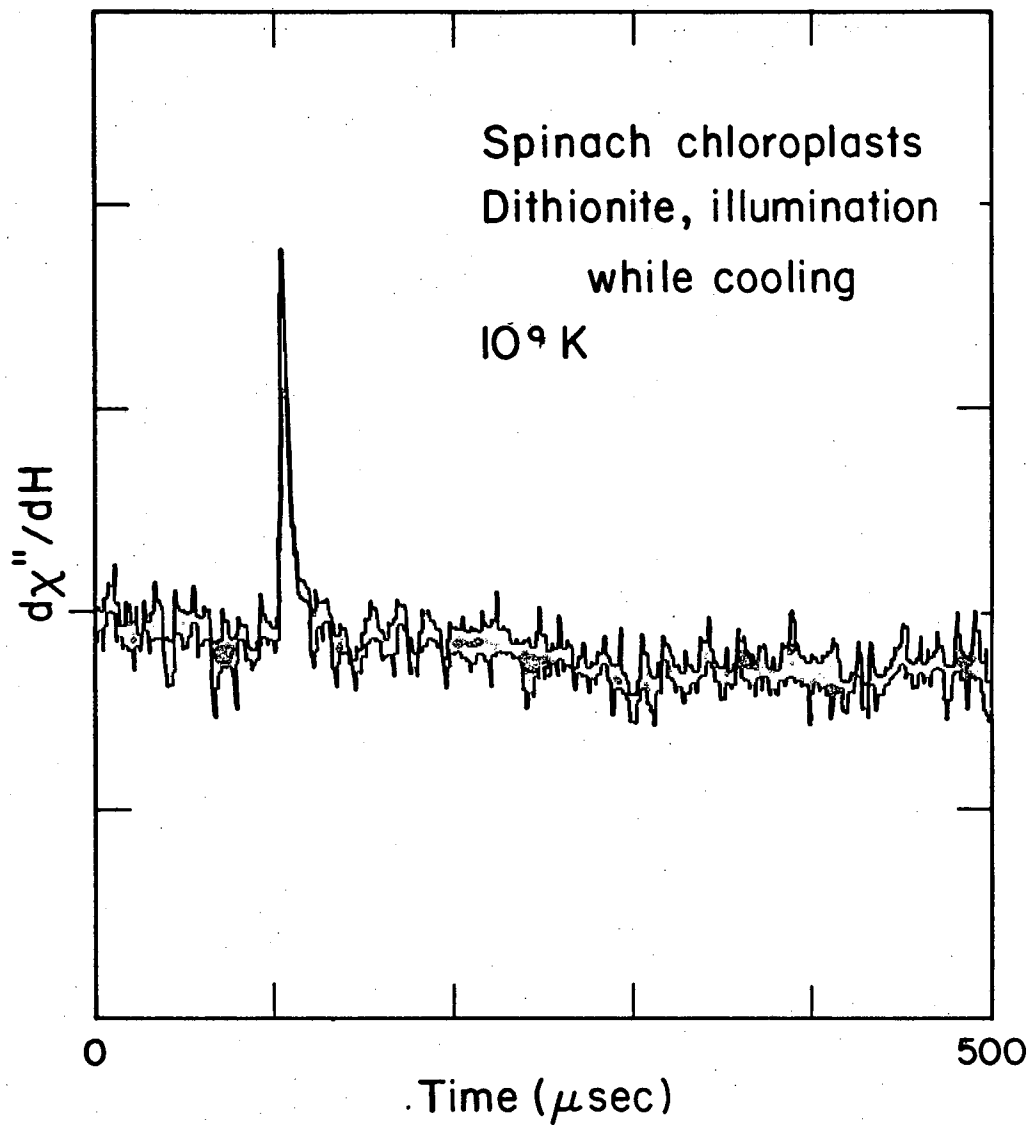


XBL 7912-5127

Power dependence of spin polarization decay kinetics

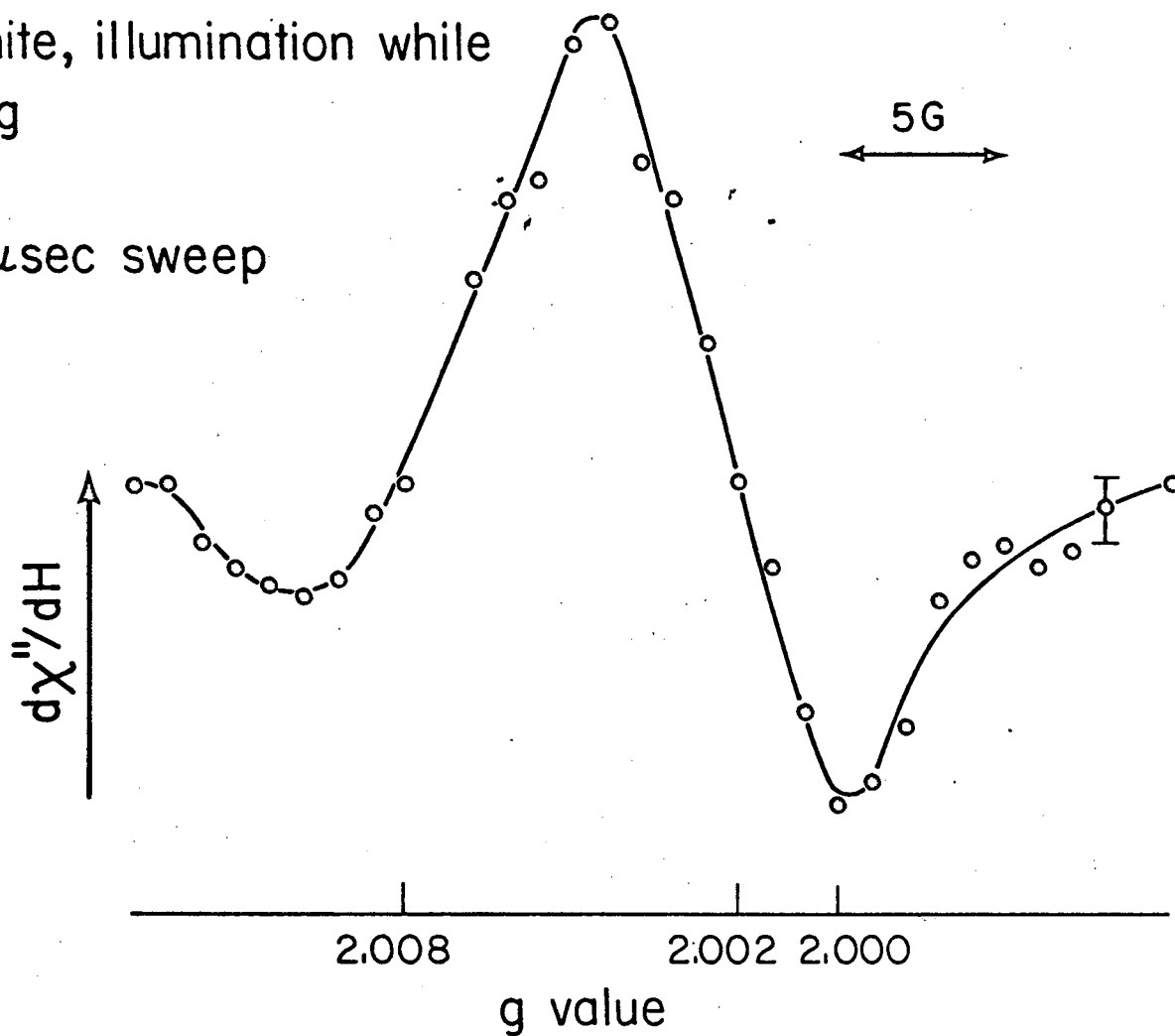


XBL 7912-5130

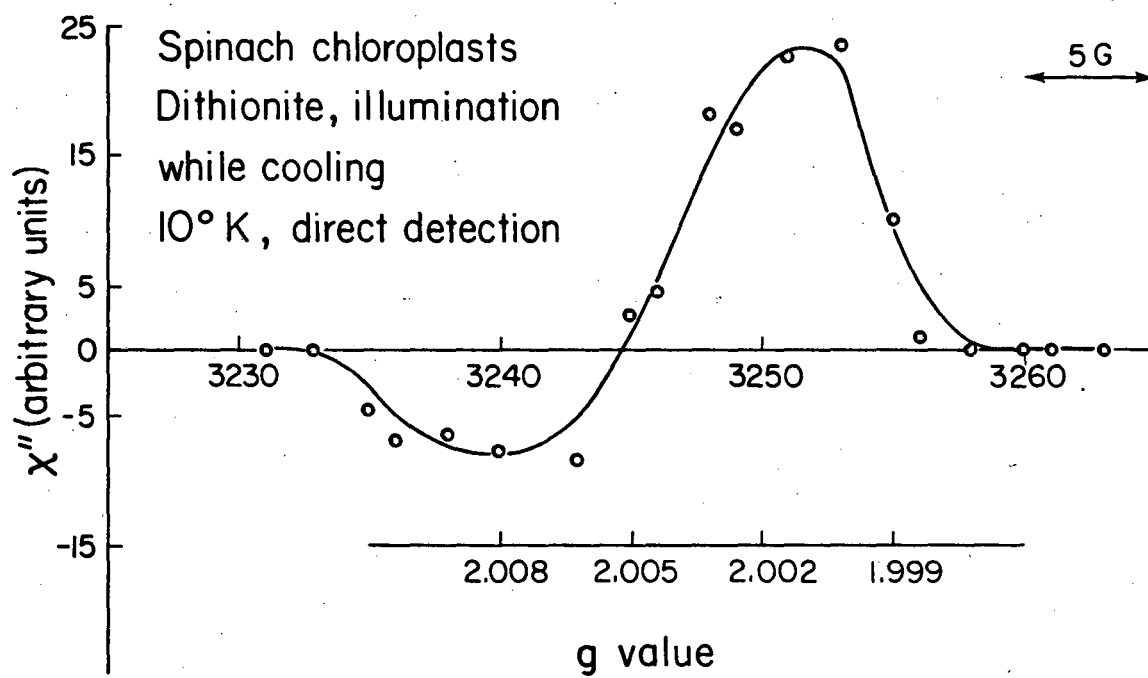


XBL 7912-5129

Spinach chloroplasts
Dithionite, illumination while
cooling
10°K,
500 μ sec sweep

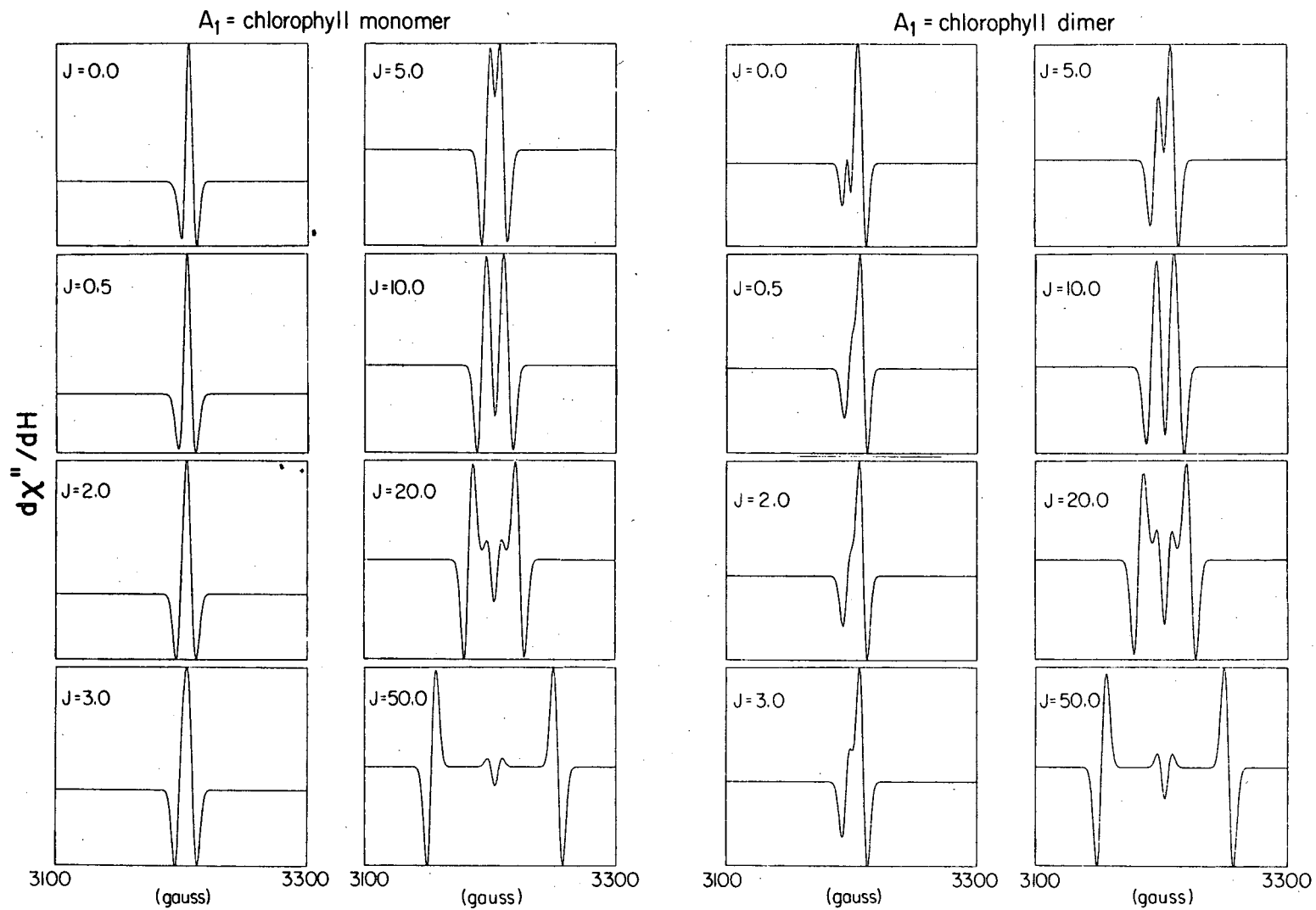


XBL 7912-5125A



XBL 812-4471

Computer Simulated
P700* A₁ Radical Pair Spectra



XBL 806-4224

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