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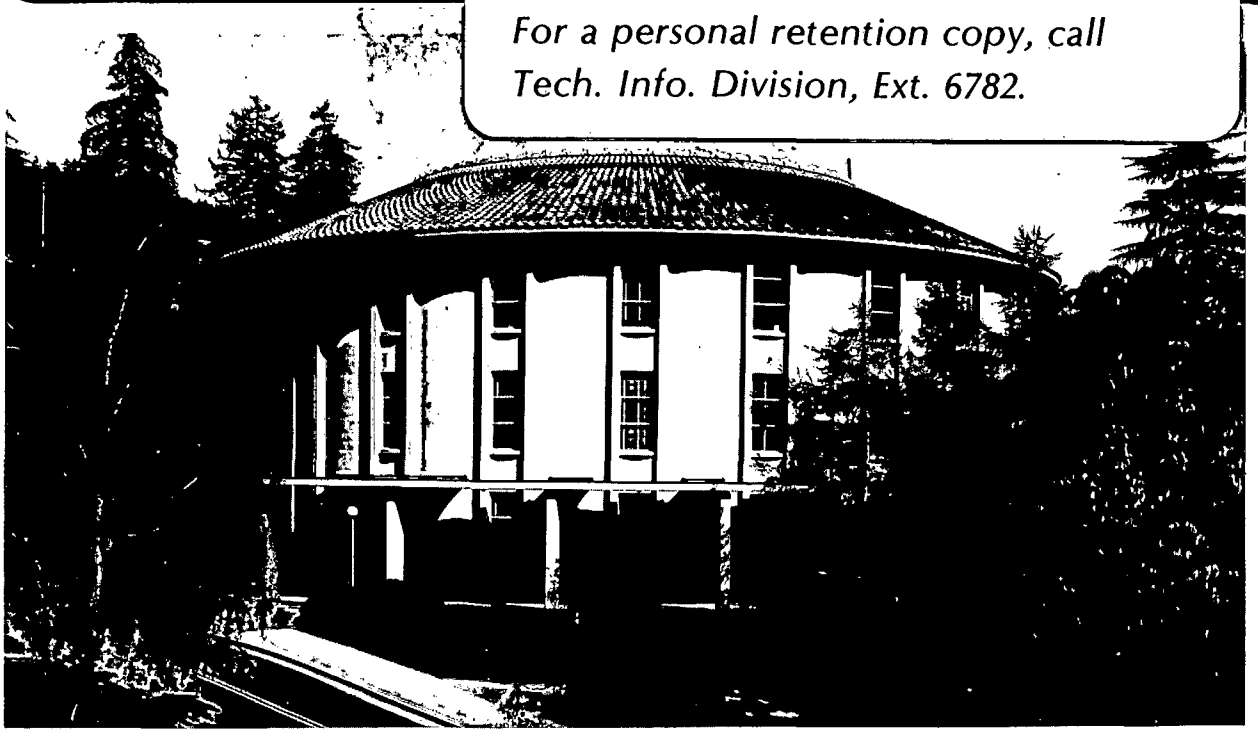

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Orientation Dependence of Radical Pair Interactions in Spinach Chloroplasts

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Key words:Photosystem 1;CIDEP;Anisotropic radical pair interaction;Electron acceptor;(Spinach chloroplast)

Abbreviations: PS1,Photosystem 1;Chl,Chlorophyll;DPPH, diphenylpicrylhydrazyl;PAS,principal axis system;CIDEP,chemically induced dynamic electron polarization;Fd, ferredoxin

ABSTRACT

Time-resolved EPR studies were done on ordered, broken spinach chloroplasts at 10K under conditions where Fe-S centers A and B were reduced. A dramatic dependence of the spin-polarized,g 2.0 EPR signals from Photosystem 1 on orientation of the thylakoid membranes in the magnetic field is demonstrated. Analysis of these data by computer simulations of the spin-polarized lineshapes supports a model for Photosystem 1 electron transport presented recently (Bonnerjea and Evans(1982)FEBS Lett. 148, 313-316; Gast, et al.,as part of Gast,P.,Ph.D. Thesis,State University,Leiden,The Netherlands,Oct.,1982). In this model there are two electron acceptor species,A_0 and A_1, operating in series between P-700, the primary electron donor, and X. Thus, the Photosystem 1 reaction center organization may be represented as P-700 A_0 A_1 X ($Fd_{B,A}$). Our results indicate that A_0^- may have a slightly anisotropic g tensor with g values ranging from 2.0026 $\pm$.0001 to 2.0031 $\pm$ .0002, depending on orientation, and that the species A_1^- has a g value of 2.0054 $\pm$ .0010 with a peak-to-peak linewidth of 8-10.5G. The model differs from those presented previously in that the spin-spin interaction between $P\text{-}700^+$ and A_0^- involves both isotropic electron exchange and electron-electron magnetic dipole coupling.

INTRODUCTION

The primary photoreactions of photosynthesis give rise to spin-polarized, transient electron paramagnetic resonance (EPR) signals. Previous studies on a variety of photosynthetic organisms have given us information about: the mechanism of the primary electron transfer reactions, the chemical identity of the species involved in these events [1-6], the dynamics of these reactions [7-9], and the structural relation and interactions between the primary photoreactants.

Previous attempts at analyzing spin-polarized EPR lineshapes obtained from photosynthetic samples have used adaptations of the standard radical pair theory of chemically induced dynamic electron polarization (CIDEP) developed for studies on reactions in solution [3,4,10]. This theory treats the spin-spin interaction in terms of isotropic exchange between the two radicals formed after electron transfer. Recent work on bacterial reaction centers has shown that there is significant electron-electron magnetic dipole coupling between the primary electron donor and acceptor after charge separation has occurred [11,12]. These experiments suggest that more details of reaction center structure and of the interactions between radical pairs that give rise to CIDEP could be deduced by studying the dependence of the spin-polarized lineshape on membrane orientation in the Zeeman field.

Earlier investigations done in our laboratory on broken spinach chloroplasts, studied under reducing conditions at 10K,

showed that when the photosystem 1 (PS1) reaction center is poised in the redox state P-700 A_1 X $(Fd_{B,A})^{=}$, a significant orientation dependence of the spin polarized EPR spectrum in the g 2.0 region is observed. These experiments were done on chloroplast thylakoids which had been aligned in a 10kG magnetic field. Recently, it has been shown that a higher degree of orientation of broken chloroplasts can be obtained by using the technique of partial dehydration on a flat surface rather than magnetic field alignment [13,14]. Therefore we have repeated the above measurements to examine the magnetic interactions and properties of $P\text{-}700^{+}$ and the reduced primary acceptors of PS1 in more detail.

We analyze these data using a model where the observed orientation dependence is accounted for by an electron-electron magnetic dipole interaction between $P\text{-}700^{+}$ and the reduced primary acceptor species, including a small g tensor anisotropy on the primary acceptor. The model also requires that there are two acceptor species with g values in the free electron region that are prior to the more stable electron acceptor known as X or A_2 [15]. Thus, supporting the recent proposal made by Gast, et al.[16] and Bonnerjea and Evans[17] that the makeup of the PS1 electron transport scheme is: P-700 A_0 A_1 X $(Fd_{B,A})$.

MATERIALS AND METHODS

Time-resolved EPR measurements were made at X-band with a Varian E-109 EPR spectrometer modified for magnetic field

modulation at 1 MHz [18]. The TE_{102} cavity used for these measurements was fitted with an optical transmission flange to allow 100% transmission of the exciting light. The output of the 1 MHz lockin amplifier was fed directly to a Nicolet Explorer IIIA digital oscilloscope equipped with a model 204 plugin. The data was then signal averaged locally on a home-built signal averager and transmitted to a VAX 11/780 computer (Digital Equipment Corp.) for analysis and storage. For low temperature operation, the spectrometer was equipped with an Air Products Heli-tran Cryostat, and temperature measurements were made with a gold/chromel thermocouple. To test for the possibility of rapid-passage distortion of the EPR signals, the experiments were repeated using direct microwave power detection. The direct detection measurements were made using a home-built superheterodyne EPR spectrometer with a response time of approximately 200 nsec. To avoid light artifacts, which are typically present in direct detection measurements, the laser pulse energy had to be kept below 5mJ.

Microwave frequency measurements were made with a Hewlitt-Packard model X532B wave meter, and g values were calibrated with 'weak pitch' and DPPH. The field modulation amplitude was calibrated using an aqueous sample of Fremy's salt (ICN Pharmaceutical/K+K Labs Inc.)[19] using the standard line broadening technique [20]. The light source for these experiments was a Phase-R DL-1400 dye laser which was operated broadband at 640 nm (Rhodamine 101(Exciton Chemical, Dayton, OH, USA) in methanol). The pulse width (FWHM) was 400 nsec., and pulse

energies of 30 mJ were utilized. Care was taken to ensure that the light pulses were always saturating (in the field modulation experiments only). The flash repetition rate was 2 Hz.

Broken spinach chloroplasts were prepared using a standard procedure [21] and then treated in one of two different ways:

(a) Treatment with ascorbate. Broken chloroplasts were suspended in 50 mM Tris buffer (pH 8.0) containing 10mM NaCl,0.1mM EDTA, and 50mM sodium ascorbate.

(b)Treatment with ferricyanide. Broken chloroplasts were suspended in 50mM Tris buffer (pH 8.0) containing 10mM NaCl, 0.1mM EDTA, and 10mM $K_3Fe(CN)_6$.

Orientation of the various chloroplast thylakoid suspensions used in these experiments was achieved by partial dehydration of the samples on mylar [14,22]. The procedure for doing this was as follows. Mylar strips (approx. 2.5mm x 50mm;thickness, .25mm) were soaked in a solution containing 1% Collodion(Mallinckrodt), 99% ethanol and allowed to dry. The thick chloroplast suspension (approx. 3 mg Chl/ml) was then pipetted onto the strips which were supported horizontally on a teflon drying rack. The rack was then placed in a 90% relative humidity environment or in a N_2 environment at 4°C and allowed to dry. When drying was achieved by flowing N_2 gas over the strips, the flow rate was adjusted so that the process took about 4 h. When the 90% relative humidity chamber was used, drying took about 36 h. In both cases, good orientation was obtained, and for this work most of the drying

was done in a N_2 gas environment. After drying, approximately 5 strips were stacked into a "sandwich" and placed in an EPR tube.

In many instances the samples were illuminated while being cooled to liquid nitrogen temperature. The light source for these treatments was a 400W tungsten lamp (Cary) filtered through a Calflex heat reflection filter (Balzers). The sample was illuminated at low intensity for 20 sec at room temperature and then immediately placed in an optical dewar containing liquid N_2 and illuminated another 20 sec.

RESULTS

1. Steady state spectra

Prior to doing flash studies on the various samples examined in this work, the steady state EPR spectra of reduced Fe-S centers A and B and of reduced X(where applicable) were examined as a function of the angle between the normal to the mylar strips(coincident with the thylakoid membrane normal) and the direction of the spectrometer's magnetic field. These observations served a dual function: (a) to determine the degree to which the thylakoid membranes were oriented in the sample; and (b) to determine the redox poise of PS1 in the various sample treatments. Fig. 1 shows the steady state EPR spectra (taken in the dark) from samples prepared so that Fe-S centers A and B are reduced. In the parallel orientation (membrane normal parallel to the magnetic field direction-Fig.1a), the ratio of the amplitude(measured from the baseline) of the signal at g 1.89 to the signal

at g 1.92 is nearly 1 while in the perpendicular orientation(Fig.1b) this ratio is typically >6. The angle dependence of the relative peak intensities at g 1.89, 1.92, 1.94, and 2.05 found in all experiments were in good agreement with those previously reported [13]. In experiments where the species X is reduced, a large signal at g 1.77 is observed in the parallel orientation in agreement with previous findings [23].

There are two experimental problems which arise when one studies EPR properties of samples dried on mylar strips. (1)Because the samples do not have cylindrical symmetry and the EPR cavity mode pattern is TE_{102}, the amount of sample in the maximum of the microwave magnetic field varies as one rotates the sample. This problem is alleviated somewhat by stacking the sample strips in a cylindrical EPR tube. (2)In the time-resolved measurements the amount of sample that can be irradiated by the laser flash also depends on sample orientation. Therefore, only the relative peak intensities at a given sample orientation have quantitative significance.

2. Time-Resolved EPR Results

Oriented samples prepared so that Fe-S centers A and B were reduced prior to flash excitation gave rise to predominantly monophasic transient EPR signals in the g 2.0 region at 10K and 50 μW microwave power. The lifetime of this decay component was approximately 50 μs (1/e time) and independent of orientation. A slower component with a decay time of 150 μs was also present at

some field positions, but its presence seemed to vary with different sample preparations. Also, the lifetime of this slow component varies over a range of 150-500 μs as a function of field position for a given sample. These observations are similar to those reported previously [4] for randomly oriented thylakoids.

The amplitude of the 50 μs component is plotted versus field position in the g 2.0 region in Fig. 2 for randomly oriented thylakoids(Fig. 2a), and for thylakoids oriented so that the membrane normals are parallel(2b), perpendicular(2c), and at 45°(2d) to the magnetic field direction. The amplitude of this kinetic component depends strongly on the orientation of the thylakoid membrane normals in the Zeeman field. In the perpendicular orientation, a mixed absorptive-emissive type signal centered about g 2.0026 is observed (Fig. 2c). When the sample is rotated to the parallel configuration, the polarization pattern is inverted and an appreciable amount of signal intensity grows in at low field causing the center of this pattern to be at g 2.0034 (Fig. 2b). The field profile obtained in the 45° orientation (Fig. 2d) is similar to that of the parallel orientation, except that the structure in the high field lobe of the spectrum is different. The trends seen in these field profiles are similar to those reported previously for magnetically aligned thylakoids under the same conditions[4], but the effects shown in Fig. 2 are much more pronounced because of the higher degree of orientation.

The possibility of distortion due to rapid passage in field

modulation experiments was tested by repeating the above measurements using direct detection of resonance. The field profile (Fig. 3) recorded for oriented thylakoids(parallel orientation) using direct detection yields a mixed emissive absorptive lineshape identical to that obtained in the field modulation experiment(Fig. 2b). This indicates that distortion of the signal amplitudes due to rapid passage is not significant. The structure seen in the field modulated data (the shoulders in the low and high field lobes of Fig. 2b) is also observed in the direct detection experiment.

Oriented samples prepared so that the species X is reduced prior to flash stimulation give rise to decays with kinetic components of 3 μs and 30 μs. The relative amplitude of each component depends on both orientation and field position. When the sample is in the perpendicular orientation, the fast (3 μs) component vanishes whereas in the 45° and parallel orientations it dominates the decays at some field positions. The effect of reducing X on the EPR transient signals is illustrated in Fig. 4.

A plot of the amplitude of the 30 μs component versus magnetic field strength gives identical results to those of Fig. 2. The field profiles of the 3 μs component in the 45° and parallel configurations give 3-lobe emissive-absorptive shapes similar to those reported previously [4]. However, the signal-to-noise ratio is poor(possibly due to time resolution constraints). We are currently investigating the orientation behavior of this signal in more detail using the direct detection system.

Time-resolved EPR measurements were done also on samples

which had been treated with 10mM $K_3Fe(CN)_6$ before drying and freezing in the dark. Steady state EPR spectra showed that P-700, the primary donor of PS1, was oxidized and that Fe-S center A could not be reduced by steady illumination with white light at 10K. Under these conditions, no rapid (sub-millisecond) EPR transient signals were observed in the g 2.0 region.

DISCUSSION

The field profile obtained when Fe-S centers A and B are reduced prior to flash excitation is highly orientation dependent (Fig. 2). In a previous paper [4], we attempted to interpret the field profile for randomly oriented thylakoids poised in this redox state using the so-called two-site model of Friesner, et al. [10]. In that model we assumed that the transient EPR signals were due entirely to $P\text{-}700^+$ and that the observed spin polarization developed as the result of two separate radical pair interactions. The first interaction was between $P\text{-}700^+$ and A_1^-, and the second between $P\text{-}700^+$ and X^- after electron transfer from A_1^- to X had occurred. In that treatment both sets of spin-spin interactions (between sucessive radical pairs) were taken to be isotropic, and the only origin of anisotropy in the spin polarized EPR spectrum of $P\text{-}700^+$ was attributed to the g-value anisotropy of reduced X.

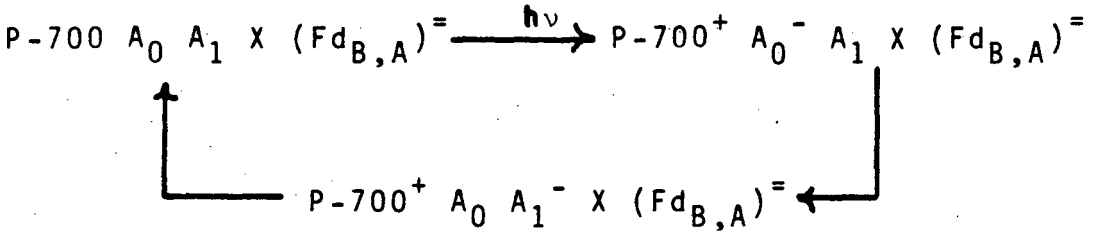
Attempts to simulate the orientation dependence shown in Fig. 2 using the above model coupled with the orientation averaging procedure described by Blum, et al. [24] and the known

orientation of the X^- g-tensor relative to the membrane normal [23] gave unsatisfactory results. In all cases the simulations gave either 3-lobe type lineshapes or emissive shapes similar to those obtained by Friesner, et al.[10] in their Fig. 1. We found that the g-anisotropy of X^- allows one to simulate intensity shifts which are only minor compared to those observed experimentally, and it never predicts inversion of the polarization pattern to the degree that we observe between parallel and perpendicular orientations (Fig. 2). Therefore, we conclude that the anisotropy in the observed spin-polarized EPR lineshape when PS1 is in this redox state is due to more than the g-anisotropy of X^-.

Recently, Gast, et al.[16] and Bonnerjea and Evans[17] have proposed a model for PS1 electron transport which differs from the standard approach. They have incorporated a new acceptor species, A_0, which precedes both A_1 and X in the electron transport chain. Their hypothesis is that the existence of A_0 is necessary to explain the steady-state and time- resolved EPR data in a consistent fashion. Further, Gast, et al.[16] propose that the the spin-polarized EPR signal observed in the g 2.0 region when Fe-S centers A and B are reduced, is due to both P-700$^+$ and A_1^-. In their model, P-700$^+$ is polarized statically via a brief radical pair interaction with A_0^-, and A_1^- is polarized dynamically upon electron transfer from A_0^- to A_1(the idea of static and dynamic polarization was put forth by Pedersen [25]). We have found that this model, modified so that the spin-spin interaction between P-700$^+$ and A_0^- involves both isotropic

exchange and electron-electron magnetic dipole interactions is capable of explaining the major features of the data in Fig. 2.

To use the above model for explaining the low temperature CIDEP data obtained from broken chloroplasts prepared with centers A and B reduced, it is necessary that electron transfer from A_1^- to X be unfavorable compared to charge recombination between P-700$^+$ and A_1^-. Thus, the dominant path of electron flow in PS1 under these conditions would be:



To do computer simulations of the data in Fig. 2 we use a model in which spin polarization develops on P-700$^+$ due to radical pair interactions with A_0^-. The radical pair hamiltonian is then:

$$H=H_{zee}^{(D)}+H_{zee}^{(A)}+H_{hf}^{(D)}+H_{hf}^{(A)}+H_{ex}+H_{dd}$$

where D represents the donor radical and A, the electron acceptor species. The Zeeman interactions (H_{zee}) and the first order nuclear hyperfine interactions (H_{hf}) on both radicals are isotropic. The forms of the exchange and dipole-dipole hamiltonians are:

$$H_{ex} = J(2\underset{\sim}{S}_D \cdot \underset{\sim}{S}_A + 1/2)$$

$$H_{dd} = D(S_z^2 - 1/3\underset{\sim}{S}^2) + E(S_x^2 - S_y^2)$$

where S_z, $\underset{\sim}{S}$, S_x and S_y are operators of the total spin; D and E are the zero field splitting parameters in the principal axis system (PAS) of the dipole-dipole interaction; and J is the value of the exchange integral. The spin polarization on $P\text{-}700^+$ is determined in the standard manner by finding the eigenvalues and eigenvectors of the radical pair hamiltonian needed to compute the time evolution of the radical-pair wavefunction, and then computing the polarization weighting for a given hyperfine state of $P\text{-}700^+$. The resulting polarization weighting is then time averaged over the radical-pair lifetime and integrated over all possible orientations of the magnetic field vector in the PAS of the dipole-dipole splitting tensor. This procedure is described in more detail elsewhere [10].

For simulations involving oriented samples, the orientational averaging technique described by Blum, et al. [24] for partially ordered membrane multilayers is used to weight various field directions with respect to the PAS of the dipolar coupling tensor. This procedure involves relating the PAS of the dipolar coupling tensor to a lab axis system described by the membrane normal via an Euler angle rotation. Various orientations of the magnetic field vector can then be weighted according to the known orientation of the vector in the lab axis system and

then related to the PAS of the tensor in question. This procedure involves the use of five angle parameters: θ and ϕ, which describe the orientation of the membrane normal in the PAS of the dipolar coupling tensor(fixed for a given calculation); ω and α, which describe the orientation of the magnetic field vector in the lab axis system; and the mosaic spread or wobble angle which describes the angle spread of the membrane normals in the sample. ω is the angle between the membrane normals and the field direction and is known from the experiment, whereas α must be averaged over 360° because there is no preferential ordering of the thylakoid membranes transverse to the membrane normal.

According to a theory put forth by Pedersen [25] the A_1^- species will be dynamically polarized because electron transfer from A_0^-(which is statically polarized) to A_1 does not selectively involve the nuclear states of either radical. Therefore, the net polarization on A_0^- is transfered uniformly over the hyperfine states of A_1^- so that the spectrum of this species will be either emissive or absorptive, but not mixed. Experimental evidence in support of this idea has been obtained from bacterial reaction centers[26,27]. The procedure for simulating the spectra of Fig. 2 involves first calculating the polarization pattern of $P\text{-}700^+$ and determining its net polarization to find the appropriate scaling factor for the spectrum of A_1^-. The spectrum for each radical is then added to give the predicted output.

Computer simulation of the random and oriented spectra of Fig. 2 involves the specification of several parameters. These

parameters are: the g-values of P-700$^+$, A_0^-, and A_1^-; the peak-to-peak linewidths of P-700$^+$ and A_1^-; the spin-spin coupling constants for the interaction between P-700$^+$ and A_0^-, J, D and E; and the angle parameters needed for the orientational averaging. The g-value of P-700$^+$ was set at 2.0026 and its linewidth at 8G, while the g-values of A_0^-,A_1^- and the linewidth of A_1^- were allowed to vary. We found that the values of D and J were dependent on one another to the extent that for a given value of D, J could be adjusted to yield good simulations of the data. Therefore, the values of D and J used in our simulations were chosen so that they fell into the range of D and J values reported for the primary radical pair of bacterial reaction centers[12]. The general approach to doing these simulations was to fix D at -50G and then vary J, the g-values of the acceptors, the linewidth of A_1^-, and E to predict the random spectrum. Then these parameter sets(there are several combinations of these parameters that yield acceptable random spectrum simulations) were fixed and the spectra of oriented samples were calculated by adjusting only the angle variables; θ , ϕ , and the mosaic spread.

The parameters that can be accurately set by simulation of the random spectrum alone are the g-value of A_1^- and its linewidth. A typical simulation of this spectrum is shown in Fig. 5(upper trace). The range of acceptable g-values for A_1^- was 2.0054±.0010 and the peak-to peak linewidth for this species must be between 8-10.5G. These values are in good agreement with those reported by Gast, et al.[16] and Bonnerjea and Evans[17]. The range of acceptable J values(if D=-50G) was from -8G to -10.5G

depending on the g-value chosen for A_0^-, which was found to have a range of acceptable values from 2.0027 to 2.0040. The effects of E on the random spectrum are slight, and good simulations can be obtained for the full range of acceptable values from zero to D/3. The lower trace in Fig. 5 shows the contribution of P-700$^+$ to the random spectrum.

The constraint that the above parameters, which allow satisfactory fits of the random spectrum, must also provide good fits for the oriented spectra narrows the range of acceptable parameter values considerably. Computer simulations of the three oriented spectra examined in this study are plotted in Fig. 6 along with the experimental data. We find that the oriented spectra can be simulated only if: (a) the g-value of A_0^- is $2.0031 \pm .0002$ for the parallel and 45° orientations; and (b) the g-value of A_0^- is $2.0026 \pm .0001$ in the perpendicular case. This slight amount of anisotropy in the g-tensor of A_0^- is needed to make the low field portion of the spectrum due to A_1^- go to zero in the perpendicular orientation(Fig. 6b). When there is no net polarization on P-700$^+$ (such as occurs when $\Delta g=0$ in the primary radical pair), there is no polarization transfered to A_1^-, and the low field contribution to the spectrum is minimized. Thus, the spectrum observed in the perpendicular orientation covers a narrower width of field values than the random spectrum and is centered at g 2.0026, the g-value of P-700$^+$. Evidence for small g anisotropy of the primary acceptor of bacterial photosynthesis has recently been found by Boxer, et al.[28]. A second way in which the spectrum in the perpendicular orientation can be

simulated is if the g-value of A_0^- is fixed at 2.0031 ± .0002 and g anisotropy and/or hyperfine anisotropy of A_1^- is incorporated into the model, so that the contribution to the spectrum due to A_1^- lies precisely on top of that due to P-700$^+$.

As stated above the random spectrum is only slightly sensitive to the value of E. Simulation of the spectra of the oriented samples requires that E be between 0 and -8G. When the dipolar coupling tensor is made more rhombic, it is no longer possible to predict the peak positions and amplitude ratios of the spectrum obtained for the 45° orientation. Because the range of acceptable g-values for A_0^- is narrowed by the oriented spectra simulations, the range of J values (assuming D=-50G) is also narrowed to J=-9.5 ± 1.0G.

Of the angle parameters used for doing the orientational averaging, the simulations are most sensitive to the values of θ and the mosaic spread. It was found that θ, the angle between the membrane normal and the z axis of the PAS of the dipolar coupling tensor, must be very close to 90° (the lower limit is 85°) to predict the spectra successfully at all three orientations. Further, the mosaic spread cannot be greater than 20°. Because the best simulations were obtained with the dipolar coupling tensor nearly axial and a θ of 90°, the dependence of the predicted spectra on ϕ(the angle between the x axis of the PAS of the dipolar coupling tensor and the membrane normal) was slight.

One shortcoming of the model presented here is that, although it is successful in predicting the peak positions and relative peak amplitudes of the random and oriented spectra, it

does not predict the structure in some of the oriented spectra(Figs. 5,6). It is possible that the structure in these spectra is due to spectral properties of A_1^- that are difficult to extract from these experiments because the spectra from A_1^- and P-700$^+$ overlap extensively at X-band. Because little is known about the A_1 species, it was assumed in our simulations that it has no g-tensor or hyperfine tensor anisotropy. Addition of either one or both of these anisotropic properties to the model presented above could account for the structure in our data. Recently, Bonnerjea and Evans[17] have obtained steady state EPR spectra which they assigned to A_0^- and A_1^-. The spectrum that they attributed to A_1^- has a g-value of 2.0051, a linewidth of 10.5G(in good agreement with our results), and has an asymmetric lineshape.

The g-value obtained in our work for A_0^- differs from the value 2.0017 ± .0006 reported by Gast, et al.[16]. The difference stems from the inclusion of dipolar coupling in our calculations, which allows simulation of the contribution of P-700$^+$ to the random spectrum without invoking a negative g-value difference between P-700$^+$ and A_0^-. The g-value that we obtain for A_0^- is consistent with that expected for a reduced chlorophyll or pheophytin species, but those measurements are not sensitive to the linewidth of A_0^- so a comparison with other EPR data concerning early PS1 acceptors[17,29,30] is not possible. In [17] the authors were able to deconvolute the g 2.0 signal from reduced PS1 particles and reported a g-value of 2.0024 for A_0^- and a linewidth compatible with a chlorophyll or pheophytin

monomer species. This g value also differs from the one we reported here; but, taking into account the uncertainty in our data and the problems which may arise in deconvoluting the asymmetric lineshapes reported in [17], these experiments are in reasonable agreement.

Two other models for predicting the data presented in this work were investigated. The first model assumed that P-700$^+$ was polarized via a one-site interaction with A_1^-(A_0 does not exist in this model) and that the observed spectra arose from contributions from both P-700$^+$ and A_1^- (both being statically polarized[25]). This model fails to predict the spectrum of the randomly oriented sample. The second alternative model involved the incorporation of A_0, but differed from that presented above in that isotropic exchange between P-700$^+$ and A_1^- was also included(a two-site model). In this model, both P-700$^+$ and A_1^- are statically polarized and the random spectrum can be simulated as long as the exchange interaction between P-700$^+$ and A_1^- is less than that between P-700$^+$ and A_0^- by a factor of 1000. In light of this result, the one-site model used in our analysis of the above data is justified.

CONCLUSIONS

In this paper, we have presented new results concerning the orientation dependence of the low temperature CIDEP signals obtained from spinach chloroplasts under reducing conditions. Our analysis involves a model capable of predicting the major

features(g-values of peaks and amplitude ratios) of both the random spectrum and the spectra for oriented thylakoids. This model is similar to those proposed in two recent works[16,17] in that it requires the existence of two acceptor species which have EPR signals (when reduced) in the g 2.0 region and are prior to the more stable electron acceptor, X. The new findings presented in this work are:

1. The low temperature CIDEP data from chloroplasts is consistant with the idea that there is an electron-electron magnetic dipole interaction between P-700$^+$ and the primary electron acceptor, A_0^-.
2. The PAS of the dipolar coupling tensor between P-700$^+$ and A_0^- is oriented such that its z axis lies in the plane of the thylakoid membranes.
3. There is additional anisotropy in the acceptor species which manifests itself in either a small amount of g tensor anisotropy on A_0^- or in g tensor anisotropy and/or hyperfine anisotropy on A_1^-.These possibilities are currently under investigation.

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FIGURE CAPTIONS

Fig.1. Steady state EPR spectra of oriented spinach chloroplast thylakoids measured in the dark with: (a) the membrane normals parallel to the field direction; and (b) the membrane normals perpendicular to the field direction. Samples were suspended in 50mM Tris buffer(pH 8.0) containing 50mM ascorbate, 10mM NaCl and .1mM EDTA, then oriented by partial dehydration on mylar and illuminated while freezing to 77K. The spectrometer settings were: microwave frequency, 9.206 GHz; field modulation amplitude, 10G; modulation frequency, 100KHz; microwave power, 10mW; sample temperature, 13K.

Fig.2. Plots of EPR transient signal amplitude (50 μs component) versus magnetic field strength for: (a) randomly oriented thylakoids, (b) oriented thylakoids in the parallel orientation, (c)perpendicular orientation, and (d) 45° orientation. Spectrometer conditions common to all measurements were: microwave power, 50 μW; field modulation frequency, 1 MHz; field modulation amplitude, 2.5G; sample temperature, 15K; and time constant, 2 μs. Each point represents the average of 500 events and the estimated error in each point is represented by the circle diameter. The microwave frequency for the traces was: (a) 9.193 GHz, (b) 9.141 GHz, (c) 9.143 GHz, and (d) 9.198 GHz. Thylakoids were prepared and reduced as in Fig.1.

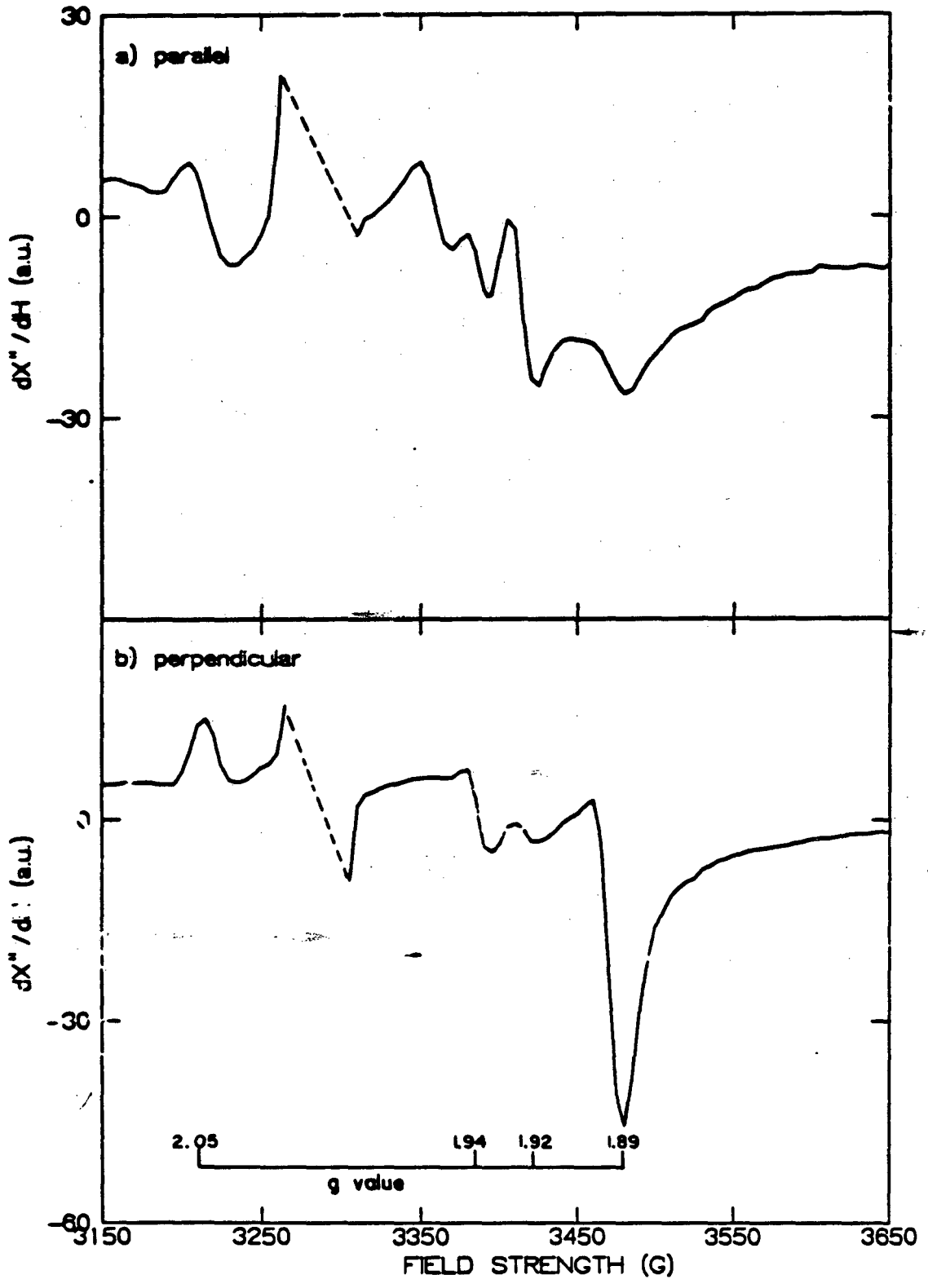
Fig.3.Plot of EPR transient signal amplitude (50 μs component) versus magnetic field strength for oriented thylakoids prepared as in Fig.1. The measurement conditions were: sample orientation, parallel; microwave frequency, 9.225 GHz; microwave power, 50 μW; time constant, 200 ns; sample temperature, 15K; and the direct detection system was utilized. Each point is the average of 500 events and the circle diameter is indicative of the estimated error.

Fig.4.Kinetic traces for oriented thylakoids prepared so that: (a) X was not reduced; and (b) X was reduced. Measurement conditions were: g=2.0041; parallel orientation; sample temperature, 15K;field modulation frequency, 1 MHz; field modulation amplitude, 2.5G; and microwave power, 50 μW. Each trace represents the average of 500 passes.

Fig.5.A comparison of the calculated and experimental EPR spectrum for randomly oriented thylakoids prepared as for Fig.2a. The lower trace represents the contribution to the spectrum by spin-polarized P-700$^+$. The parameters for the simulation were: g-value of P700$^+$, 2.0026; linewidth of P-700$^+$, 8G;g-value of A_0^-, 2.0031; g-value of A_1^-, 2.0054; linewidth of A_1^-, 9.0G; J=-9.35G; D=-50G ; E=0G; microwave frequency, 9.193 GHz.

Fig.6.A comparison of calculated and experimental EPR spectra for oriented thylakoids prepared as in Fig. 2b-d. The g-values and linewidths of P-700$^+$ and A_1^-, as well as the values of J, D, and

E were identical to those used to simulate the random spectrum(Fig.5). The microwave frequency used in simulating each spectrum was identical to that used in the experiment(Fig.2). Other simulation parameters were: (a) angle between the membrane normal and the magnetic field direction, 0°; g-value of A_0^-, 2.0031; (b) orientation angle, 90°; g-value of A_0^-, 2.0026; and (c) orientation angle, 45°; g-value of A_0^-, 2.0031. The mosaic spread of the membrane normals was 20°, θ was set at 90° and ϕ was 10°.


a) parallel
b) perpendicular
dX"/dH (a.u.)
dX"/dH (a.u.)
30
0
-30
-30
-60
2.05
1.94
1.92
1.89
g value
3150
3250
3350
3450
3550
3650
FIELD STRENGTH (G)

XBL 831-7501

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Fig. 2a,b

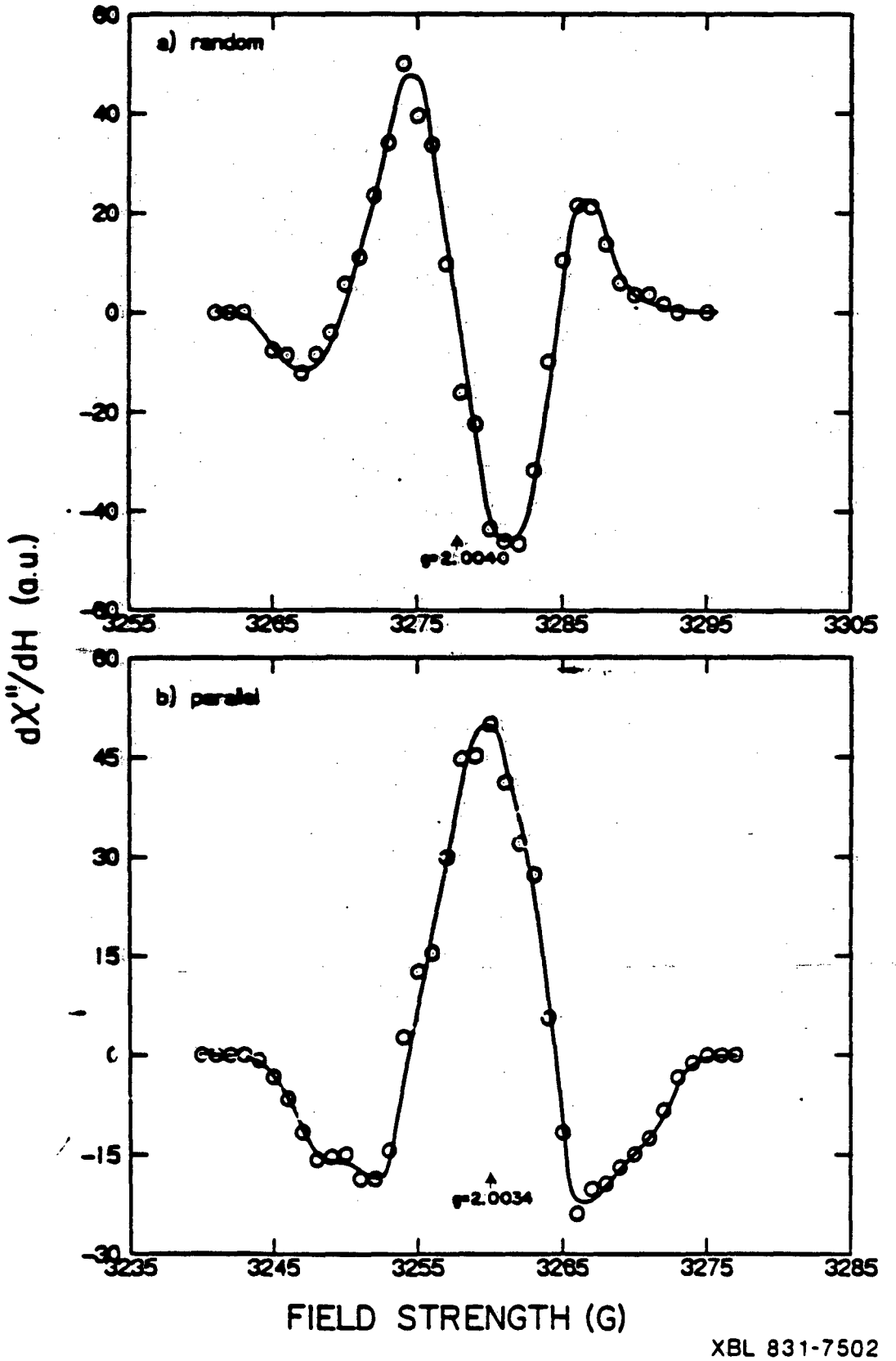
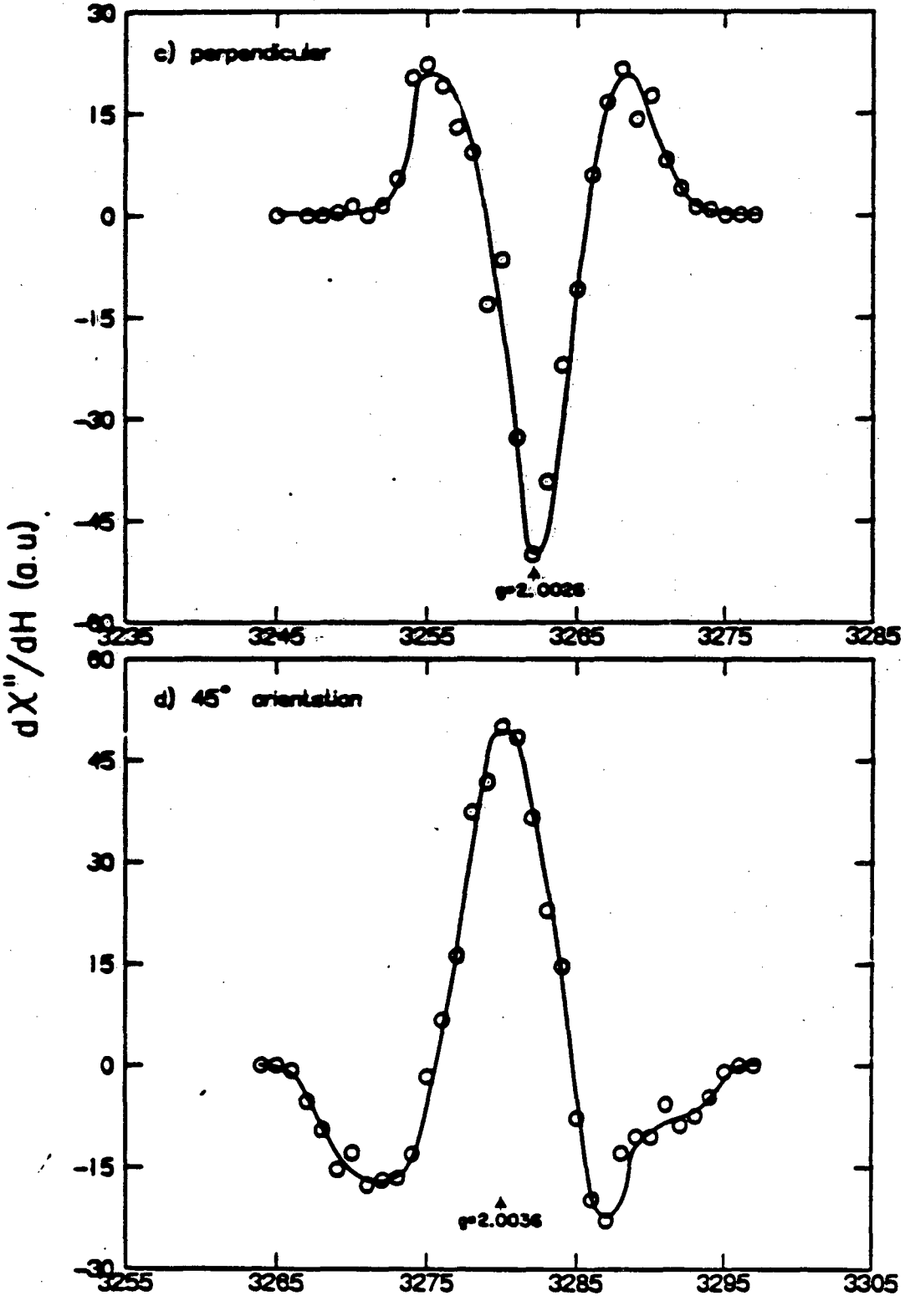


Fig. 2c,d



XBL 831-7503

Fig. 3

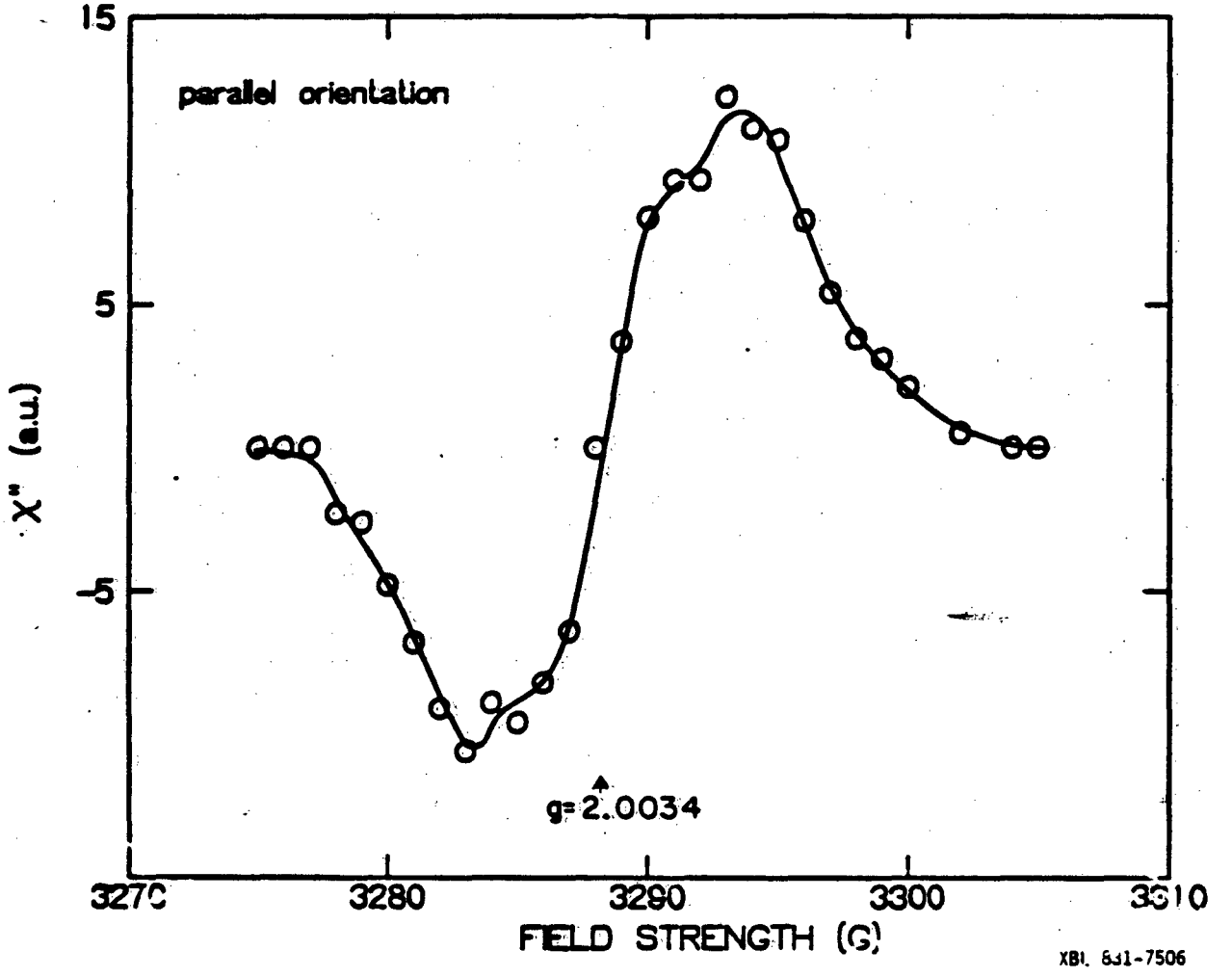
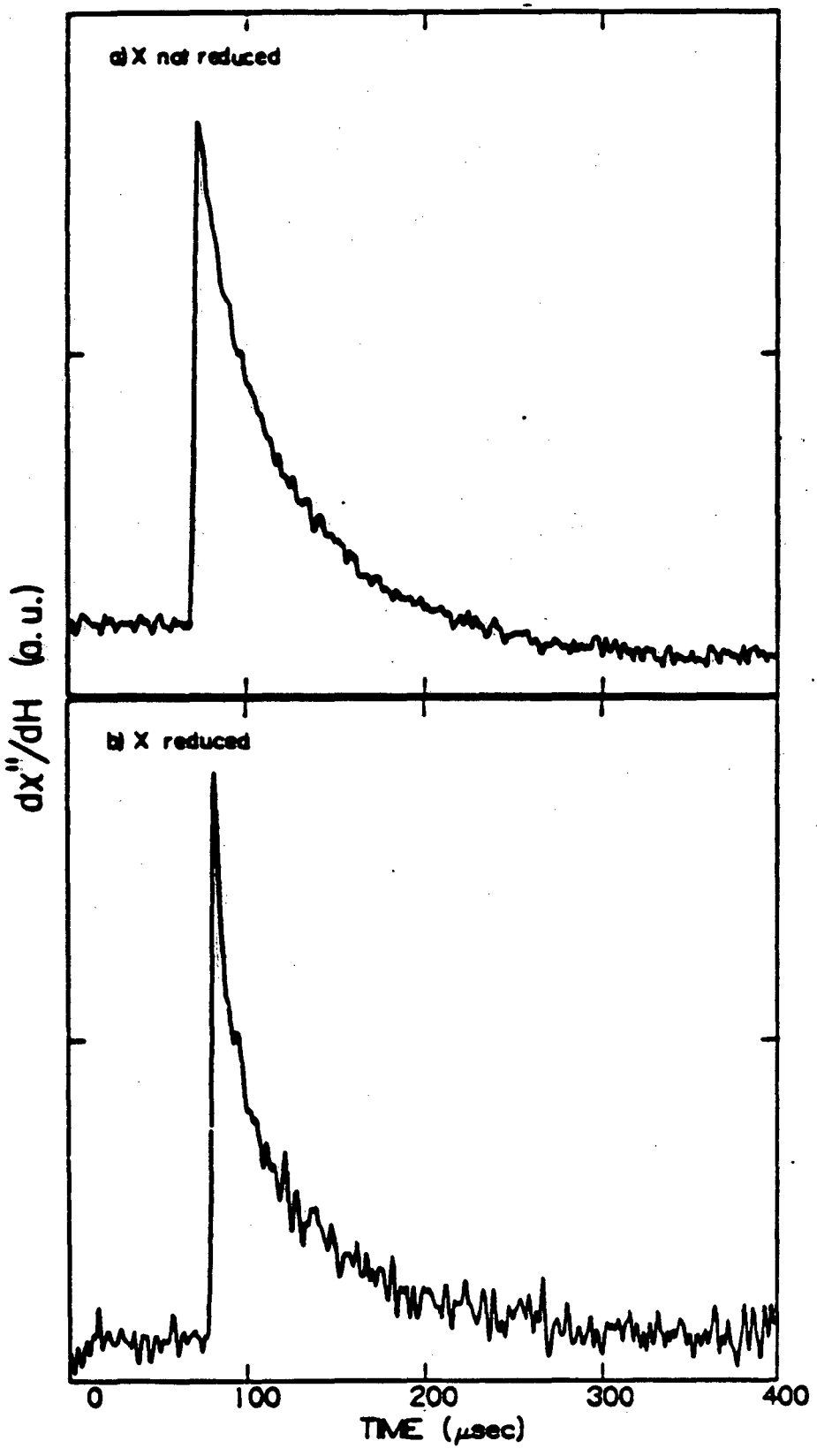

15
5
-5
parallel orientation
χ'' (a.u.)
g=2.0034
3270
3280
3290
3300
3310
FIELD STRENGTH (G)
XBL 851-7506

Fig. 4



XBL 831-7504

Fig. 5

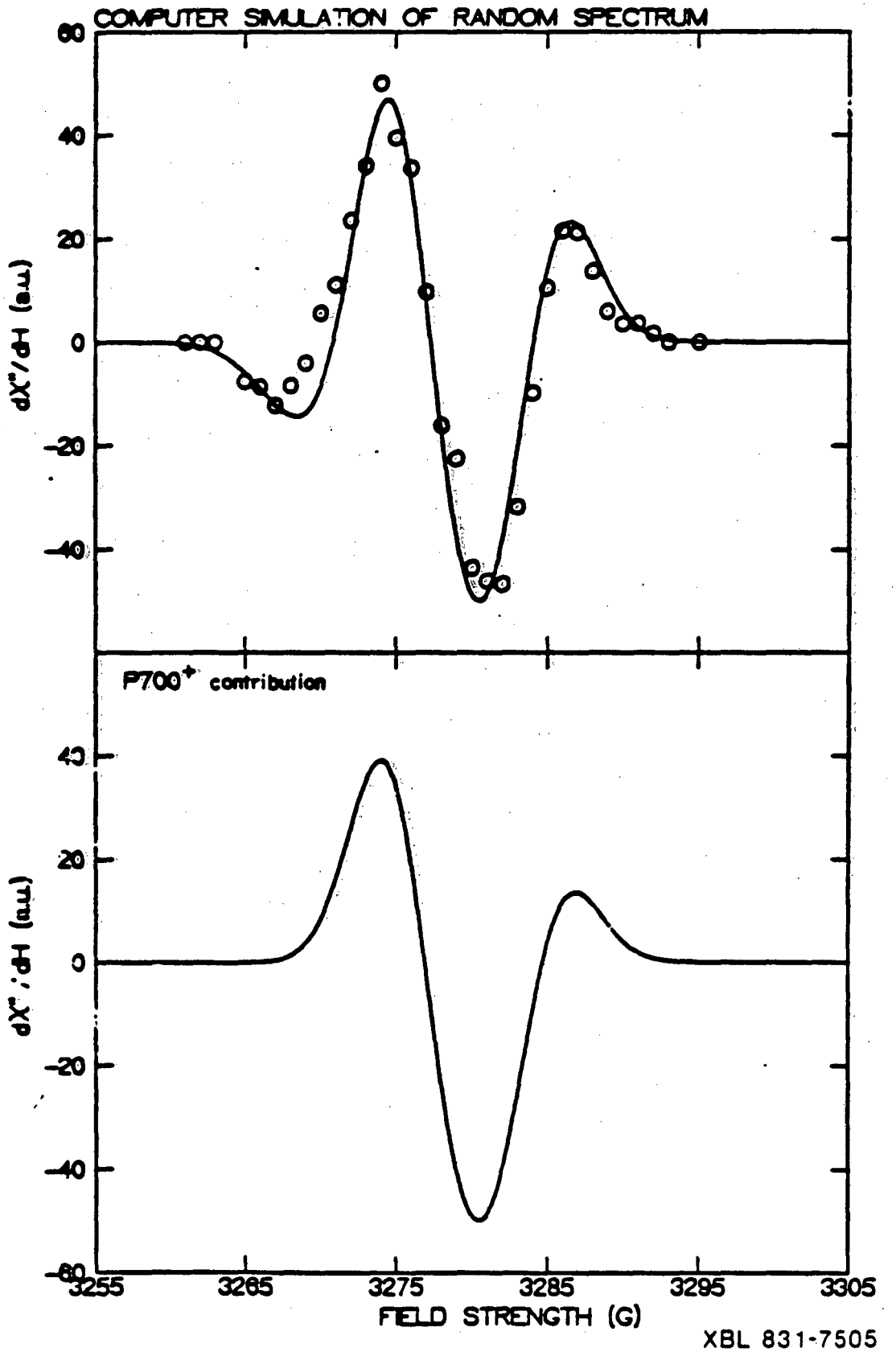
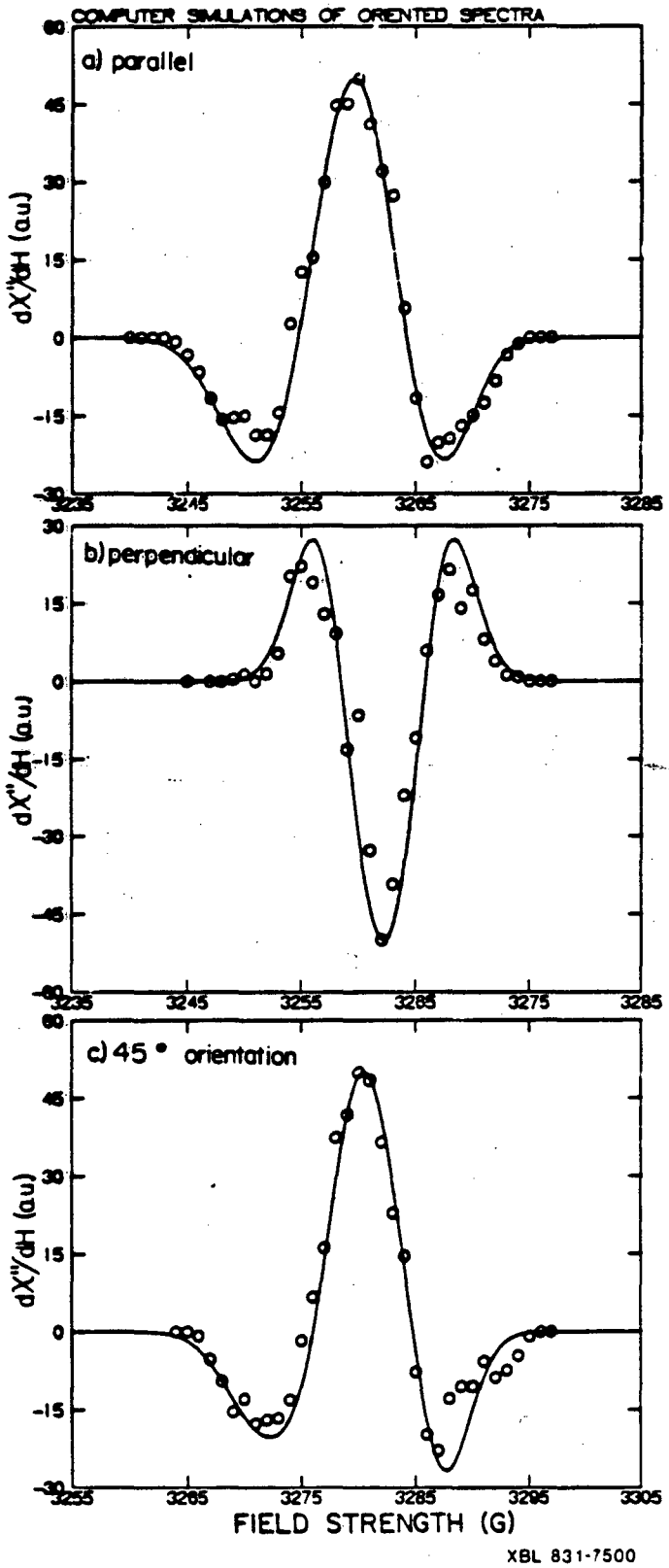

COMPUTER SIMULATION OF RANDOM SPECTRUM
dX"/dH (a.u.)
60
40
20
0
-20
-40
P700+ contribution
dX"/dH (a.u.)
40
20
0
-20
-40
-60
3255
3265
3275
3285
3295
3305
FIELD STRENGTH (G)
XBL 831-7505

Fig. 6


COMPUTER SIMULATIONS OF ORIENTED SPECTRA
a) parallel
b) perpendicular
c) 45 ° orientation
dX''/dH (a.u.)
FIELD STRENGTH (G)
XBL 831-7500

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