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Publication Date

1981-06-01

LBL-12957
Preprint

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CHEMICAL BIODYNAMICS DIVISION

Submitted to Biochimica et Biophysica Acta

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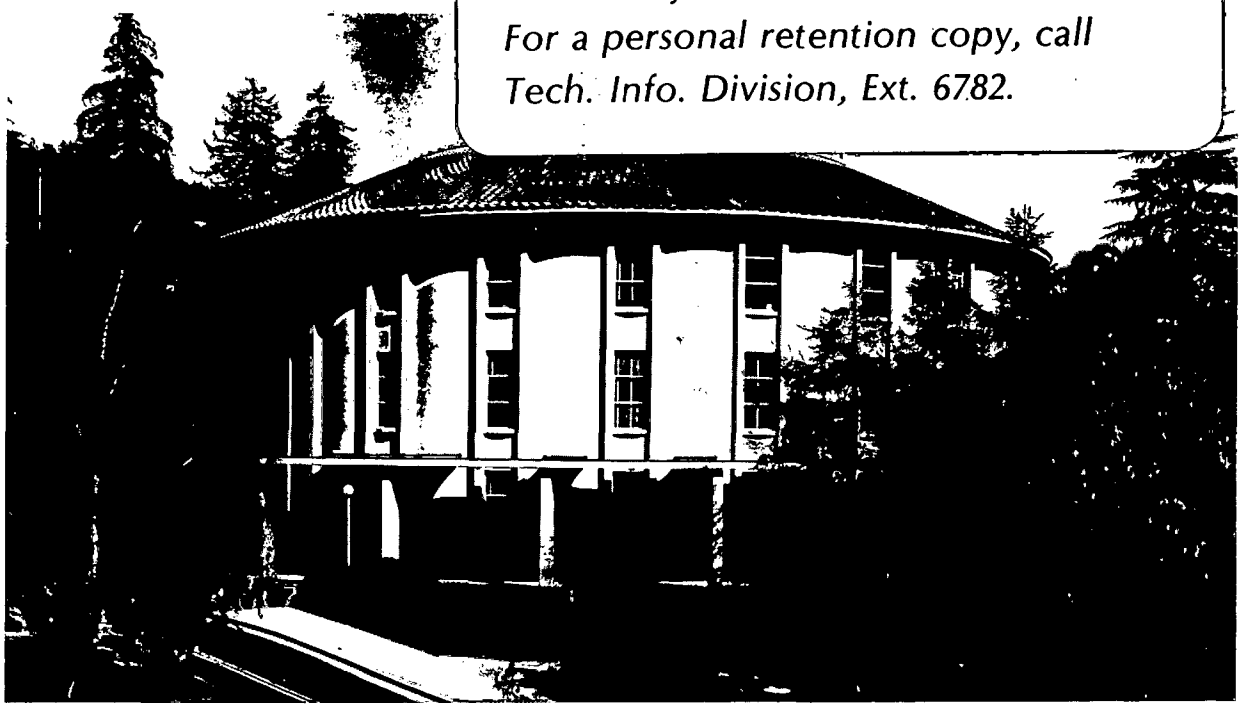
June 1981



Prepared for the U.S. Department of Energy under Contract W-7405-ENG-48

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LBL-12957

The Dependence of Reaction Center and Antenna Triplets on the Redox State of Photosystem I

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Key Words: Photosynthesis, Electron Paramagnetic Resonance, Chlorophyll

This research was supported, in part, by the Basic Energy Division of the U.S. Department of Energy under Contract W-7405-ENG-48 and, in part, by a grant from the National Science Foundation (PCM79-11251).

SUMMARY

The formation of chlorophyll triplet states during illumination of photosystem I reaction center samples depends upon the redox states of P700, X and ferredoxin Centers A and B. When the reaction centers are in the states $P700^+A_1$ X Fd_B Fd_A^- and P700 A_1 X $Fd_B^-Fd_A^-$ prior to illumination, we observe electron paramagnetic resonance (EPR) spectra from a triplet species which has zero field splitting parameters(|D| and |E|) larger than those of either chlorophyll a or chlorophyll b monomer triplet, and a polarization which results from population of the triplet spin sublevels by an intersystem crossing mechanism. We interpret this triplet as arising from photoexcited chlorophyll antenna species associated with reaction centers in the states $P700^+Fd_A^-$ and $P700^+X^-$, respectively, which undergo de-excitation via intersystem crossing. When the reaction centers are in the states P700 A_1 X $Fd_B^-Fd_A^-$ and P700 A_1 $X^-Fd_B^-Fd_A^-$ prior to illumination, we observe a triplet species with a polarization which results from population of the triplet spin sublevels by radical pair recombination, and which has a ¶D¶ value similar to that of chlorophyll a monomer. We interpret this triplet (the RPP triplet) as arising from $^3P700$ which has been populated by the process $P700^+A_1^- \longrightarrow {}^3P700\ A_1$. We observe both the RPP triplet and the chlorophyll antenna triplet when the reaction centers are in the state P700 A_1 X Fd_B^- Fd_A^-, presumably because the processes

$P700^+A_1^-X \longrightarrow P700^+A_1\ X^-$ and $P700^+\ A_1^-X \longrightarrow {}^3P700\ A_1\ X$

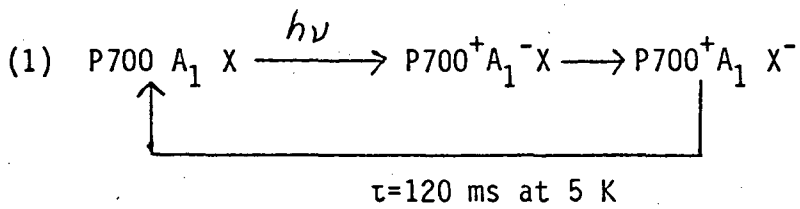
have similar rate constants when Centers A and B are reduced,

i.e., the forward electron transfer time from A_1^- to X is apparently much slower in the redox state P700 A_1 X $Fd_B^-Fd_A^-$ than it is in the state P700 A_1 X Fd_B Fd_A.

The amplitude of the RPP triplet does not decrease in the presence of a 13.5 Gauss wide EPR signal centered at g = 2.0 which was recorded in the dark prior to triplet measurements in samples previously frozen under intense illumination. This g = 2.0 signal, which has been attributed to phototrapped A_1^- (Heathcote et al(1979)FEBS Lett. 101,105), corresponds to as many as 12 spins per P700 and can be photo-generated during freezing without causing any apparent attenuation of the RPP triplet amplitude. We conclude that species other than A_1^- contribute to the g = 2.0 signal.

INTRODUCTION

The transfer of electrons from P700 to iron sulfur Centers A and B during primary photochemistry in photosystem I appears to involve two intermediate acceptors. MacIntosh et al [1] and Evans et al [2] observed EPR signals at liquid helium temperatures from photooxidized P700 and a photoreduced acceptor, denoted X, during continuous illumination of samples in which Centers A and B had been chemically reduced prior to illumination. Evidence that X is not the only acceptor was obtained from studies of the kinetics of optical absorption changes by Sauer et al [3], and of optical and EPR changes by Shuvalov et al [4]. In both cases it was observed that photoinduced charge separation decays biphasically below 100 K in samples in which Centers A and B are reduced prior to measurement. The slower phase, which Shuvalov et al [4] determined to have a halftime of 120 ms at 5 K, was attributed to the charge recombination between P700 and X. The faster phase has a halftime of about 1 ms at 5 K and was attributed to charge recombination between P700 and an additional acceptor, denoted A_1. The simplest interpretation of these data is that when X is not reduced prior to measurement, one observes the process



whereas when X is reduced prior to illumination, one observes

the process

(2) $P700\ A_1\ X^- \longrightarrow P700^+ A_1^- X^-$

τ=1 ms at 5 K

However, this hypothesis now appears to be questionable, partly because the mechanism of recovery by process (2) to the ground state P700 A_1 X has been shown to involve more than a single step. Frank et al [5] reported evidence that the molecular triplet state of P700 functions as an intermediate in the $P700^+ A_1^-$ back reaction. They observed an EPR triplet from P700 whose polarization indicates that it has been populated during primary photochemistry from a radical pair precursor[5]. In addition, the kinetic data are suggestive that photo-reduced A_1^- does not always transfer its electron to X when X is not reduced prior to photoexcitation. Sauer et al [3] reported that the 1 ms phase was observed regardless of whether X was reduced prior to measurement, and Shuvalov et al [4] observed the 1 ms phase in samples poised at -625 mV, a potential at which X is not reduced prior to measurement. Thus, under the conditions of these measurements, primary photochemistry would appear to be more complicated than the simple serial transfer of electrons from P700 to A_1 and then to X depicted in process (1).

With these mechanistic questions in mind, we studied the relation between the state of reduction of photosystem I acceptors and the amplitude of the radical pair polarized (RPP)

EPR triplet signal from P700. For these studies we used photosystem I reaction centers isolated using Triton X-100 detergent. In these preparations we do not observe a triplet that is seen in chloroplasts and in digitonin photosystem I subchloroplasts[5] and which Frank et al [6] determined to arise from a carotenoid species. However, in addition to the RPP triplet of P700, we now observe a triplet species which appears to become populated when P700 is oxidized prior to illumination; thus, it also depends on the redox state of photosystem I. We observe the RPP triplet in samples in which X is not reduced prior to illumination. We believe that it arises when A_1^- undergoes radical pair recombination with $P700^+$ in preference to forward electron transfer to X. Our results therefore suggest that the processes

$$P700^+ A_1^- X \longrightarrow P700^+ A_1 X^- \quad \text{and}$$

$$P700^+ A_1^- X \longrightarrow {}^3P700 A_1 X$$

are in competition when Centers A and B are reduced prior to triplet measurements. We observe no correlation between the amplitude of the RPP triplet and the amplitude of a g = 2.0 signal present in the dark in samples which have been frozen under illumination prior to triplet measurements. This g = 2.0 signal was attributed by Heathcote et al [7] to A_1 which has been phototrapped in its reduced state. Our results suggest that the species giving rise to the g = 2.0 signal is not the primary electron acceptor in photosystem I.

MATERIALS AND METHODS

Triton X-100 Photosystem I Fraction(PIF): A subchloroplast photosystem I fraction was isolated from spinach chloroplasts using Triton X-100 detergent in the presence of mono- or di-valent cations as follows. Chloroplasts were isolated from market spinach in 0.4 M sucrose/0.1 M NaCl/ 0.05 M Tris (pH 8) buffer and centrifuged at 7000Xg for 5 min. The pellets were washed in 0.7 mM EDTA to remove any Mn^{+2} EPR signal and centrifuged for 10 min at 15000Xg. The pellets were then resuspended to a chlorophyll concentration of 0.3 to 0.5 mg/ml in 0.05 M Tris pH 8/0.1 M NaCl or 0.01 M $MgSO_4$ and allowed to incubate 1 hr at $4^{o}C$ to assure clean fractionation of photosystem I from photosystem II [8]. Triton X-100 was stirred into the chloroplast suspension to give 1% detergent, and the mixture was incubated in the dark at $4^{o}C$ for 2-24 hours. The supernatant of a 30 min spin at 30,000Xg contained photosystem I complexes with the characteristics: chlorophyll a/chlorophyll b = 5-12, chlorophyll/P700 = 175-200. The photosystem I fraction was concentrated by dialysis against crystalline sucrose before it was used in EPR experiments or subjected to further purification.

Triton X-100 Photosystem I Reaction Centers(PIRC): Photosystem I reaction centers were isolated from PIF by the procedure of Golbeck et al[9]. Concentrated PIF (25ml) put through a 500 ml Sephacryl S-300 column yielded reaction centers with the characteristics chlorophyll a/chlorophyll b >9, chlorophyll/P700 = 35-65,that eluted with the column void volume.

Photosystem I reaction centers were concentrated for EPR studies by dialysis against crystalline sucrose.

Assays: Chlorophyll was assayed by the method of Arnon[10]. P700 was assayed by the ferricyanide-ascorbate absorbance difference at around 700 nm, assuming Ke's differential extinction coefficient of 64 mM^{-1} cm^{-1} [11]. Carotenoids were assayed qualitatively by thin layer chromatography as described by Barr and Crane [12].

EPR Sample Preparation: Samples of PIF or PIRC were prepared for EPR studies as previously described[5] and had final chlorophyll concentrations of about 0.5 or 0.1 mg/ml, respectively. The samples contained 1 mM phenazine methosulfate (PMS) or neutral red, as noted below. Samples which were illuminated before and during freezing were cooled to 156 K using a Varian liquid nitrogen temperature controller with a reproducible cooling cycle. Cooling from 273 to 156 K took about 30 s. Samples were illuminated with a Cary High Intensity tungsten lamp operating at or below 150 W. The EPR samples were placed in a cold finger dewar located 40 cm from the lamp, and we did not place any lenses between the sample and the lamp. Photodamage was avoided by keeping the sample temperature below 0 ^{o}C during illumination and by filtering the light beam through 20 cm of water. We found that at room temperature up to 100% of the chlorophyll was irreversibly photobleached within 60 s of illumination by an unfiltered 150 W tungsten beam.

EPR Measurements: EPR measurements were made using a Varian E-109 spectrometer operating at X band. All measurements utilized 100

kHz field modulation and a TE-102 cavity that was fitted with an optical transmission flange. Light modulation experiments were performed at 33 Hz, as described previously[5] except that a Princeton Applied Research Model HR-8 lock-in amplifier was used for phase sensitive detection. The light source was a Cary High Intensity tungsten lamp operating at 410 W. A Bell Model 811A digital gaussmeter was used to calibrate the magnetic field. An Air Products Helitran cryostat was used for liquid helium temperature EPR measurements.

RESULTS

The EPR spectra of Figs 1-3 were obtained from four identical aliquots of PIRC which were treated so as to produce four different redox states of photosystem I reaction center, as follows:

Sample (a)- frozen in the dark and then illuminated at 10 K.

Sample (b)- frozen in the dark in the presence of dithionite and PMS.

Sample (c)- same as sample (b), but illuminated 60 s at 0^{o} C and then frozen under illumination at low (25 W) lamp intensity.

Sample (d)- same as sample (c), but with lamp operating at higher (110W) intensity.

Figs 1 and 2 show the EPR spectra recorded in the dark at 12 K of samples (a)-(d). Illumination of sample (a) at 10 K resulted in the irreversible photooxidation of P700 (Fig 2a) and photoreduction of Center A (Fig 1a), as reported by Bearden and

Malkin [13]. In sample (b), ferredoxin centers A and B are reduced by dithionite, but X is not (Fig 1b), and there is not much signal in the g = 2.0 region present in the dark(Fig 2b). In sample (c), component X has been phototrapped in its reduced state(Fig 1c), and there is a small signal centered at g = 2.0 with a linewidth of 13.5 Gauss (Fig 2c). In sample (d), component X has been phototrapped in its reduced state, and a much larger 13.5 Gauss wide signal is present at g = 2.0 (Fig 2d) than is present in sample (c). We shall frequently refer to this photo-generated signal as 'the g = 2.0 signal', to distinguish it from the photoinduced, 8 Gauss wide $P700^+$ signal in samples like sample (a), which we shall call 'the $P700^+$ signal'.

Figure 3 shows the EPR triplet spectra observed in samples (a)-(d) during 33 Hz light-modulation experiments. The phase of the lock-in amplifier was calibrated using the P^R triplet from a sample of photosynthetic bacteria in which quinones had been removed by sodium dodecyl sulfate treatment[14], and which was kindly provided by W.W. Parson and V.A. Shuvalov. The rise and decay times for the P^R state in this sample are known to be 10 ns and 120 μs[15], respectively, so this signal is a convenient zero-phase reference for 33 Hz experiments. The triplet spectra in Fig 3 were recorded at zero phase with respect to the maximum amplitude of the P^R triplet. Figs 3a and 3d show the two distinct triplet spectra which we observe in PIRC; the spectra in Figs 3b and 3c are convolutions of these two triplets. Fig 3d shows the RPP triplet spectrum reported previously [5]. It has the polarization pattern AEEAAE and zero field splitting (zfs)

parameters $|D|$ =0.0278 cm-1 and $|E|$=0.0038 cm-1. Fig 3a shows the EPR spectrum of a triplet with a polarization pattern EEEAAA. This triplet is evidently populated by an intersystem crossing mechanism [5]. Its zfs parameters are $|D|$=0.0301 cm-1 and $|E|$=0.0039 cm-1. The latter D value is larger than that reported for chlorophyll a or chlorophyll b in vitro[16,17] or of the chlorophyll a triplet observed in spinach chloroplasts by Uphaus et al[16]. However, it seems likely that this triplet arises from a chlorophyll antenna species, and so we shall refer to it as 'the antenna triplet' for clarity. Table 1 gives the zfs parameters of the RPP and antenna triplets as well as those reported elsewhere [5,16,17] for chlorophyll and pheophytin triplets.

The results presented in Figs 1-3 typify those observed in measurements on eight similar sets of samples of PIRC. The results from five sets of PIF were essentially the same. We did not observe the carotenoid triplet which was observed in whole chloroplasts and in digitonin photosystem I subchloroplasts[5], despite the fact that we found substantial amounts of carotenoid in PIF by TLC analysis.

The g = 2.0 signal in Fig 2d has the same characteristics as the signal Heathcote that et al [7] observed in Triton X-100 reaction centers which had been frozen under intense illumination. They attributed this signal to A_1 which had been phototrapped in its reduced state. Fig 4 shows the results of experiments intended to correlate the number of spins represented by the g = 2.0 signal with the total reaction center

concentration and with the amplitude of light-inducible RPP triplet. Fig 4 shows the results of three separate trials. In each trial, a set of EPR samples was prepared from identical aliquots of PIRC or PIF in 0.1 M glycine buffer at pH 10. Several aliquots in each set were treated with dithionite and PMS and were then illuminated before and during freezing, each with a different light intensity, in order to vary the amplitude of the phototrapped g = 2.0 signal. Each set of samples included a sample which did not contain dithionite and PMS, so that P700 could be irreversibly photooxidized at low temperatures, e.g. sample (a) above. The number of $P700^+$ spins in the latter sample was assumed to be equimolar with the total reaction center concentration. The $P700^+$ signal in the latter sample, and the g = 2.0 signals in the other samples in the set were recorded in the dark at 100 K at non-saturating microwave powers. Under these conditions, the integrated areas of the signals are a direct measure of the number of spins,N, giving rise to the g = 2.0 signal and of the number of spins giving rise to the $P700^+$ signal, N_{P700}. The ratios N/N_{P700} relate the moles of the g = 2.0 species to the moles of P700. EPR triplet spectra were then recorded using an 11 Hz chopper, a frequency at which the antenna triplet makes a minimal contribution to the observed triplet spectra. The amplitude of the low field z peak of the RPP triplet was used in Fig 4 to represent the RPP triplet amplitude. Fig 4 shows that up to 12 spins per reaction center may be photo-generated in the g = 2.0 signal without having any apparent effect on the amplitude of light-inducible RPP triplet.

DISCUSSION

On The ZFS Parameters of the RPP and Antenna Triplets

In bacterial systems, the $|D|$ value of the P^R triplet is 20% smaller than that of bacteriochlorophyll monomer[18]. This effect is well accounted for by the argument that it results from a delocalization of the P^R triplet over two bacteriochlorophyll molecules in the reaction center special pair[17,18]. The evidence presented here and elsewhere [5,16,19] suggests that such a simple relationship does not hold between observed zfs parameters and the aggregation state of chlorophylls *in vivo* or *in vitro*. Table 1 allows comparison of the zfs parameters of the RPP and antenna triplets with those previously reported [16,17] for chlorophyll and pheophytin triplets. Although P700 is also thought to be a dimer [20], the RPP triplet $|D|$ value is not significantly different from that of chlorophyll *a* monomer. This is consistent with the observation of Uphaus *et al* [16] that the $|D|$ values of chlorophyll *a* dimer and chlorophyll *b* oligomer were within 3% of their respective monomeric values. The question of the correlation between the zfs parameters and chlorophyll environment is also raised by the antenna triplet $|D|$ value, which is significantly larger than that of either chlorophyll *a* or chlorophyll *b* monomer, and smaller than that of pheophytin. We cannot exclude the possibility that the antenna triplet arises from chlorophyll *b* or pheophytin on the basis of its $|D|$ value. However, it is now generally thought that chlorophyll *b* and pheophytin do not play a role in photosystem I, so that it is likely that the antenna triplet arises from chlorophyll *a*.

On the Redox Dependence of the RPP and Antenna Triplets

Figs 3a-d show that the amplitudes of both the RPP triplet and of the antenna triplet depend on the state of reduction of photosystem I acceptors at cryogenic temperatures.

The antenna triplet is not observed in samples in which X has been reduced prior to illumination (Figs 3c and 3d), and is observed together with the RPP triplet in samples in which centers A and B are reduced but X is not reduced prior to illumination (Fig 3b). These results are consistent with the assignment of the antenna triplet to an excited antenna species which undergoes intersystem crossing when P700 is in its oxidized state during the illumination period. The antenna triplet has its maximum amplitude in sample (a), in which presumably the entire population of reaction centers has undergone irreversible charge separation to the state $P700^{+}Fd_{A}^{-}$, as indicated by the spectra in Figs 1a and 2a. Antenna chlorophyll molecules excited during illumination at low temperature cannot transfer their excitation to reaction centers in this state, so they undergo intersystem crossing to the triplet state. In sample (b), illumination results in charge separation to the state $P700^{+}X^{-}$ and we observe the EPR signals from $P700^{+}$ (Fig 2b) and X^{-} (data not shown), as reported by Evans et al [2] . Figs 2a and 2b show that the amplitude of light-induced $P700^{+}$ EPR signal in sample (b) is equal to that observed in sample (a), so that both signals represent the same number of spins. Because the $P700^{+}$ in sample (a) arises from an irreversible light- induced electron

transfer to center A in all of the reaction centers, it appears that all of the reaction centers in sample (b) can achieve the state $P700^+X^-$ during illumination. The 120 ms [4] lifetime of the state $P700^+X^-$ is slow compared to the 33 Hz chopper period, so there will appear to be a steady-state population of reaction centers in the state $P700^+X^-$ during experiments using 33 Hz phase sensitive detection. This steady-state population of reaction centers in which P700 is oxidized during the illumination period will give rise to the antenna triplet. In samples (c) and (d), X is reduced prior to measurement. Illumination results in charge separation to the state $P700^+A_1^-$, which has a lifetime more than an order of magnitude shorter than the 33 Hz illumination period. In this case, no significant steady-state population of reaction centers in the state $P700^+$ is achieved, and the antenna triplet is not observed.

In sample (b), we observe a convolution of the RPP triplet with the antenna triplet. We that believe this is evidence, in addition to the kinetic evidence [3,4] discussed in the Introduction, that the processes

$$P700^+A_1^-X \longrightarrow {}^3P700\ A_1\ X$$

and

$$P700^+\ A_1^-X \longrightarrow P700^+A_1\ X^-$$

have similar rate constants when centers A and B are reduced, for the following reasons. We have argued that the observation of the antenna triplet results from a steady-state build-up of reaction centers in the state $P700^+$. The observation that the photo-induced $P700^+$ EPR signal has the same amplitude in sample (b) as it has in sample (a) suggests that the reaction

centers can achieve the state $P700^+X^-$ during illumination of sample (b). However, the state $P700^+X^-$ has a 120 ms lifetime [4] and so the reaction centers return periodically to the ground state. The observation of the RPP triplet in sample (b) suggests that every time a reaction center is excited, it has some probability that A_1^- will not transfer its electron to X but will instead undergo radical pair recombination to $^3P700$. This can happen only if the two rate constants are similar. Our argument is, of course, based on the assumption that the RPP triplet arises from $P700^+A_1^-$ pair recombination rather than from $P700^+ X^-$ pair recombination. The latter process cannot give rise to a polarized triplet on P700 if the spin states of $P700^+X^-$ become Boltzmann populated before charge recombination populates the triplet on P700. The X^- EPR signal is not saturated at 100 mW at 10 K, which suggests that its spin lattice relaxation time is not longer than a few microseconds. The spin lattice relaxation time of $P700^+$ was reported to be 800 μs at 10 K [21]. Thus, spin lattice relaxation will destroy any polarization on $P700^+X^-$ within the 120 ms lifetime. Our argument is also based on the assumption that all of the light-induced $P700^+$ signal in sample (b) arises from the $P700^+X^-$ state, rather than from $P700^+A_1^-$. McCracken et al [22] reported the observation during kinetics measurements of a flash-induced, polarized EPR signal centered around g = 2.0, which they attributed to the $P700^+A_1^-$ radical pair. A convolution of significant amounts of the polarized $P700^+A_1^-$ EPR signal with the unpolarized $P700^+$ signal from $P700^+X^-$ in sample (b) would not give rise to an EPR

signal which looks like the light-induced signal in Fig 2b. On the other hand, if $P700^+A_1^-$ gives rise to a Boltzmann-populated EPR signal which contributes to the light-induced g = 2.0 signal in Fig 2b, then it cannot be the precursor of a polarized triplet on P700 after radical pair recombination. The observation of the antenna triplet and of the full extent of $P700^+$ steady-state photooxidation in sample (b) suggest that the reaction centers are able to achieve the state $P700^+X^-$ during continuous illumination. The observation of the RPP triplet, on the other hand, indicates that the reaction centers also undergo charge separation followed by radical pair recombination between $P700^+$ and A_1^-, which populates the RPP triplet. This suggests to us that when Centers A and B are reduced, the forward electron transfer to X and radical pair recombination between $P700^+$ and A_1^- are in competition, and that the reaction kinetics may be represented by the scheme:

$$P700\ A_1\ X\ Fd_B^-Fd_A^- \longrightarrow P700^+A_1^-\ X\ Fd_B^-Fd_A^- \xrightarrow{k_f} P700^+A_1\ X^-Fd_B^-Fd_A^-$$

$$\uparrow \qquad \downarrow k_b$$

$$^3P700\ A_1\ X\ Fd_B^-Fd_A^-$$

where $k_f \sim k_b$. Our results are thus in agreement with the results of Sauer et al [3] and of Shuvalov et al [4] who observed the 1 ms phase associated with $P700^+A_1^-$ recombination in samples in which X was not reduced prior to measurement. There is indeed evidence that this situation exists

also at room temperature. In room temperature kinetics measurements, Shuvalov et al [23] observed a biphasic decay of optical changes attributed to $P700^+A_1^-$ radical pair formation under conditions where Centers A and B were reduced prior to measurement. They assigned a 10 nsec component at 694 nm to the recovery of changes in the spectrum of A_1 due to radical pair recombination to a triplet state, presumably $^3P700$. They also observed a 3 μs component which they attributed to the $^3P700$ lifetime. However, they noted that the amplitude and decay rate of the 10 ns component were the same regardless of whether X was reduced by exogenous donors and background illumination during the kinetics measurements. This result again suggests a similarity between the two rate constants for the processes of $P700^+A_1^-$ pair recombination and forward electron transfer from A_1^- to X. If we take this argument seriously, then it would appear that when Centers A and B are reduced, the forward electron transfer time from A_1^- to X is on the order of 10 ns, which is 50 times slower than the 200 ps forward transfer time when Centers A and B are not reduced prior to illumination [23]. Thus, our results suggest that the reduction of Centers A and B affects the kinetics of primary events in photosystem I. If so, then the kinetics involving P700, A_1 and X occurring during primary photochemistry under physiological conditions will need to be studied in some other way, such as by selectively removing Centers A and B from the reaction center. So far, however, any treatment which removes Centers A and B also removes X [9,19].

In the case of samples (c) and (d) we observe that the

reduction of the species giving rise to the g = 2.0 EPR signal shown in Fig 2d has no apparent effect on the amplitude of the RPP triplet. This suggests that the g = 2.0 radical is not the primary photosystem I acceptor in its reduced state. If this radical were the fully reduced primary acceptor, then charge separation could not occur and we would not observe the RPP triplet. Fig 4 shows that up to 12 spins per P700 may be photo-generated and contribute to the g = 2.0 signal without having any apparent effect on the amplitude of the RPP triplet. We suggest that photooxidized antenna chlorophyll contributes amplitude to the g = 2.0 signal. Recently, Rutherford and Mullet [19] reported studies of the EPR triplets in photosystem I reaction centers in which X and Centers A and B had been denatured by treatment with lithium dodecyl sulfate. They observed the RPP triplet in samples which contained dithionite and neutral red and were frozen in the dark. After these samples had been thawed and refrozen during illumination, the g = 2.0 signal attributed to A_1^- was present and the RPP triplet amplitude was smaller, suggesting that charge separation is not occurring when the g = 2.0 radical is phototrapped. After the latter samples had been thawed and refrozen in the dark, the g = 2.0 signal was smaller, and the original amplitude of the RPP triplet was restored. Our results (data not shown) with photosystem I reaction centers that have been treated with urea and ferricyanide by the method of Golbeck et al [9] to remove iron from X and Centers A and B are in agreement with the results of Rutherford and Mullet[21]. It makes sense that A_1^-

should be easier to phototrap in preparations in which X and Centers A and B are not present. When these secondary acceptors are active, they must be reduced before A_1^- can be phototrapped, and A_1 must then be reduced in close proximity to three negatively charged sites. It seems likely that in these preparations, some of the g = 2.0 signal present in samples which have been frozen during illumination arises from A_1^-. However, the results presented in Fig 4 show that there are species other than A_1^- contributing amplitude to the g = 2.0 signal. Until further quantitative work settles this point, the g = 2.0 signal should not be interpreted as the EPR spectrum of A_1^-.

ACKNOWLEDGEMENTS

We are indebted to Mr. John McCracken for many helpful discussions, and to Dr. Harry Frank for getting us interested in triplet states. This research was supported, in part, by the Basic Energy Division of the U.S. Department of Energy under Contract W-7405-ENG-48 and, in part, by a grant from the National Science Foundation (PCM 79-11251).

Figure Captions

Figure 1: EPR spectra of samples (a)-(d) recorded in the dark at 10 K. Photosystem I reaction centers were: (a) frozen in the dark in glycine buffer at pH = 10 and then illuminated at 10 K;(b)frozen in the dark in the presence of 25 mM dithionite and 1 mM phenazine methosulfate at pH 10;(c)sample identical to sample (b) which was frozen under low intensity illumination; (d)sample identical to sample (b) which was frozen under higher intensity of illumination. EPR spectrometer conditions: gain, 12,500 (Fig 1a) and 10,000 (Fig 1b-1d); modulation amplitude, 20 Gauss; microwave power, 20 mW; microwave frequency, 9.16 GHz; scan rate, 250 Gauss min^{-1}.

Figure 2: EPR spectra in the g = 2.0 region of samples (a)-(d), which are as described in Fig 1. (a) spectrum recorded during illumination of sample (a); (b, solid line) spectrum of sample (b) recorded in the dark before and after illumination; (b, dotted line) spectrum of sample (b) recorded during illumination; (c) and (d) spectra of samples (c) and (d), respectively, recorded in the dark. All spectra were recorded at 10 K. Spectrometer conditions:gain, 20,000;modulation amplitude, 4 Gauss; microwave power, 10 μW; microwave frequency, 9.16 GHz; scan rate, 25 Gauss min^{-1}.

Figure 3: Light-modulated EPR triplets observed in samples a-d at zero degrees phase with respect to the maximum of the bacterial P^R triplet amplitude, as described in the text. All spectra were recorded at 10 K. Spectrometer conditions: 33 Hz light modulation; gain,200; modulation amplitude, 40 Gauss; microwave power, 100 μW; microwave frequency, 9.16 GHz; scan rate, 63 Gauss/min.

Figure 4: Plot of the ratio N/N_{P700} vs. the amplitude of the RPP triplet, where N is the number of spins represented by the g = 2.0 signals (e.g. Fig 2d) which are photo-generated by freezing aliquots of reaction centers in the presence of dithionite and phenazine methosulfate during illumination of varying intensity, and N_{P700} is the number of spins represented by the $P700^+$ signal (e.g. Fig 2a) in an identical reaction center aliquot which does not contain dithionite or phenazine methosulfate but has been illuminated at 77 K. (○), (◇) and (□) represent three different sets of measurements. See Results section for details. Conditions for triplet measurements are as in Fig 3, except that 11 Hz light modulation frequency was used. The g = 2.0 EPR signal measurements were made at 77 K and 10 μW microwave power.

Table 1

Species	ZFS Parameters [a]	
	D (cm^{-1})	E (cm^{-1})
RPP	0.0278	0.0038
Antenna	0.0301	0.0039
In vitro [b]		
chlorophyll a monomer	0.0275	0.0036
chlorophyll b monomer	0.0287	0.0037
pheophytin a	0.0339	0.0033
In vivo		
spinach chloroplasts[b]	0.0284	0.0039
spinach chloroplasts[c]	0.0278	0.0039

[a]Error limits 0.0002 cm^{-1}

[b]from ref. [16]

[c]from ref. [5]

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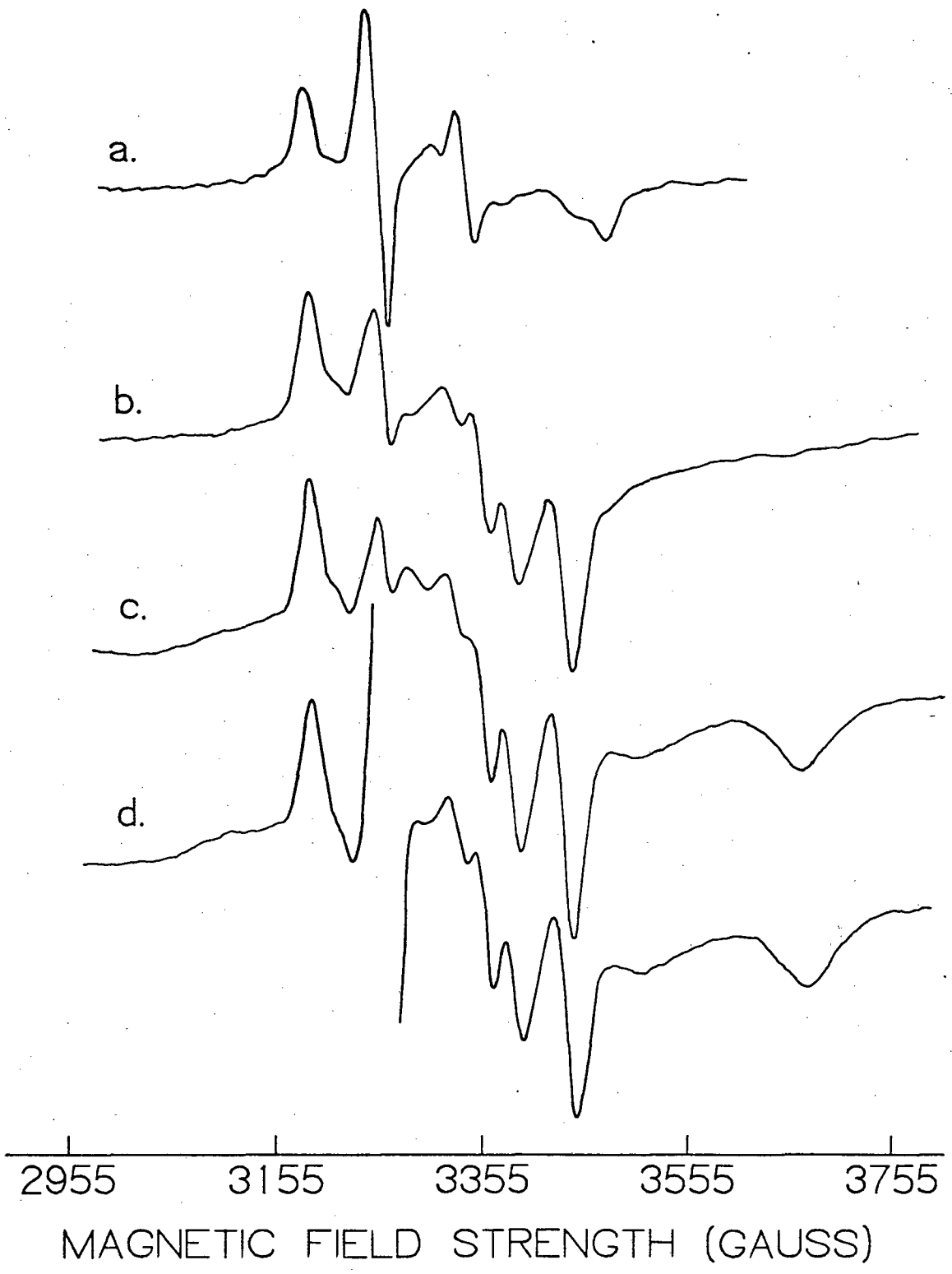
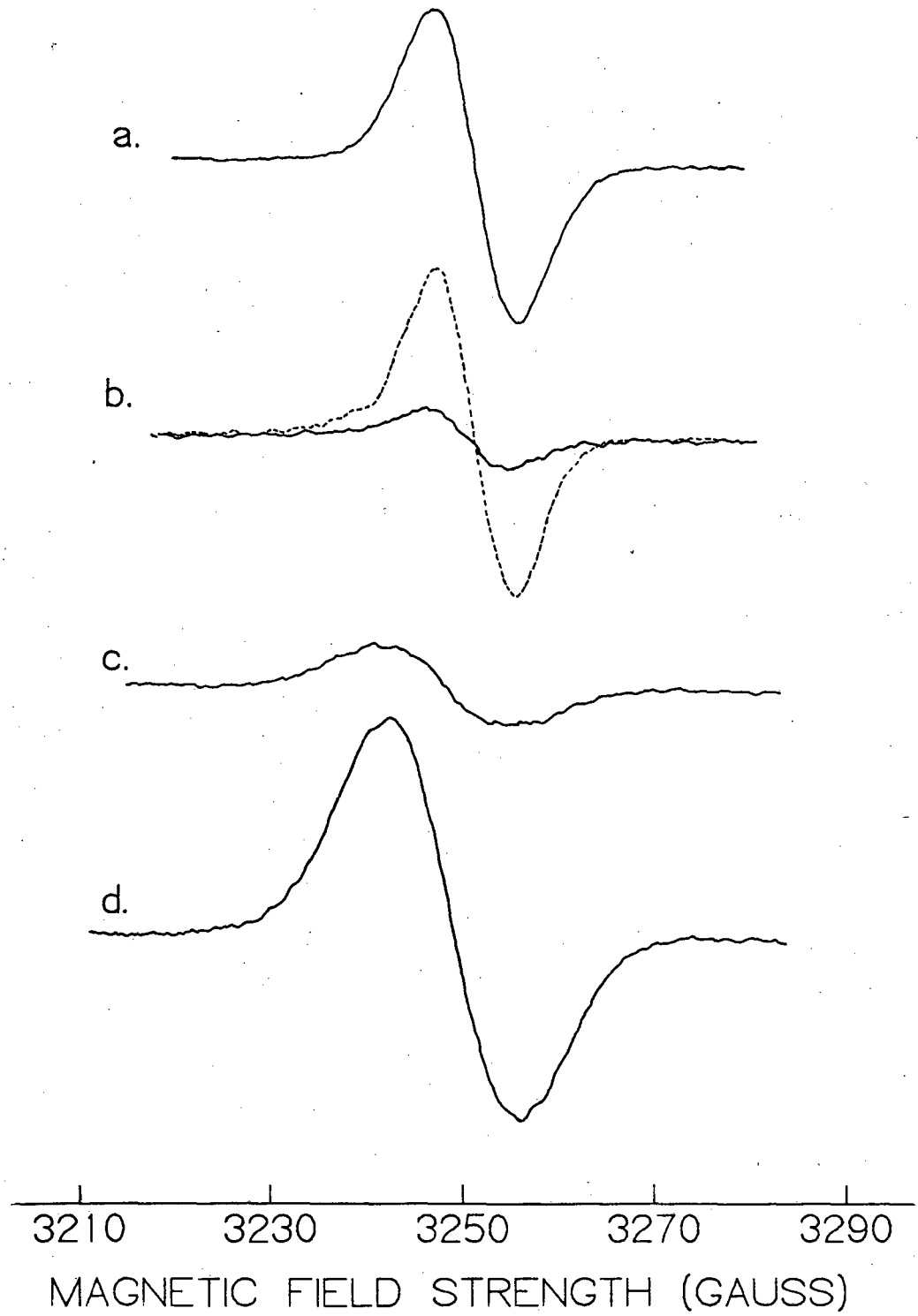


XBL 816-4644

McLean and Sauer

Figure 1



XBL 816-4642

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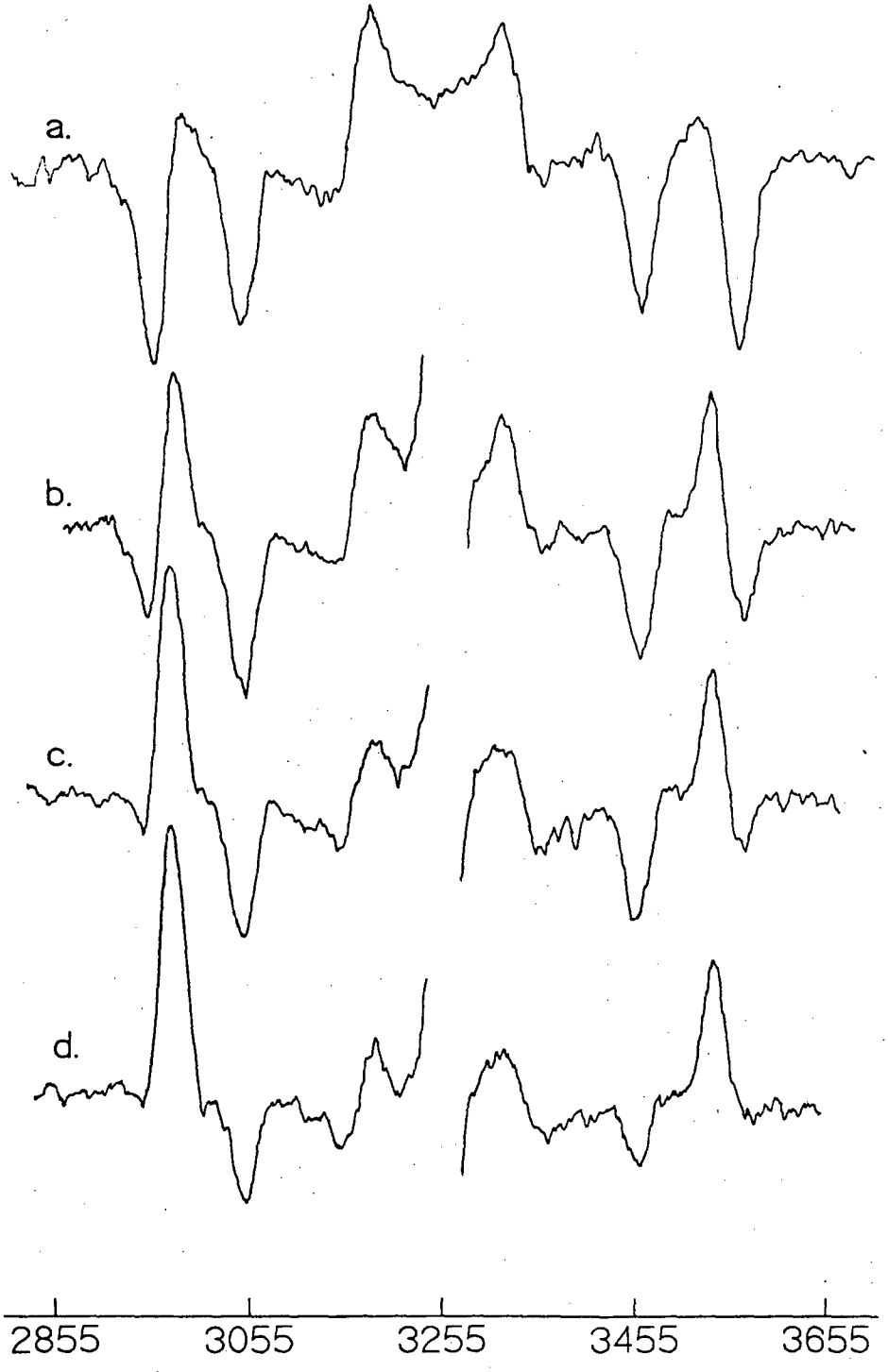
Figure 2



XBL 816-4643

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Figure 3

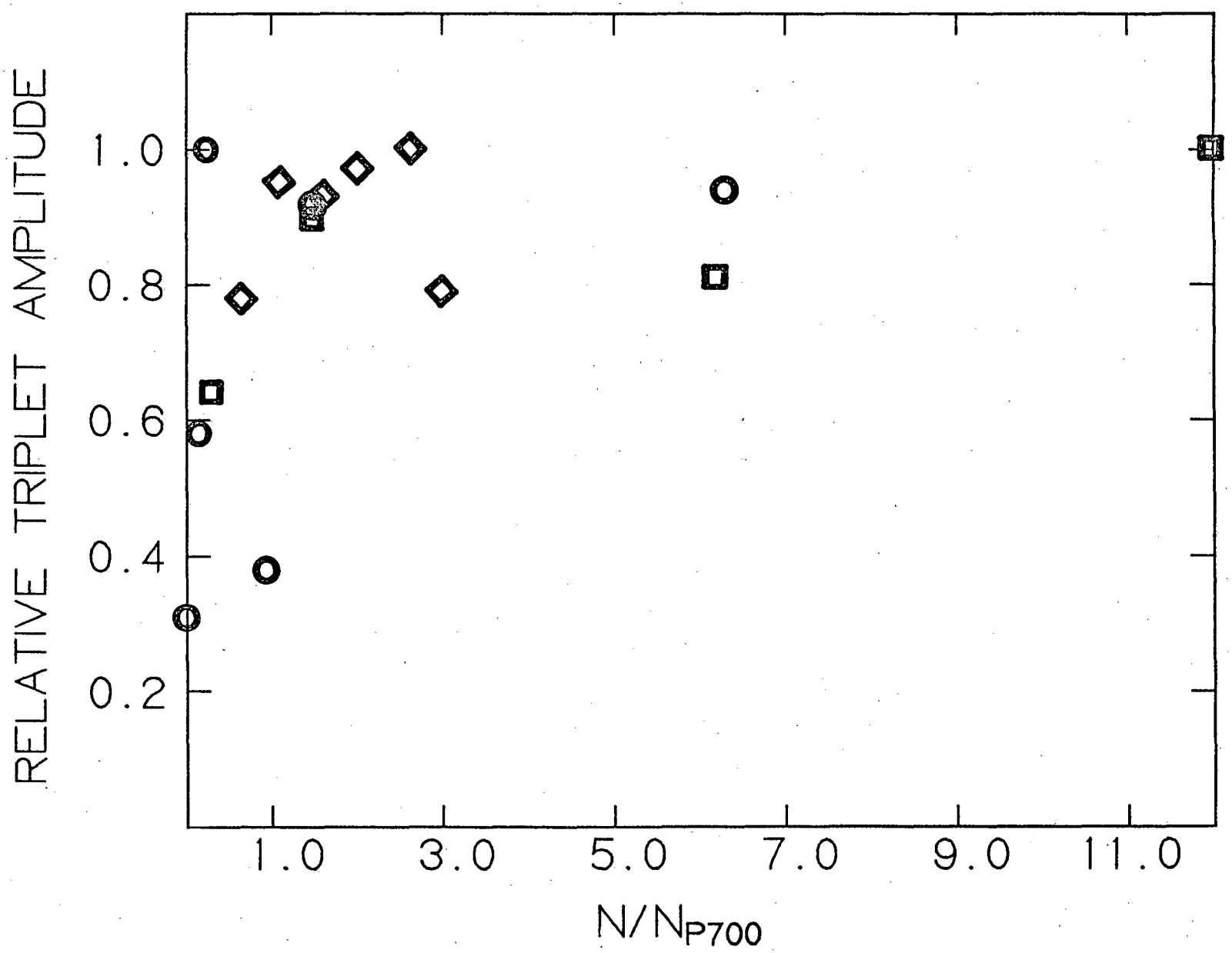

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Figure 4

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