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# Location of the Iron–Sulfur Clusters $F_A$ and $F_B$ in Photosystem I: An Electron Paramagnetic Resonance Study of Spin Relaxation Enhancement of $P_{700}^{+\dagger}$

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**ABSTRACT:** Photosystem I (PS I) mediates electron-transfer from plastocyanin to ferredoxin via a photochemically active chlorophyll dimer ( $P_{700}$ ), a monomeric chlorophyll electron acceptor ( $A_0$ ), a phylloquinone ( $A_1$ ), and three [4Fe-4S] clusters ( $F_{X/A/B}$ ). The sequence of electron-transfer events between the iron–sulfur cluster,  $F_X$ , and ferredoxin is presently unclear. Owing to the presence of a 2-fold symmetry in the PsaC protein to which the iron–sulfur clusters  $F_A$  and  $F_B$  are bound, the spatial arrangement of these cofactors with respect to the  $C_2$ -axis of symmetry in PS I is uncertain as well. An unequivocal determination of the spatial arrangement of the iron–sulfur clusters  $F_A$  and  $F_B$  within the protein is necessary to unravel the complete electron-transport chain in PS I. In the present study, we generate EPR signals from charge-separated spin pairs ( $P_{700}^{+}-F_{X/A/B}^{\text{red}}$ ) in PS I and characterize them by progressive microwave power saturation measurements to determine the arrangement of the iron–sulfur clusters  $F_{X/A/B}$  relative to  $P_{700}$ . The microwave power at half saturation ( $P_{1/2}$ ) of  $P_{700}^{+}$  is greater when both  $F_A$  and  $F_B$  are reduced in untreated PS I than when only  $F_A$  is reduced in mercury-treated PS I. The experimental  $P_{1/2}$  values are compared to values calculated by using  $P_{700}-F_{A/B}$  crystallographic distances and assuming that either  $F_A$  or  $F_B$  is closer to  $P_{700}^{+}$ . On the basis of this comparison of experimental and theoretical values of spin relaxation enhancement effects on  $P_{700}^{+}$  in  $P_{700}^{+}$  [4Fe-4S]<sup>−</sup> charge-separated pairs, we find that iron–sulfur cluster  $F_A$  is in closer proximity to  $P_{700}$  than the  $F_B$  cluster.

Photosynthetic reactions involve a series of photoinduced charge separations coupled with rapid electron transfer in the reaction centers photosystems I and II (PS I and II, respectively). Photosystem II catalyzes water oxidation on the luminal side of the thylakoid membrane while photosystem I is a chlorophyll-multisubunit protein complex that catalyzes the second photochemical reaction in electron-transfer from water to  $\text{NADP}^{+}$ . PS I is known to contain three [4Fe-4S] clusters ( $F_{X/A/B}$ ) as well as other redox active cofactors including a photochemically active chlorophyll dimer ( $P_{700}$ ), a monomeric chlorophyll electron acceptor ( $A_0$ ), and a phylloquinone ( $A_1$ ) that participate in electron transfer. Although the presence of electron transfer from the primary donor,  $P_{700}$ , to ferredoxin via the redox cofactors  $A_0$ ,  $A_1$  and  $F_X$  is well established, the precise sequence of electron-transfer events between the iron–sulfur cluster,  $F_X$ , and ferredoxin remains unclear (1).

A recently published 4.0 Å resolution X-ray crystallography structure by Krauss et al. (2, 3) unambiguously assigns the location of the  $P_{700}$  chlorophylls, the accessory chlorophylls, the electron acceptor ( $A_0$ ), and the iron–sulfur cluster  $F_X$  in PS I. Although it is possible to determine the separation between the terminal iron–sulfur electron-acceptors  $F_A$  and  $F_B$  (12 Å) at this resolution, the two centers are indistinguishable in this structure due to a pseudo 2-fold symmetry in the PsaC protein to which they are bound. The electron density map shows that the  $F_A$ – $F_B$  vector is 52° with respect to membrane plane suggesting sequential electron transfer from  $F_A$  to  $F_B$  (2, 3). There have been a number of studies conducted to probe indirectly the sequence of electron transfer between  $F_X$  and ferredoxin. Kinetics of electron transfer in Hg-treated PS I suggest that  $F_B$  is an intermediate donor to ferredoxin and flavodoxin (4, 5) and electrogenicity studies on the acceptor side suggest that  $F_A$  (rather than  $F_B$ ) is proximal to  $F_X$  (6), indicating that  $F_B$  may be the electron donor to ferredoxin. The EPR properties of in vitro and in vivo mutants of the cysteine ligands to the [4Fe-4S] clusters provide a biochemical argument that PsaC is oriented so that  $F_A$  is proximal to, and  $F_B$  is distal to,  $F_X$  (7). Studies involving selective destruction of  $F_B$  by either the use of urea-ferricyanide (8) or Hg-treatment (9–13) also imply that  $F_B$  is closer to the protein surface, increasing the possibility of attack by such reagents. Contrary to this picture, mutagenesis studies show that the D9A mutant does not bind  $F_X$ , which might be an indication that there is a preferential

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arrangement in the reaction center where  $F_B$  is closer to  $F_X$  than to the ferredoxin (14). Historically, the midpoint potentials of the three iron–sulfur clusters and increased photoreduction of  $F_A$  over  $F_B$  at cryogenic temperature have been invoked to suggest electron-transfer events that involve a linear sequence of transfer from  $F_X$  to  $F_A$  via  $F_B$  where  $F_A$  is the terminal acceptor in the electron-transfer chain (15) (reviewed in ref 1).

To date, there remains a lingering uncertainty regarding the identity of the terminal acceptor in the electron transport chain to ferredoxin as well as the kinetic rates of the discrete electron-transfer steps involved. Also, the issue of whether both clusters are really essential for electron-transport to ferredoxin is presently unresolved, i.e., is the flow of electrons serial through  $F_A$  and  $F_B$  (as implied by midpoint potentials) or is it parallel from  $F_X$  to either  $F_A$  or  $F_B$ . An unambiguous determination of the spatial arrangement of the iron–sulfur clusters  $F_A$  and  $F_B$  within the protein is key to understanding the chain of terminal electron-transport events in PS I. Spin relaxation behavior of an EPR-active species provides information on magnetic environments and interspin distances between spatially connected paramagnetic species. In the present study, we generate EPR signals from charge-separated spin pairs ( $P_{700}^{+}F_{X/A/B}^{-}$ ) in PS I and characterize them via microwave power saturation measurements to determine the arrangement of the iron–sulfur clusters  $F_{X/A/B}^{-}$  relative to  $P_{700}^{+}$ . The experimental data are compared to values calculated by using  $P_{700}F_{A/B}$  crystallographic distances and assuming that either  $F_A$  or  $F_B$  is closer to  $P_{700}^{+}$ . These results show that  $F_A$  is proximal to  $P_{700}$ .

## MATERIALS AND METHODS

**Preparation of Charge-Separated  $P_{700}^{+}$ -[4Fe-4S] $^{-}$  Pairs in PS I.** Wild-type PS I complexes were isolated from *Synechococcus* sp. by previously published procedures (16). Mercury-treated  $F_B$ -depleted PS I was prepared by the procedure of Jung et al. (13), which is a modification of published techniques (9) suited for cyanobacteria.  $F_A/F_B$ -depleted PS I was prepared by the method of Parrett et al. (17). Unless otherwise mentioned, the samples were resuspended in a buffer containing 50 mM Tris(hydroxymethyl)-aminomethane (Tris), 0.05% Triton X-100, and 20% glycerol at pH 7.0.  $P_{700}^{+}F_A^{ox}F_B^{ox}F_X^{ox}$  was chemically induced in wild-type PS I by oxidation with minimal concentrations (<0.05 mM) of potassium ferricyanide [ $K_3Fe(CN)_6$ ] at pH 7.0.  $P_{700}^{+}F_A^{red}$  was photoinduced in wild-type PS I and Hg-treated,  $F_B$ -depleted PS I by illumination at 77 K following the addition of 1 mM sodium ascorbate and 1 mM tetramethyl-*p*-phenylenediamine (TMPD) at pH 7.0.  $P_{700}^{+}F_A^{red}F_B^{red}$  was photoinduced by illumination at 77 K in wild-type PS I in a buffer containing 0.1 M glycine, 0.05% Triton X-100, 50 mM dithionite, and 20% glycerol at pH 10.0. Here, dithionite reduction produced  $P_{700}^{+}F_X^{ox}F_A^{red}F_B^{red}$  (~90%) and  $P_{700}^{+}F_X^{ox}F_A^{ox}F_B^{ox}$  (~10%), which upon photoreduction yields  $P_{700}^{+}F_X^{red}F_A^{red}F_B^{red}$  and  $P_{700}^{+}F_X^{red}F_A^{ox}F_B^{red}$ , respectively. However,  $P_{700}^{+}F_X^{red}$  charge-separated pairs decay in the dark resulting in a mixture of  $P_{700}^{+}F_X^{ox}F_A^{red}F_B^{red}$  and  $P_{700}^{+}F_X^{ox}F_A^{ox}F_B^{red}$  so that the  $P_{700}^{+}$  species observed in the EPR experiment is magnetically coupled to the  $F_A^{red}F_B^{red}$  pair.  $P_{700}^{+}F_X^{red}$  was photoinduced by illumination at 20.0 K in  $F_A/F_B$ -depleted PS I at pH 7.0 following the addition of 1 mM

sodium ascorbate and 1 mM TMPD. EPR data acquisition for the  $P_{700}^{+}F_A^{red}/F_B^{red}/F_A^{red}F_B^{red}$  samples was conducted in the dark to avoid interference from  $P_{700}^{+}F_X^{red}$  pairs.  $P_{700}^{+}F_X^{red}$  measurements in  $F_A/F_B$ -depleted PS I were conducted under continuous illumination due to the short lifetime of this charge-separated state in the absence of light. Chlorophyll concentrations in all the samples were typically ~1.0 mg/mL except for the  $F_B$ -depleted mercury-treated PS I sample (0.4 mg/mL).

**EPR Spectroscopy.** All EPR measurements were performed on a Varian E-9 spectrometer (operating at a frequency of 9.28 GHz) equipped with a TE<sub>102</sub> cavity and a helium flow cryostat (Oxford Instruments). PS I samples were loaded into 4 mm quartz tubes (Wilma Glass) and the samples were repeatedly degassed prior to EPR measurements to ensure the absence of relaxation enhancement due to triplet oxygen (18) (unless otherwise mentioned). All power saturation measurements were carried out at 20 K. Typically 4–6 spectra were acquired per data point in the microwave power saturation curves and the intensities were measured from the peak-to-trough heights of the  $P_{700}^{+}$  EPR signals.

**Spin–Lattice Relaxation Enhancement Measurements.** Long-range interactions ( $r > 10$  Å) between a pair of electron spins ( $i$  and  $j$ ) are mainly comprised of dipolar couplings. The field experienced by the species  $i$  is a combination of the applied magnetic field,  $B_0$ , and a local fluctuation caused by spin flips of the species  $j$ . If the species  $j$  has a rapid spin–lattice relaxation rate,  $T_{1j}$ , the local field fluctuations enhance the spin–lattice relaxation rate of the species  $i$  as given in eq 1,

$$1/T_{1i,observed} = 1/T_{1i,intrinsic} + 1/T_{1i,dipolar} \quad (1)$$

where,  $T_{1i,observed}$  is the measured spin–lattice relaxation rate of the  $i$  spins in the presence of the fast-relaxing  $j$  spins and  $T_{1i,intrinsic}$  and  $T_{1i,dipolar}$  are the intrinsic and dipolar-enhanced relaxation rates of the  $i$  spins. The spin–lattice relaxation enhancement observed for the species  $i$  due to a dipolar interaction with the fast-relaxing species  $j$  will display an  $r^{-6}$  distance dependence.

Progressive microwave power saturation is a well-established technique to estimate spin–lattice relaxation enhancement of a slow-relaxing species in the presence of a fast-relaxing species (19, 20). The half-power at which an EPR-active species saturates ( $P_{1/2}$ ) is a direct reflection of its spin–lattice relaxation rate ( $T_1$ ) as given in eq 2,

$$P_{1/2i} \propto 1/(\gamma^2 T_{1i} T_{2i}) \quad (2)$$

where  $\gamma$  is the electron magnetogyric ratio. The EPR signal amplitude ( $A$ ) is related to the observe microwave power ( $P$ ) by eqs 3 and 4 (21):

$$A = K\sqrt{P/(1 + P/P_{1/2i})^{b/2}} \quad (3)$$

$$\log(A/\sqrt{P}) = -b/2 \log(P_{1/2i} + P) + \text{constant} \quad (4)$$

Thus, a least-squares curve fit of  $\log(A/\sqrt{P})$  as a function of  $\log P$  would yield an estimate of the half-power at which the species  $i$  saturates ( $P_{1/2i}$ ). Furthermore, because

$$P_{1/2i,observed} = P_{1/2i,intrinsic} + P_{1/2i,dipolar} \quad (5)$$

the  $P_{1/2i,dipolar}$  values can be estimated experimentally by measuring the power saturation of the slow-relaxing species  $i$  in the presence ( $P_{1/2i,observed}$ ) and absence ( $P_{1/2i,intrinsic}$ ) of the fast-relaxing species  $j$ . A direct comparison of the relative magnitudes of  $P_{1/2i}$  of the slow-relaxing species ( $P_{700}^+$ ) in the presence of different fast-relaxers ( $F_{X/A/B}^{red}$ ) yields a qualitative estimate of the interspin distance between the various charge-separated pairs in PS I using eq 6:

$$P_{1/2i,dipolar} = \text{constant}/r^6 \quad (6)$$

## RESULTS AND DISCUSSION

In the present study, we utilize the technique of progressive power saturation to measure the spin relaxation enhancement of  $P_{700}^+$  centers due to dipolar interactions with reduced iron–sulfur clusters in charge-separated  $P_{700}^+$   $[4Fe-4S]^-$  pairs in PS I. On the basis of the recent X-ray crystallography structure published by Fromme and co-workers (2), the special pair of chlorophyll moieties constituting the primary donor ( $P_{700}$ ) in PS I is fairly exposed to the external surface of the protein making them readily accessible to the bulk solvent (and dissolved paramagnetic molecules). Thus, in addition to spin–lattice relaxation-enhancement of the  $P_{700}^+$  radicals due to dipolar interactions with reduced iron–sulfur clusters within the charge-separated pairs in PS I,  $P_{700}^+$  radicals may also exhibit additional relaxation enhancement due to the presence of chemical oxidants or triplet oxygen in these samples (18). To determine the magnitude of these effects, we compare progressive power saturation curves of  $P_{700}^+$  radicals (both chemically induced and photoinduced) under aerobic and anaerobic conditions. Shown in Figure 1 is a comparison of the power saturation behavior of photoinduced  $P_{700}^+$  in the charge-separated pair  $P_{700}^+F_A^{red}F_B^{ox}F_X^{ox}$  (in wild-type ascorbate-treated PS I) (Figure 1a) and chemically induced  $P_{700}^+$  (in the presence of 1, 2, and 10 mM ferricyanide) (Figure 1, panels b–d) under aerobic and anaerobic sample conditions. As can be seen in this figure, the spin relaxation rates of the  $P_{700}^+$  radical in the anaerobic samples are lower than those observed in the aerobic samples. This indicates that the  $P_{700}^+$  radicals experience significant spin relaxation enhancement due to dipolar and exchange interactions with paramagnetic triplet oxygen present in the aerobic PS I samples. Thus, to isolate the relaxation effects expected from the presence of the reduced iron–sulfur clusters in the various charge-separated pairs, it is critical that the progressive power saturation measurements be conducted under anaerobic conditions.

To facilitate comparison between the  $P_{700}^+$  relaxation enhancement due to the presence of the iron–sulfur clusters  $F_A$ ,  $F_B$ , and  $F_X$  in PS I, it is important to determine the intrinsic relaxation rate of a noninteracting  $P_{700}^+$  radical in the native protein environment. This can be achieved by chemically generating a  $P_{700}^+$  radical by the addition of an external oxidant (ferricyanide). However, the presence of excess paramagnetic ferricyanide ions in the system could potentially enhance the spin–lattice relaxation rates of  $P_{700}^+$  and interfere with the observation of relaxation effects solely due to the presence of fast-relaxing reduced iron–sulfur clusters. Shown in Figure 1e is a plot of the  $P_{1/2}$  values of chemically induced  $P_{700}^+$  in wild-type PS I samples as a function of the ferricyanide concentration used. A best linear

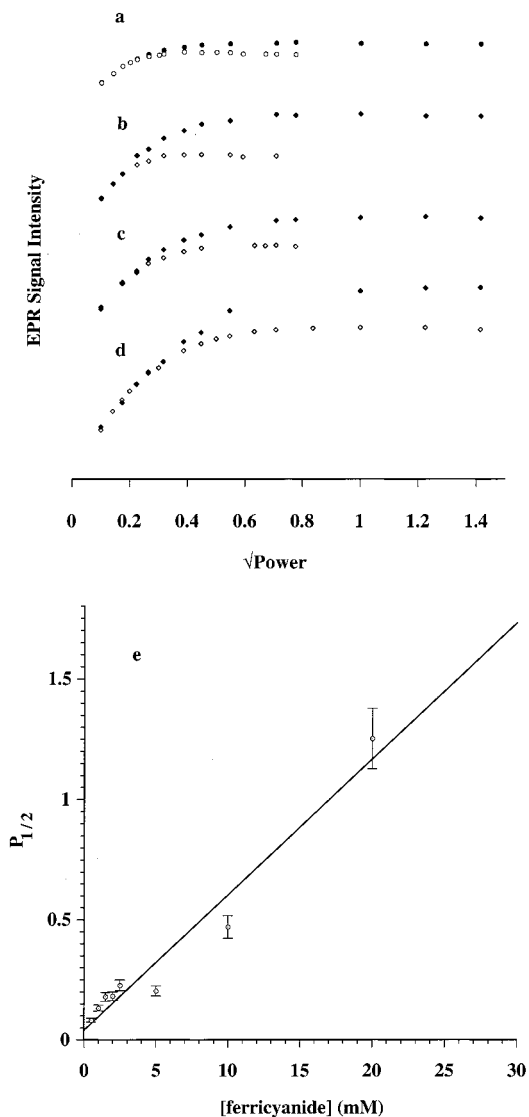


FIGURE 1: Progressive microwave power saturation measurements of  $P_{700}^+$  in aerobic (closed symbols) and anaerobic (open symbols) PS I samples: (a) photoinduced  $P_{700}^+$  in the charge-separated pair  $P_{700}^+F_A^{red}F_B^{ox}F_X^{ox}$  (in wild-type ascorbate-treated PS I) and chemically generated  $P_{700}^+$  in wild-type PS I containing (b) 1 mM, (c) 2 mM or (d) 10 mM ferricyanide, and (e) the dependence of  $P_{1/2}$  values of chemically induced  $P_{700}^+$  in PS I on the ferricyanide concentration in the medium. The data in parts a–d have been offset vertically for clarity. Indicated on the data points in part e are 10% error bars that account for small variations observed in the least-squares fits that yield experimental  $P_{1/2}$  values as well as errors in technique. The solid line in part e is the best weighted linear fit obtained for the ferricyanide concentration dependence of  $P_{700}^+ P_{1/2}$  values. All the  $P_{1/2}$  values shown in part e were obtained under anaerobic sample conditions.

fit for the data points indicates that, at concentrations of 0.05 mM ferricyanide or less, contributions to relaxation enhancement of  $P_{700}^+$  from ferricyanide ions are negligible.

In a progressive power saturation measurement, the half-power at which an EPR-active species saturates ( $P_{1/2}$ ) is a direct reflection of its spin–lattice relaxation rate. We expect enhancement of the intrinsic spin–lattice relaxation rate of a slow-relaxing  $P_{700}^+$  radical in the presence of a fast-relaxing  $[4Fe-4S]^-$  cluster in isolated charge-separated pairs in the PS I reaction centers. The magnitude of the enhancement observed depends on the interspin distance ( $r$ ). A measure of  $P_{1/2}$  values for  $P_{700}^+$  in the various charge-separated pairs



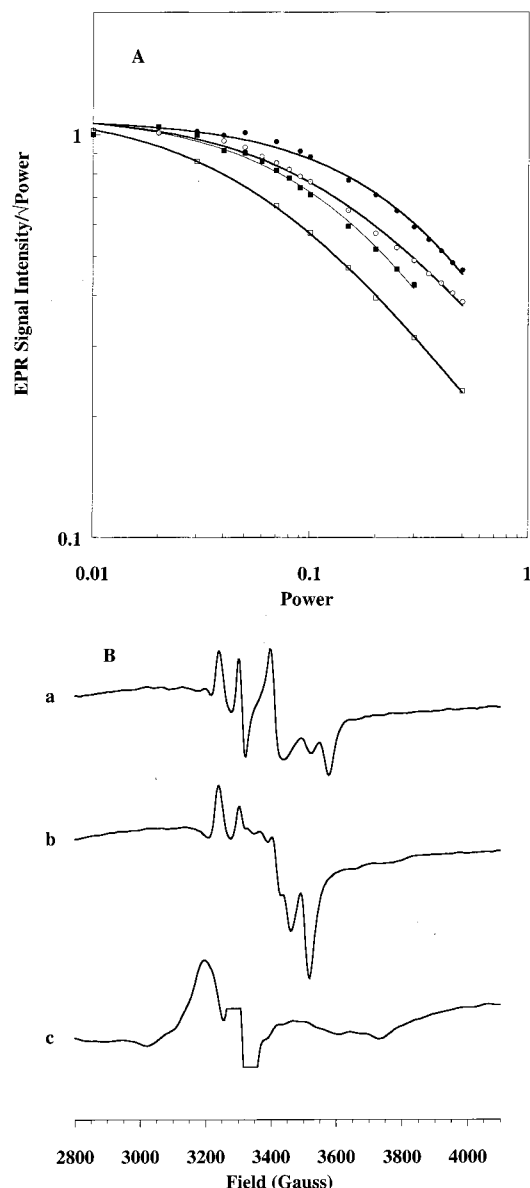


FIGURE 2: (A) Progressive power saturation measurements of chemically generated  $P_{700}^+$  ( $\square$ ) and  $P_{700}^+$  in the charge-separated pairs  $P_{700}^+F_A^{\text{red}}$  ( $\blacksquare$ ),  $P_{700}^+F_A^{\text{red}}F_B^{\text{red}}$  ( $\circ$ ), and  $P_{700}^+F_X^{\text{red}}$  ( $\bullet$ ). Solid lines indicate the least-squares curve fits of  $\log(A/\sqrt{P})$  as a function of  $\log P$  (eq 4) and yield an estimate of the  $P_{1/2}$  values of  $P_{700}^+$  in the absence and presence of the iron-sulfur clusters. (B) EPR spectra of the iron-sulfur clusters in the photoinduced charge-separated pairs: (a)  $P_{700}^+F_A^{\text{red}}$  in wild-type ascorbate-treated PS I, (b)  $P_{700}^+F_A^{\text{red}}F_B^{\text{red}}$  in wild-type dithionite-treated PS I, (c)  $P_{700}^+F_X^{\text{red}}$  in  $F_A/F_B$ -depleted PS I at pH 7.0. The central region of the spectrum in part c has been deleted due to interference from the  $P_{700}^+$  EPR signal. Experimental parameters: microwave frequency = 9.28 GHz; magnetic field modulation frequency = 100 kHz; modulation amplitude = 20 G; microwave power = 20.0 mW for parts a and b and 40 mW for part c, respectively; temperature = 7.8, 20.0, and 6.0 K for parts a, b and c, respectively.

will yield a qualitative estimate of distances (21) between the chlorophyll primary donor and [4Fe-4S] clusters. Shown in Figure 2A is a logarithmic plot of the ratio of  $P_{700}^+$  EPR signal intensities to the square-root of observe microwave power as a function of the observe microwave power used. The  $P_{1/2}$  values were determined by least-squares computer fits to the experimental data (eq 4) (21). As can be seen in Figure 2A, the intrinsic spin relaxation rate of a noninter-

Table 1: Experimental and Calculated  $P_{1/2}$  Values of  $P_{700}^+$

species	$P_{1/2}$ , exptl (mW)	$P_{1/2}$ , calcd <sup>a</sup> (mW)	$P_{1/2}$ , calcd <sup>b</sup> (mW)
$P_{700}^+$ (ferricyanide-treated PS I)	0.080		
$P_{700}^+F_A^{\text{red}}$ (Hg-treated $F_B$ -depleted PS I)	0.122		
$P_{700}^+F_A^{\text{red}}F_B^{\text{red}}$ (wt dithionite-treated PS I)	0.145	0.140	0.478
$P_{700}^+F_X^{\text{red}}$ ( $F_A/F_B$ -depleted PS I)	0.213		

<sup>a</sup> Assuming that the iron-sulfur cluster  $F_A$  is proximal to  $P_{700}$ .  
<sup>b</sup> Assuming that the iron-sulfur cluster  $F_A$  is distal to  $P_{700}$ .

acting chemically generated  $P_{700}^+$  radical is slower than that in the charge-separated pairs  $P_{700}^+F_A^{\text{red}}$  (in Hg-treated  $F_B$ -depleted PS I),  $P_{700}^+F_A^{\text{red}}F_B^{\text{red}}$  (in dithionite-treated PS I), and  $P_{700}^+F_X^{\text{red}}$  (in  $F_A/F_B$ -depleted PS I). The  $P_{1/2}$  values shown in Table 1 indicate that  $P_{700}^+$  does indeed exhibit enhanced relaxation rates in the presence of the fast-relaxing iron-sulfur clusters  $F_A$ ,  $F_B$ , and  $F_X$ . Comparison of individual progressive power saturation curves for  $P_{700}^+$  in the charge-separated  $P_{700}^+F_A^{\text{red}}$ ,  $P_{700}^+F_A^{\text{red}}F_B^{\text{red}}$ , and  $P_{700}^+F_X^{\text{red}}$  pairs indicates a maximum spin relaxation enhancement in  $P_{700}^+F_X^{\text{red}}$  consistent with crystallographic data showing that  $F_X$  is in much closer proximity to  $P_{700}$ . Observation of the  $P_{700}^+F_X^{\text{red}}$  pair in  $F_A/F_B$ -depleted PS I isolates the relaxation enhancement solely due to the presence of  $F_X^{\text{red}}$ . A different approach was used in previous relaxation measurements (22) where the enhancement due to  $F_X^{\text{red}}$  was determined in the presence of all three iron-sulfur clusters,  $F_A^{\text{red}}$ ,  $F_B^{\text{red}}$ , and  $F_X^{\text{red}}$ , and it was assumed that the relaxation enhancement due to  $F_A^{\text{red}}$  and  $F_B^{\text{red}}$  is negligible in comparison to  $F_X^{\text{red}}$ . Thus, the spin-lattice relaxation rate enhancement and  $P_{700}^+-F_X$  distance determined previously in the presence of  $F_A^{\text{red}}$  and  $F_B^{\text{red}}$  are approximate values. A comparison between the power saturation data for  $P_{700}^+F_A^{\text{red}}$  and  $P_{700}^+F_A^{\text{red}}F_B^{\text{red}}$  shows that  $P_{700}^+$  relaxation rates in the presence of  $F_A^{\text{red}}$  alone and  $F_A^{\text{red}}F_B^{\text{red}}$  together are comparable. Given that the spin-lattice relaxation rates of the  $F_A^{\text{red}}$  and  $F_B^{\text{red}}$  iron-sulfur clusters are comparable (22) and the two clusters display similar microwave power saturation behavior (J. H. Golbeck, unpublished results), this is direct evidence for  $F_A$  being closer to  $P_{700}$  than  $F_B$ .

Shown in Figure 2B are EPR signals of the iron-sulfur clusters in each of the charge-separated states that identify the individual [4Fe-4S] species present, namely,  $P_{700}^+F_A^{\text{red}}$ ,  $P_{700}^+F_A^{\text{red}}F_B^{\text{red}}$ , and  $P_{700}^+F_X^{\text{red}}$ . Upon comparison with previously published experimental and simulated EPR spectra of iron-sulfur clusters in PS I (1), it can be seen that the samples used in the present study contain high photoinduced yields of the individual charge-separated pairs  $P_{700}^+F_A^{\text{red}}$  (in wild-type ascorbate-treated PS I),  $P_{700}^+F_A^{\text{red}}F_B^{\text{red}}$  (in wild-type dithionite-treated PS I), and  $P_{700}^+F_X^{\text{red}}$  (in  $F_A/F_B$ -depleted PS I). The EPR spectra of  $P_{700}^+F_A^{\text{red}}$  and  $P_{700}^+F_A^{\text{red}}F_B^{\text{red}}$  in Figure 2B exhibit  $g$ -values of 1.86, 1.94, and 2.05 and 1.89, 1.92, 1.94, and 2.05, which are typical of noninteracting  $F_A^{\text{red}}$  and interacting  $F_A^{\text{red}}$  and  $F_B^{\text{red}}$  iron-sulfur clusters, respectively. Furthermore, the absence of a peak at  $g = 1.86$  in part b indicates that there is no contribution from  $P_{700}^+F_A^{\text{red}}F_B^{\text{ox}}$

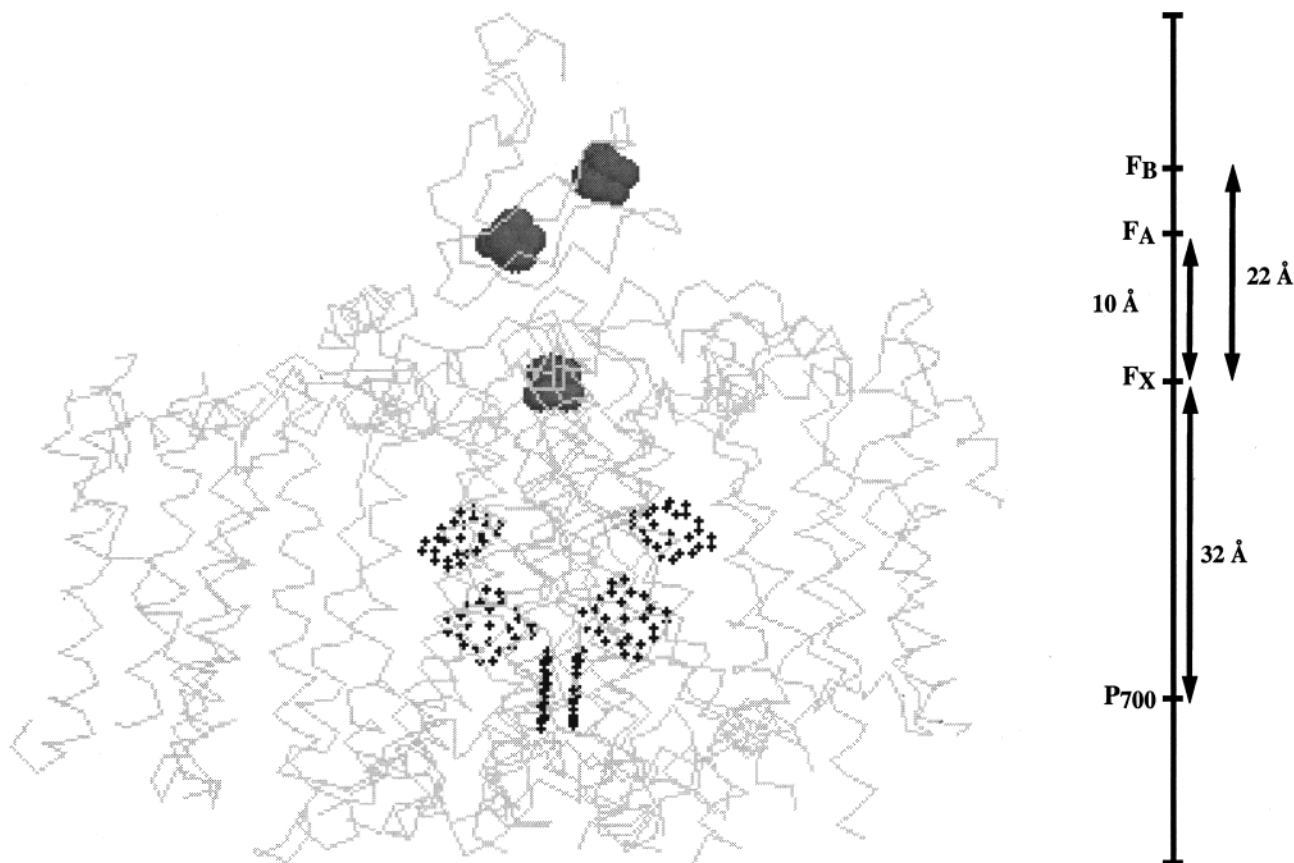


FIGURE 3: A combined model based on the X-ray coordinates<sup>1</sup> (3) of PS I and the arrangement of cofactors based on EPR spin relaxation enhancement studies. The  $P_{700}$   $F_X$ ,  $P_{700}$   $F_A$  and  $P_{700}$   $F_B$  distances are from the X-ray coordinates and the spatial assignment of  $F_A$  and  $F_B$  is based on the  $P_{1/2}$  values calculated in the present study.

charge-separated pairs in the dithionite-treated photoreduced PS I samples.

Shown in Table 1 are the experimental  $P_{1/2}$  values for  $P_{700}^+$  radicals in the presence of  $F_A^{\text{red}}$ ,  $F_B^{\text{red}}$ , and  $F_X^{\text{red}}$ . Also shown are calculated  $P_{1/2}$  values of  $P_{700}^+$  radicals interacting with  $F_A^{\text{red}}$   $F_B^{\text{red}}$  (in dithionite-treated PS I). The  $P_{700}^+$   $P_{1/2}$  value for  $P_{700}^+ F_A^{\text{red}}$  is used as a calibration point in this table for the calculated  $P_{1/2}$  value of  $P_{700}^+ F_A^{\text{red}} F_B^{\text{red}}$ . Using the evidence that the spin-lattice relaxation rates of the  $F_A^{\text{red}}$  and  $F_B^{\text{red}}$  iron-sulfur clusters are comparable (22), the  $r^{-6}$  dependence of the spin-relaxation enhancement (eq 6) and the relative  $P_{700}$ - $F_{A/B}$  distances (42 and 54 Å, respectively) from the X-ray crystallography data (2), we estimate the expected magnitude of spin-lattice relaxation enhancement effects on the  $P_{1/2}$  values of  $P_{700}^+$  in the  $P_{700}^+ F_A^{\text{red}} F_B^{\text{red}}$  charge-separated state. As can be seen in Table 1, the predicted value of  $P_{1/2}$  of  $P_{700}^+$  in the  $P_{700}^+ F_A^{\text{red}} F_B^{\text{red}}$  state is in good agreement with the experimentally determined value when it is assumed that  $F_A$  is closer to  $P_{700}$ . The calculation using the reverse assumption that the  $P_{700}$ - $F_A$  and  $P_{700}$ - $F_B$  distances are 54 and 42 Å, respectively, yields a value that is not in agreement with the experimentally observed  $P_{1/2}$  value obtained in this study.

Single-crystal EPR data on the iron-sulfur clusters,  $F_A$  and  $F_B$ , in conjunction with structural data on bacterial 2[4Fe-4S] ferredoxins and electron density maps from Fromme and co-workers (2, 3) have been used to model the orientation of the PsaC protein that contains  $F_A$  and  $F_B$  (23, 24). Unfortunately, the local pseudo  $C_2$ -symmetry axis inherent

to the ferredoxin model leaves a 2-fold ambiguity in their conclusions. Kamrowski et al. (23) conclude that either  $F_A$  or  $F_B$  could be the proximal to  $F_X$ . Previous spin-lattice relaxation and transient EPR measurements on PS I have focused on the distances between  $P_{700}$ - $F_X$ ,  $A_1$ - $F_X$ ,  $A_0$ - $F_X$  (22, 25, 26), and  $P_{700}$ - $A_1$  (27). The distances determined in these studies mainly address the spatial location of the  $P_{700}$ ,  $A_1$ ,  $A_0$  cofactors relative to the  $F_X$  iron-sulfur cluster in PS I. In the present study, we unambiguously determine the relative spatial orientation of the three iron-sulfur clusters in PS I. On the basis of progressive microwave power saturation data, we conclude that  $F_A$  is the cluster that is proximal to  $F_X$  and  $F_B$  is the terminal cluster.

Figure 3 shows a schematic of the 4.0 Å X-ray coordinates<sup>1</sup> determined by Fromme and co-workers (2, 3). As can be seen in this structure,  $F_X$  is at a distance of 32 Å from the chlorophyll special pair,  $P_{700}$ . Combining the "EPR ruler" obtained from the progressive microwave power saturation data in the present study with the X-ray crystallography structure, we conclude that  $F_A$  is in closer proximity to  $F_X$  and is at a distance of 42 Å from  $P_{700}$  and  $F_B$  is placed such that it is the terminal iron-sulfur cluster in PS I (54 Å from  $P_{700}$ ). Saturation-recovery EPR measurements are currently in progress to measure precise spin-lattice relaxation rates

<sup>1</sup> Coordinates for the theoretical model of the photosystem I reaction center were taken from the protein data bank (28, 29) (entry, 2PPS; N. Krauss, W.-D. Schubert, O. Klukas, P. Fromme, H. T. Witt, W. Saenger, 1996) (2) and were visualized using the software program "Swiss-PDB viewer" (Glaxo Wellcome Experimental Research, Geneva, Switzerland).

of  $P_{700}^{+}$  in the charge-separated pairs and thus determine precise distances between the special pair of chlorophylls and  $F_X/F_A$  using EPR spectroscopy.

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