A High-Field EPR Study of $P_{700}^{+\ast}$ in Wild-Type and Mutant Photosystem I from *Chlamydomonas reinhardtii*†‡

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**ABSTRACT:** High-frequency, high-field EPR at 330 GHz was used to study the photo-oxidized primary donor of photosystem I ($P_{700}^{+\ast}$) in wild-type and mutant forms of photosystem I in the green alga *Chlamydomonas reinhardtii*. The main focus was the substitution of the axial ligand of the chlorophyll $a$ and chlorophyll $a'$ molecules that form the $P_{700}$ heterodimer. Specifically, we examined PsA-H676Q, in which the histidine axial ligand of the A-side chlorophyll $a'$ ($P_A$) is replaced with glutamine, and PsAB-H656Q, with a similar replacement of the axial ligand of the B-side chlorophyll $a$ ($P_B$), as well as the double mutant (PsA-H676Q/PsAB-H656Q), in which both axial ligands were replaced. We also examined the PsA-T739A mutant, which replaces a threonine residue hydrogen-bonded to the 13-keto group of $P_A$ with an alanine residue. The principal $g$-tensor components of the $P_{700}^{+\ast}$ radical determined in these mutants and in wild-type photosystem I were compared with each other, with the monomeric chlorophyll cation radical (Chl$^+$) in photosystem II, and with recent theoretical calculations for different model structures of the chlorophyll $a^+$ cation radical. In mutants with a modified $P_B$ axial ligand, the $g_{zz}$ component of $P_{700}^{+\ast}$ was shifted down by up to $2 \times 10^{-4}$, while mutations near $P_A$ had no significant effect. We discuss the shift of the $g_{zz}$ component in terms of a model with a highly asymmetric distribution of unpaired electron spin in the $P_{700}^{+\ast}$ radical cation, mostly localized on $P_B$, and a deviation of the $P_B$ chlorophyll structure from planarity due to the axial ligand.

The initial electron donor in photosystem I (PS1) is $P_{700}^{•}$, the excited state of a pair of excitonically coupled chlorophyll (Chl) molecules (Chl $a'$ and Chl $a$; see Figure 1), which are coordinated by subunits PsA and PsAB, respectively ($I, 2$). The first stage of electron transfer forms the cation radical $P_{700}^{+\ast}$. The electronic properties of $P_{700}^{+\ast}$ are determined by the nature of electronic states of the oxidized Chl dimer, as well as its interaction with the protein environment. Surprisingly, the 2.5-Å resolution crystal structure of PS1 from *Thermosynechococcus elongatus* ($I$) revealed that $P_{700}$ is not a Chl $a$ dimer, as had been assumed, but a heterodimer of Chl $a$ ($P_A$) and Chl $a'$ ($P_B$), the 13$^\text{2}$-epimer of Chl $a$ (see Figure 1). The side chain of a threonine residue (PsA-Thr739) is in position to donate a hydrogen bond to the 13-keto oxygen. (The numbering system refers to the polypeptides of *Chlamydomonas reinhardtii*.) The hydroxyl group of this Thr residue also seems to participate in a hydrogen bonding network involving a bound water molecule, which donates a H-bond to the 13$^\text{3}$-methyl ester oxygen of $P_A$. This water has three other potential hydrogen bond partners around it: hydroxyls of PsA-Ser607 and the backbone oxygen of PsA-Gly739. However, $P_B$ has no comparable H-bond network. Thus, $P_{700}$ is fundamentally asymmetric, and the H-bond to the 13$^\text{3}$-keto oxygen would seem to be the most important functional feature of this asymmetry, since this keto group is part of the conjugated $\pi$ system. These structural features would seem to be consistent with electron nuclear double resonance (ENDOR) measurements of $P_{700}^{+\ast}$ that estimated a $g_{zz}$ localization of unpaired electron spin on $P_B$ ($3$). However, Fourier transform infrared (FTIR) difference spectra of $P_{700}$ have been interpreted in terms of a model in which the positive charge is shared equally between the two Chls ($4$) (see ref $5$ for an alternate interpretation).

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‡ Protein Data Bank entries 1PSS (RC of *Rhodobacter sphaeroides*, 1JB0 (PS1 of *Thermosynechococcus elongatus*), and 1PPR (peridinin—chlorophyll protein of *Amphidinium carterae*) were used.

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† Abbreviations: BChl, bacteriochlorophyll; Chl, chlorophyll; ENDOR, electron nuclear double resonance; EPR, electron paramagnetic resonance; FTIR, Fourier transform infrared; hfc, hyperfine coupling; HF-EPR, high-field (frequency) EPR; $P_A$, chlorophyll $a'$ on the A-side of $P_{700}$, $P_B$, chlorophyll $a$ on the B-side of $P_{700}$, PS1, photosystem I; PS2, photosystem II; RC, reaction center; WT, wild type.
The P700 caused shifts of proton hyperfine couplings (hfcs) to the lines. Bratt hyperfine couplings that would otherwise broaden the EPR deuteration of the sample, which eliminates the large proton GHz. Use of a sufficiently high field and/or frequency allows deuterated PS1 from environment is that of the axial ligand and the central hole was primarily localized on P B. Interestingly, mutation of the axial ligands could perturb P700 (6-8). While mutation of PsaB-His656 caused shifts of proton hyperfine couplings (hfcs) to the P700** radical cation, as seen by ENDOR spectroscopy, mutation of the ligand to P A had almost no effect upon the ENDOR spectrum (6, 8). Thus, it was concluded that the hole was primarily localized on P A. Interestingly, mutation of PsaA-His656 to Gln did have a noticeable effect upon the P700*/P700 FTIR difference spectrum in the region assigned to the 13^-keto group (7, 9). Mutations of axial ligands on either side had significant effects upon the P700*/P700 visible difference spectrum and increased the midpoint potential of P700*/P700 (7, 8).

The use of high-frequency, high-field EPR enables the complete resolution of the g-tensor of slightly anisotropic radicals, such as organic cofactors. Prisner et al. (10) were the first to completely resolve the g-tensor of P700**, using deuterated PS1 from T. elongatus and a frequency of 140 GHz. Use of a sufficiently high field and/or frequency allows resolution of the g-tensor without having to resort to deuteration of the sample, which eliminates the large proton hyperfine couplings that would otherwise broaden the EPR lines. Bratt et al. (11) reported the complete resolution of the P700** EPR spectrum at 330 GHz using protonated PS1 from spinach. They found essentially identical g-tensor principal components as in the previous study, but also found a temperature dependence for g_e, indicating that the P700** radical may become more anisotropic at lower temperatures. In this study, we have used high-field EPR to resolve completely the g-tensor components of P700** in wild-type PS1 and four mutants in which amino acids serving as axial ligands or H-bond donors have been targeted.

**EXPERIMENTAL PROCEDURES**

The axial ligand mutants (7) and the PsaA-T739A mutant (5) have been described. Isolation of thylakoid membranes from C. reinhardtii was performed as described previously (7). To generate the P700** radical, concentrated thylakoid membranes were mixed with a small amount of ferricyanide in a Teflon cup and illuminated with strong white light for 10 s before being placed in liquid nitrogen under continuous illumination. The Chl** sample has been described previously (12) and was remeasured at the same time as the P700** samples.

The HF-EPR spectrometer, as well as a procedure for simulation spectra, has been described previously (13). Spectra were recorded at a magnetic field of 11.6–11.7 T and a temperature of 10 K. We used a P-doped Si standard ([P] ~ 10^15 spins/cm^3) for field calibration and measurement of sample g-tensor principal values. Spectra of P700** were recorded before and after addition of the g standard; the standard was added through the waveguide tube so that the sample would not be moved. The P-Si standard g value was calibrated using Mn(II) in MgO at 240 GHz. We have used the effective g value of Mn^2+ in MgO standard (g_eff = 2.00101 ± 0.00005 (14)) and obtained the g value of our reference P-Si standard (g = 1.99854 ± 0.00005), which is in good agreement with the literature value of 1.99850 ± 0.00010 (15). The simulations were fits to the data made via a home-written program that allows for an admixture of dispersion and absorption, and optimization of the g values and line widths. All fits were done using a Gaussian line shape only. In some spectra, we observed lines from Mn(II) contaminating the samples. When possible, these were simulated as well (not shown).

**RESULTS AND DISCUSSION**

Figure 2 shows the high-field EPR spectra recorded for C. reinhardtii P700** cation radicals from the wild type (WT), from single mutants PsaA-T739A, PsaA-H676Q, and PsbB-H656Q, and from double mutant PsaA-H676Q/PsbB-H656Q. All spectra were simulated, and the simulation parameters are given in Table 1.

When the g values for the wild-type and mutant PS1s were compared, a relationship was clearly seen between the side of the P700 radical perturbed by the mutation and the g_z component. Only mutation of the axial ligand to P B (PsaB-H656Q and PsaA-H676Q/PsbB-H656Q mutants) caused a significant shift in the g_z value. The slight differences between the g_z value in the P A mutants (PsaA-H676Q and PsaA-T739A) and in WT were within the limits of experimental error.

Results of previous ENDOR studies supported the model in which the unpaired electron spin distribution within the P700** radical cation is strongly localized to P B (see ref 16 for a recent review). The structural inequivalence of P A and P B is determined first by the difference in their configurations, with P A being the 13^-epimer of Chl a. Second, P A accepts hydrogen bonds from side chains of PsaA-ThrA739 and PsaA-Tyr731 and from a water molecule, while there are no hydrogen bonds to P B. The electronic states of P700**...
are also perturbed by interaction of the Chls with their axial ligands, and the perturbations may not be equal across the two sides. These structural and environmental differences are thought to influence the spin density distribution in P$_{700}^{\cdot\cdot}$ such that most of it is localized on one of the P$_{700}$ Chls, consistent with the results of hyperfine spectroscopy (3, 17–19). The fact that mutation of the P$_{B}$ axial ligand affected the P$_{700}^{\cdot\cdot}$ $^1$H ENDOR spectrum (primarily by increasing the hfcs assigned to the 12-methyl protons), while mutation of the P$_{A}$ axial ligand had no effect, was used to identify P$_{B}$ as the chlorophyll on which the spin was localized in P$_{700}^{\cdot\cdot}$ (6, 8). However, one might well ask what the assignment would have been had mutants in the H-bond donor (PsaA-Thr739) been the first ones analyzed, as mutation of this residue caused a decrease in the same hfc (20, 21). It is not straightforward to rationalize the effect of a mutation on one side of P$_{700}$ (13$^1$-keto group of P$_{A}$) upon the hfc from a group on the other side (12-methyl group of P$_{B}$). Although a decrease in the hfc from the 12-methyl protons of P$_{B}$ is expected if spin density is redistributed from P$_{B}$ to P$_{A}$ after removal of the H-bond to P$_{A}$, one might also expect a decrease in all the hfcs assigned to P$_{B}$ and a corresponding increase in the hfcs assigned to P$_{A}$; these were not observed (20, 21). While one might argue that “indirect effects” could explain this, such an argument would undermine the claim that ENDOR spectroscopy is the “method of choice” for obtaining information about spin localization. By its nature, hyperfine spectroscopy will be sensitive to differences in local spin density; however, the g-tensor is more of a global measure of spin distribution within the system, and high-field EPR is therefore a complementary method for examining environmental effects upon a radical. On the basis of our analysis of mutations in the axial ligands to P$_{A}$ and P$_{B}$ and in the H-bond donor to the 13$^1$-keto of P$_{A}$, we also conclude that the spin is strongly localized on P$_{B}$, consistent with the previous conclusions from hyperfine measurements.

Mutation of the P$_{B}$ axial ligand provoked a decrease in $g_{z\cdot\cdot}$, while mutation of the axial ligand or H-bond donor to P$_{A}$ had no large effect. While we can explain the specificity of the effect by localization of the spin on P$_{B}$, the effect itself (shift of $g_{z\cdot\cdot}$) requires some discussion. Previously, changes in $g_{\alpha\cdot\cdot}$ caused by mutation of H-bond donors have been observed and theoretically explained (see ref 22 for a recent example). To the best of our knowledge, this is the first time that a shift in $g_{z\cdot\cdot}$ of a cofactor radical caused by a mutation in a nearby residue has been observed, and it was not expected. We now turn to a discussion of this effect and its possible origins.

It is known from density functional theory (DFT) quantum chemical calculations that the optimized structure of Chl $\alpha$ is very planar in vacuo (23, 24). However, this planarity is seriously perturbed in P$_{B}$ (2, 25) due, at least in part, to its interaction with the histidine axial ligand. In P$_{B}$, the position of the central Mg(II) deviates from the mean plane of the four nitrogen atoms by 0.4 Å (see Figure 3A). There is also a significant deviation of atoms 13$^1$ and 13$^2$ of ring V from the macrocycle mean plane. Moreover, the peripheral vinyl group at position 3 (see Figure 1) is also rotated perpendicular to the main plane of the macrocycle. This same dihedral angle characterizes the position of the acetyl group in the P$_{B}$ bacteriochlorophyll found in the X-ray structure of the P$_{655}$ special pair in reaction centers from Rhodobacter sphaeroides [PDB entry 1PSS (26)]. This out-of-plane position of the 3-vinyl group of P$_{B}$ might explain the tilt between the $z$-axis of the P$_{700}^{\cdot\cdot}$ g-tensor and the molecular $z$-axis of P$_{B}$ (27). The study of the spin-correlated P$_{700}^{\cdot\cdot}$+$^1$H ENDOR radical pair by Zech et al. (27) preceded publication of the high-resolution PS1 structure (1); they expected that the 3-vinyl group would be in plane, since it could not participate in hydrogen bonding, unlike the 3-acetyl group of BCHl. A similar explanation was put forward (28) for the tilt between the $z$-axis of the g-tensor of P$_{655}^{\cdot\cdot}$ and the molecular $z$-axes of the BCHls (29).

The perturbation of the planarity of the electronic $\pi$-system could explain changes in $g_{z\cdot\cdot}$. In the PsaB-H656Q mutant (and PsaA-H676Q/PsaB-H656Q), we might expect a return of P$_{B}$ toward a more planar structure after mutation of the axial ligands.
ligand histidine. Therefore, in accordance with the argument described above and assuming that in \( P_{700}^{+\ast} \) the unpaired electron is mostly on \( P_b \), \( g_z \) of \( P_{700}^{+\ast} \) in WT should be different from \( g_z \) of \( P_{700}^{+\ast} \) in mutants of the axial ligand of \( P_b \).

The deviation of \( Chl \ a^{+\ast} \) from planarity would shift \( g_z \) from the value in planar \( Chl \ a^{+\ast} \). There is a question of which kind of deviation would contribute most heavily to the shift of \( g_z \): the deviation of Mg(II) from the macrocycle plane or the vinyl group rotation. There was a difference of \( 22 \times 10^{-3} \) in the values of \( g_z \) calculated by RHF-INDO (13), based on either the optimized \textit{in vacuo} structure of \( Chl \ a \) or the chlorophyll in the peridinin–chlorophyll antenna protein from \textit{Amphidinium carterae} (30) used as a model for a monomeric Chl species (see Table 1). Examination of the \( A. carterae \) structure reveals that, unlike the \textit{planar in vacuo} structure, this chlorophyll actually deviates from planarity due to axial ligation by a water molecule of the central Mg(II) such that its position deviates from the mean plane of the four nitrogen atoms by 0.36 Å (Figure 3B), although the 3-vinyl group remains in plane. The same effect of deviation of Mg(II) from the mean plane of chlorophyll and bacteriochlorophyll cation free radical models was shown in a DFT study of axial Mg ligation by water molecules (31).

Water molecules also serve as axial ligands to the central Mg(II) of both accessory chlorophylls in PS1 (32), and they exhibit a similar deviation from planarity (Figure 3C). Because of the similar deviation of Mg(II) observed in both \( P_b \) of PS1 and the antenna chlorophyll of \( A. carterae \), we might expect a similar value for \( \Delta g_z \) in \( P_{700}^{+\ast} \) of WT PS1, assuming that the unpaired electron in \( P_{700}^{+\ast} \) is mostly localized on one of the Chls. The maximal experimental difference that we observed is \( \sim 15 \times 10^{-5} \) (Table 1), which is not far from the theoretical estimation of \( 22 \times 10^{-5} \) by RHF-INDO (13).

It should be noted that the effect observed here consists of not only a shift of the \( g_z \) component but also a conservation of the \( g_{xx} \) and \( g_{yy} \) values. As we can see from the data in Table 1, the RHF-INDO calculations (13) did not predict conservation of the \( g_y \) component. One possible reason for that is a difference in vinyl group orientation; it is in plane in the antenna chlorophyll of \( A. carterae \) and out of plane in \( P_b \) of PS1. However, it is also possible that semiempirical calculations (13), and the theory (33) on which these methods are based, are not always sufficiently reliable. The effects on \( g \) factor calculations should also be considered at the DFT level of theory (34–37). Although application of DFT to \( g \) factor calculations of large systems suffers from quantitative inaccuracy, it can be quite successful in prediction of general trends. In recent \( g \) factor DFT calculations (37) for a model of the BChl cation radical with perturbed planarity of the electronic \( \pi \)-system due to the rotation of the 3-acetyl group, variation of the \( g \)-tensor anisotropy (as measured by \( \Delta g = g_{xx} - g_z \)) was observed. Variation of \( \Delta g \) during such rotation was within the limits of \( \sim 170 \times 10^{-5} \), with a maximum value at \( \sim 30–40^\circ \) out of plane, and shifts in \( g_z \) mostly accounted for this variation. It is difficult to make a semi-quantitative estimation of that effect in our case based on the results published in ref 37, because there are many undetermined factors that can influence the result. For example, the data published in ref 37 do not allow one to determine the relative contribution of paramagnetic (spin–orbital/orbital Zeeman) and diamagnetic (relativistic mass correction and gauge correction) terms. In the recent very precise DFT

**Table 1:** Experimental \( g \)-Tensor Components of Primary Donor Cation Radical \( P_{700}^{+\ast} \) in Wild-Type and Mutant Photosystem I of \( C. reinhardtii \) and Theoretical \( g \)-Tensor Components for Model \( Chl \ a^{+\ast} \) Structures

<table>
<thead>
<tr>
<th>Species</th>
<th>( g_{xx} )</th>
<th>( g_{yy} )</th>
<th>( g_{zz} )</th>
<th>( g_{xx} - g_{yy} )</th>
<th>( g_{zz} - g_{xx} )</th>
<th>( g_{zz} - g_{yy} )</th>
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</thead>
<tbody>
<tr>
<td>WT</td>
<td>2.00304</td>
<td>2.00262</td>
<td>2.00220</td>
<td>84.7</td>
<td>41.6</td>
<td>43.1</td>
</tr>
<tr>
<td>( PsbA-H676Q )</td>
<td>2.00305</td>
<td>2.00260</td>
<td>2.00224</td>
<td>80.4</td>
<td>45.1</td>
<td>35.3</td>
</tr>
<tr>
<td>( PsbB-H656Q )</td>
<td>2.00301</td>
<td>2.00260</td>
<td>2.00205</td>
<td>96.2</td>
<td>40.8</td>
<td>55.4</td>
</tr>
<tr>
<td>( PsbA-H676Q/PsbB-H656Q )</td>
<td>2.00300</td>
<td>2.00261</td>
<td>2.00210</td>
<td>89.8</td>
<td>38.3</td>
<td>51.5</td>
</tr>
<tr>
<td>( PsbA-T739A )</td>
<td>2.00301</td>
<td>2.00255</td>
<td>2.00220</td>
<td>80.3</td>
<td>45.9</td>
<td>34.4</td>
</tr>
<tr>
<td>( Chl ) c2 ( \rightarrow ) (PS2) ( \rightarrow )</td>
<td>2.00304</td>
<td>2.00252</td>
<td>2.00213</td>
<td>91</td>
<td>52</td>
<td>39</td>
</tr>
<tr>
<td>( Chl \ a^{+\ast} ) model a</td>
<td>2.00291</td>
<td>2.00258</td>
<td>2.00197</td>
<td>94</td>
<td>33</td>
<td>61</td>
</tr>
<tr>
<td>( Chl \ a^{+\ast} ) model b</td>
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<td>2.00278</td>
<td>2.00219</td>
<td>71</td>
<td>12</td>
<td>59</td>
</tr>
</tbody>
</table>

- \( a \): Experimental error of \( 3 \times 4 \times 10^{-5} \). The difference \( (g_z - g_y) \) is multiplied by a factor of \( 10^4 \). From ref 12. \( b \): This represents a structure of chlorophyll optimized in the \( \textit{in vacuo} \) (13). This represents a structure of a monomer chlorophyll in \( A. carterae \) (13).

**Figure 3:** Structures of various chlorophylls with distorted planarity. In each case, the chlorophyll is shown from the side as a ball-and-stick model using CPK colors (central Mg in green). (A) \( P_b \) of \( T. elongatus \) PS1 (molecule 1012 of PDB entry 1JB0), including the axial ligand, \( PsbA-His660 \) (homologous with His656 of \( C. reinhardtii \)). (B) Antenna chlorophyll in the peridinin–chlorophyll protein of \( A. carterae \) (molecule 601 of PDB entry 1PPR), including the water molecule serving as the axial ligand, coordinated by His66. (C) Chlorophyll ec2A of \( T. elongatus \) PS1 (molecule 1021 of PDB entry 1JB0), including the water molecule serving as the axial ligand, coordinated by \( PsbA-Asn591 \).
calculations of the g-tensor for quinones (35), it was shown that the contribution to the $g_{zz}$ component could range from domination by the spin–orbit coupling term to domination by the diamagnetic term for different quinones and model solvated complexes of quinones. At the same time, at the qualitative level, we might expect a similar effect for the shift of $g_{zz}$ in $P_B$, as well as conservation of $g_{xx}$ and $g_{yy}$ due to the rotation of the 3-vinyl group, as was seen for rotation of the 3-acetyl group in $BChl$ +. However, because of the difference in spin–orbit coupling constants between the acetyl oxygen in $BChl$ and the vinyl carbon in Chl $a$ (33), we might also expect that the magnitude of $\Delta g_{zz}$ caused by rotation of the corresponding substituent at position 3 would be smaller in Chl than in $BChl$.

Thus, a model of localization of unpaired electron spin in the $P_{700}^{**}$ cation radical on $P_B$, coupled with its nonplanar structure, may reasonably explain the experimentally observed negative shift of $g_{zz}$ seen in the PsABH656Q mutant by a return of $P_B$ toward a planar (or near-planar) structure after mutation of the axial ligand histidine. Small changes in the distribution of unpaired electron spin between $P_A$ and $P_B$, due to changes in the protein environment, might also change the energy difference between the two chlorophylls in $P_{700}^{**}$, with a resulting influence upon the reduction potential of $P_{700}^{+}/P_{700}^{**}$ (38, 39) and $\Delta g_{zz}$, but these are likely less significant. Our conclusions on the differences between the $g_{zz}$ components of $P_{700}^{**}$ in PsABH656Q and PsAAH676Q/PsABH656Q (as well as among WT, PsAAH676Q, and PsAA-T739A) are limited by the error of measurement of the $g$-tensor principal values with respect to the reference sample $(\pm 3 \times 10^{-4})$. It should also be noted that the quality of the spectrum for the double mutant was lower than the others, due to the $\sim$10-fold reduction in the $P_{71}$ content of this mutant (7).

In Figure 2, the $P_{700}^{**}$ EPR spectra are also compared to that of Chl$Z^{**}$ from PS2 (12, 40, 41), which has been used as a representative monomeric chlorophyll cation radical. We note that the $g_{zz}$ value of Chl$Z^{**}$ is similar to that of $P_{700}^{**}$ in the mutants affecting the axial ligand of $P_B$. However, the relevance of this fact is uncertain, as the current limited resolution of the PS2 structures (42, 43) precludes a similar analysis of this Chl in terms of its planarity or coordination. Similar observations of a difference in the $g_{zz}$ values of $P_{700}^{**}$ and Chl$Z^{**}$ have been made by Poluektov and colleagues (44) in their study of the electronic structure of these radicals in deuterated PS1 and PS2 at 130 GHz; they found that the $g_{zz}$ of $P_{700}^{**}$ was higher than that of Chl$Z^{**}$ by $21 \times 10^{-5}$. Our data are in agreement with their results, except for a shift in the absolute $g$ values, which is due to the use of a different $g$ value for Mn(II) in the MnO/MgO standard.

CONCLUSIONS

In this work, we report for the first time the effect of mutations upon the high-field EPR (330 GHz) spectra of the $P_{700}^{**}$ radical cation. The only mutations to cause significant changes were those that affected the axial ligand of $P_B$, which allowed an independent identification of $P_B$ as the spin-carrying chlorophyll of $P_{700}^{**}$, consistent with prior interpretations of hyperfine spectroscopy. Furthermore, contrary to expectations, we have observed shifts in the $g_{zz}$ component caused by mutation of the $P_B$ axial ligand. Comparison of these results with those of theoretical estimations of the $g$ factor for different models of the Chl $a^{**}$ radical cation led us to explain the changes in the $g_{zz}$ component in terms of a deviation of the Chl structure from planarity.

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