Exploring Electron Spin−Spin Interactions of Paramagnetic Iron and Radical Cations of Bacteriochlorophyll from Oxidized LH1 in the Presence of Electron Transfer in the Frozen State†

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Cation free radicals of bacteriochlorophyll ($BChl^+$) are formed in the light harvesting complex 1 (LH1) of photosynthetic bacteria upon oxidation by potassium ferricyanide. Unusually narrow EPR line widths are observed for $BChl^+$ in the frozen state. These narrow line widths are consistent with a molecular-wire behavior where rapid electron/hole transfer occurs between the BChl constituents of the pigment array responsible for light harvesting in bacterial photosynthesis. However, in addition to electron/hole transfer, two distinct types of spin−spin exchange could contribute the EPR line width narrowing, thus obfuscating the determination of LH1 as a molecular wire. First, because excess ferricyanide ion is always present during the EPR measurements, electron spin−spin interactions between the paramagnetic ferricyanide and $BChl^+$ could be a major source of the EPR line width changes previously attributed solely to electron/hole hopping within the array of BChl molecules in a LH1 unit. Fixing the potential of the ferricyanide/ferrocyanide redox couple gives a constant concentration of paramagnetic iron as the amount of BChl oxidized in LH1 changes. As long as the fraction of oxidized BChl in LH1 remains the same, the EPR line width is found independent of the concentration of the ferricyanide oxidant. Additionally, the trend in EPR line width as a function of temperatures depends only on the fraction of oxidized BChl and not on the concentration of ferricyanide ion. Second, spin−spin exchange interactions between $BChl^+$s within LH1 rings could also change the EPR line width. Using LH1 preparations containing at most a few BChl cations per LH1 complex also eliminates the occurrence of significant electron spin−spin exchange as a cause of the observed line width narrowing in minimally oxidized LH1. This investigation of the two types of electron spin−spin exchange interactions demonstrates (1) that electron/hole hopping can take place in oxidized LH1 without involvement of paramagnetic ferricyanide or spin−spin exchange between $BChl^+$s and (2) that LH1 maintains a molecular-wire nature at cryogenic temperatures.

Introduction

Light harvesting complex 1 (LH1) of *Rhodobacter sphaeroides*, also known as photosynthetic antenna, is an integral membrane protein containing bacteriochlorophyll pigments (BChls). LH1 constitutes either an open or closed ring that surrounds the photosynthetic reaction center (RC) protein of purple photosynthetic bacteria.[1−10] Altogether, a total of about 28 membrane-spanning helices, in pairs of two, anchor the BChls to form the complete LH1 of *Rb. sphaeroides*.[6] The photosynthetic role of the LH1 complex is to absorb photons and transfer the energy to the core RCs where the initial charge separation occurs. Energy transfer occurs without photo-oxidation of BChl so that the integrity of the light harvesting protein complex is maintained. Consequently, during the energy transfer process, the LH1 remains diamagnetic. Although visible and near-infrared light cannot photo-oxidize LH1, appropriate chemical oxidation even in the absence of light can produce BChl radical cations readily observable by conventional continuous wave EPR.[2]

Recent research on the LH1 structure using an 8.5 Å resolution cryo-EM projection map revealed that LH1 is a closed S-shape molecule composed of two rings attached to each other containing 28 α/β $BChl_2$ subunits.[6] Each ring has a diameter of about 125 Å and possesses about 16 $\alpha\beta$ $BChl_2$ subunits where several subunits are shared between rings. Given that the ring size of the circular part of LH1 has a diameter of about 125 Å, the approximate distance between closest BChls in LH1 must be about 11 Å. Such a short distance between BChls is consistent with not only very fast energy migration, as required by the photosynthetic process, but also relatively slower electron/hole transfer within an array of BChls in the oxidized LH1 complex. Of course, only the diamagnetic energy transfer process is intrinsic to the native process of photosynthesis. However, if electron/hole migration occurs in chemically oxidized LH1 containing BChl cation free radicals, then LH1 is of special interest because the protein complex constitutes a molecular wire in the sense as defined by Ratner et al.[11]

Using conventional continuous wave EPR, Gingras et al.[2] investigated electron transfer in the purple photosynthetic bacterium *Rhodospirillum rubrum* after chemical oxidation of a fraction of the bacteriochlorophyll (BChl) contained in LH1.

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A single, approximately Gaussian line shape with a line width of 3.8 G was observed for oxidized LH1 at room temperature.[1,3−5,7−10] The EPR line width of LH1 was considerably narrower than that of a monomeric free radical of BChl. Gingras et al. concluded that the EPR line narrowing was the result of electron/hole migration or hopping within the array of BChl associated with the LH1. Using the same model that explained the narrow EPR line width of the primary donor special pair dimer of the photosynthetic RC,[12] they concluded that LH1 contained six units of dimeric BChla.[2]

Additional studies of LH1 at low temperature have revealed that the narrowed EPR line width of oxidized LH1 remains in the frozen state but monotonically increases with decreases in temperature. It was proposed that the monotonic line width increase with decreases in temperature occurs because the electron/hole hopping rate slowed in the LH1 system, as expected by Marcus theory.[13−16] Another observation was that at constant temperatures below about 250 K, the EPR line width decreases with increased oxidation, raising the possibility that Heisenberg spin exchange is responsible for the narrowing of the EPR line width.

EPR line width narrowing in polymers is not limited to LH1. At the extreme of strong coupling among the members of a paramagnetic polymer,[17−20] the electron spins are extensively delocalized, resulting in narrow EPR line widths.[20] In contrast to LH1, a hopping model is probably not appropriate for describing the EPR spectra of these polymers. Susumu et al.[20] have shown in an elegant study of the cation of meso-to-meso ethyne-bridged (porphinato)zinc(II) oligomers that the EPR line width is constant from 300 to 8 K. The constant EPR line width as a function of temperature is indicative of the extensive delocalization associated with the strong coupling limit. These covalently linked models are important to the field of molecular wires and illustrate extreme delocalization of electron spins, an example far from the hopping process appropriate for LH1.

In contrast, in the weak coupling case, the EPR line width varies considerably as a function of temperature. As examples of the weak-coupling limit other than LH1, changes in the EPR line width have also been observed in oxidized, covalently linked arrays of porphyrins.[21,22] Seth et al. chemically synthesized artificial light harvesting arrays of porphyrins and explored their oxidized states using EPR.[21,22] In liquid solution, the EPR spectral envelope was narrowed in width compared to the EPR line width of the monomer cation radical and was easily explained by the model[12] used to describe the EPR of the RC.[22] Their results demonstrated that rapid hole/electron hopping occurred among monomer members of the oxidized array. As revealed by the line width reverting to that of the monomer, in the case of artificial antenna, the changes in EPR line width attributable to electron/hole migration essentially stopped when the solvent was frozen. In certain limited cases, the line width remained narrow and was explained by the occurrence of electron spin−spin exchange interactions. However, when the solution containing LH1 was frozen, surprisingly, the EPR line width remained narrower than the line width of monomeric $BChl^+$ cation free radical even at temperatures as low as 10 K.[13−15] Such line narrowing indicates that electron transfer in LH1 works in the frozen state and that LH1 is exhibiting molecular-wire properties different from those of oxidized solid solutions of covalently linked porphyrin arrays. In all these investigations, oxidation of the LH1 complex was accomplished using paramagnetic potassium ferricyanide, $K_3Fe(CN)_6$, raising the possibility that the decrease of line width could also arise from spin−spin exchange between paramagnetic ferricyanide and $BChl^+$, or as in the covalent arrays, between cation free radicals residing on the same array fragment. Thus the EPR line width change would not solely be the result of electron/hole transfer in the frozen state. As another possibility, perhaps the ferricyanide acts in some superexchange capacity that promotes electron transfer in the solid state.

Consequently, determining the role this paramagnetic oxidizing agent plays in the EPR line narrowing process is necessary to certify the molecular-wire nature of LH1. In principle, a simple means to determine if ferricyanide contributes to the EPR line width narrowing is to use a diamagnetic oxidizing agent or at least a different paramagnetic oxidizing agent. However, the choice of oxidizing agent is limited by the labile nature of BChl, other pigment molecules and the protein found in LH1. In addition, because LH1 must function in photosynthesis without photo-oxidation occurring, the BChl of the LH1 is difficult to oxidize. As a result, the LH1 complex exhibits some instability under the redox extremes necessary to oxidize the BChl. For these reasons the chemical oxidation of the BChls of the LH1 has been almost exclusively accomplished using potassium ferricyanide. This high redox potential of BChl in LH1 requires excess ferricyanide and therefore results in the presence of a significant amount of unreacted ferricyanide in the EPR experiments.

Ferricyanide is paramagnetic while ferrocyanide is not. Numerous studies exist on the interactions between paramagnetic ferric ions in porphyrins and enzymes with spin labels such as nitroxide radicals.[23−25] For example, low spin ferric ion, a paramagnetic species with fast electron spin−lattice relaxation time (T_1), interacts with other electron spins that are within about 15 Å. Accordingly, the T_1 of the nearby electron spins can also change. When $1/T_1$ is equal to or greater than the electron−electron spin−spin splitting, the splitting will get narrower and coalesce to show partially resolved splitting even at 4 K.[25] In view of that, direct interaction between paramagnetic ferricyanide iron and $BChl^+$ and interaction between BChl cations induced by the presence of nearby iron can potentially contribute to the mechanism by which the EPR line width changes.

In previous studies, to decrease the observed EPR line width, the concentration of potassium ferricyanide was always increased to boost the amount of oxidized BChl in LH1. This oxidation procedure always increased the amount of paramagnetic ferricyanide and BChl radicals at the same time and in principle the change in paramagnetic iron concentration could affect or even control the LH1 line width. That such spin−spin interactions induced by paramagnetic species other than the oxidized $BChl^+$ could influence the EPR line width was not adequately ruled out in previous studies. In this study, to investigate the potential role of ferricyanide in the EPR line narrowing process for oxidized LH1, the ferricyanide/ferrocyanide ratio was changed while the concentration of ferricyanide remained constant. If the concentration of ferricyanide is fixed, changes in the EPR line width at a particular temperature must arise from mechanisms not dependent on ferricyanide concentration.

The consequences of the EPR study based on a fixed ferricyanide concentration have specific as well as broader implications. In the scope of oxidized LH1, the results of this study are to determine if the physical process behind the EPR line width narrowing with increasing temperature in oxidized LH1 is due solely to electron/hole transfer within the LH1 ring and is independent of the presence of the paramagnetic iron. Clearly, knowing whether the molecular-wire property of LH1 in the frozen state is possible only because of nearby ferricyanide ions is essential. In the broader sense, for similar concentrations,

can spin−spin interactions between low spin ferric iron and oxidized organic doublet species be neglected in the presence of electron transfer in a polymer? To eliminate the involvement of spin−spin interactions between paramagnetic iron and oxidized BChl in the LH1 system, the oxidation of LH1 is conducted as an equilibrium redox reaction between ferric ions and the circular-like array of BChl in the photosynthetic antenna. Once ferricyanide ion is eliminated as a source of EPR line narrowing, the role of spin−spin exchange between $BChl^+$'s in the frozen state in oxidized LH1 is easily pursued. Since spin−spin exchange between $BChl^+$'s depends on the concentration of $BChl^+$, it can be prevented using minimally oxidized LH1. If the EPR line width were to remain narrow in minimally oxidized LH1 and if the ferric ions were known not to be involved, then electron/hole hopping in LH1 would be established at low temperatures.

Experimental Methods

The LH1 protein complex was prepared from the purple bacterium *Rb. sphaeroides puc*705-BA, a strain without the LH2 protein complex. The bacterial culture and isolation of the LH1 complex was conducted by following a procedure from previous work.[13−15] The final, detergent-isolated LH1 was suspended in 10 mM Tris−HCl Buffer pH 7.9, 0.8% BOG, 10 μM EDTA. Radical cations of the LH1 complexes were generated by oxidation with a solution containing both potassium ferricyanide, $K_3Fe(CN)_6$, and potassium ferrocyanide, $K_4Fe(CN)_6$, under ambient conditions. Stock solutions of oxidizing reagent were prepared in 10 mM Tris−HCl Buffer pH 7.9, 0.8% BOG, 10 μM EDTA and used immediately. The oxidation of the BChl in the LH1 protein complexes was accomplished as follows. Solutions containing only potassium ferricyanide were prepared with initial concentrations of 150 or 5 mM. Additionally, new mixed solutions with 150 mM potassium ferricyanide combined with a series of different potassium ferrocyanide concentrations were prepared. Then, 10 μL of the oxidizing solution were added to 290 μL of the LH1 sample in an Eppendorf tube for the preparation of a series of oxidized LH1 samples where the oxidant concentration is diluted 30 times due to the final volume. For example, the concentrations of 150 and 5 mM potassium ferricyanide became 5 and 0.167 mM, respectively. Following this step, the detergent isolated proteins were vortexed for 45 s. After the UV/visible spectra of the oxidized proteins were recorded, the samples were transferred to 4 mm outer diameter Pyrex EPR tubes and immediately frozen in liquid nitrogen. The entire procedure, from the time of the addition of the oxidizing solution until freezing, typically took about 4 min.

Optical absorbance spectra for the LH1 complexes were measured under ambient conditions with a Shimadzu UV-1601 spectrophotometer. Quartz cuvettes of 1 mm path length were used for all measurements. The fraction of oxidized BChl, χ, was determined by the bleaching of the near-IR band characteristic of the BChl bound in the LH1 complex. Specifically, the optical absorbance spectrum at 880 nm was measured before and after oxidation of the complexes giving the fraction oxidized as $\chi = (A_{before} - A_{after})/A_{before} = \Delta A/A_{before}$. The absorbance after oxidization was corrected for the decrease due to dilution. The 5 mM and 0.167 mM potassium ferricyanide solutions yielded 15.25% and 2% oxidized LH1, respectively. A second LH1 sample with 2% fraction oxidized but a different concentration of potassium ferricyanide was prepared by trial and error. Several batches of LH1 complex were treated with mixed solutions containing different amounts of potassium ferricyanide and potassium ferrocyanide. The desired 2% fraction oxidized was achieved by adding 10 μL of the 150 mM potassium ferricyanide and 15 mM potassium ferrocyanide mixed solution to 290 μL of LH1. Including the effect of dilution, the mixed solution had a concentration of 5 mM potassium ferricyanide and 0.5 mM ferrocyanide solution before reaction with the LH1 complex. As a consequence, this second sample with 2% of the BChl oxidized contains a larger concentration paramagnetic ferricyanide than the sample prepared by oxidation only with the 0.167 mM ferricyanide solution.

The continuous-wave EPR measurements were conducted with a Bruker ELEXSYS E580 X-band EPR spectrometer equipped with an NMR gaussmeter and microwave frequency counter. A Bruker Super-High-Q-cavity (ER 4122 SHQE) in conjunction with a helium finger cryostat was used. Temperature control to ±0.1 K was achieved with a continuous flow of liquid helium and an Oxford ITC-403 temperature controller. The microwave power and the magnetic-field modulation amplitude were typically 0.2 mW and 1 G, respectively.

Results and Discussion

As rationalized by crystal field theory, CN^- produces a strong ligand field, which upon binding to ferric iron, forms a paramagnetic low spin complex $Fe(CN)_6^{3-}$ with electron spin $^1/_2$. Upon oxidation of LH1, $Fe(CN)_6^{3-}$ is reduced to $Fe(CN)_6^{4-}$, which is a diamagnetic species. The oxidation potential of potassium ferri/ferrocyanide is comparable to the 0.61 eV versus NHE estimated for the BChl of LH1 from *Rb. sphaeroides*.[26] In the oxidation of LH1, the concentration of ferricyanide far exceeds the concentration of $BChl^+$ produced by the redox chemistry. Since only a very small fraction of ferricyanide reacts to oxidize BChl in the LH1 ring, an excess of paramagnetic ferricyanide relative to $BChl^+$ remains in the sample. The presence of excess ferricyanide is the main issue that gives credence to the possibility that spin−spin interactions between paramagnetic irons and oxidized BChl may contribute to the EPR line width changes observed in oxidized LH1. Two possible types of spin exchange mechanisms involving nearby paramagnetic ferricyanide and BChls are possible. (1) The first mechanism is the direct spin−spin exchange between nearby ferricyanide and $BChl^+$, where the unpaired electron of each molecule flips as illustrated next.

$$BChl_1^+(\downarrow) + Fe^{3+}(\uparrow) \rightleftarrows BChl_1^+(\uparrow) + Fe^{3+}(\downarrow)$$

(2) The second mechanism is the indirect spin−spin exchange between two BChl radicals mediated by the presence of ferricyanide as in the following equation.

$$BChl_1^+(\downarrow) + Fe^{3+}(\uparrow) + BChl_2^+(\uparrow) \rightleftarrows BChl_1^+(\uparrow) + Fe^{3+}(\downarrow) + BChl_2^+(\uparrow) \rightleftarrows BChl_1^+(\uparrow) + Fe^{3+}(\uparrow) + BChl_2^+(\downarrow)$$

In this second mechanism, the presence of fast relaxing paramagnetic ferricyanide reduces the effective separation between two far BChl radicals and makes possible the otherwise unlikely spin−spin exchange between two distant BChl radicals.

To probe the possibility of the spin exchanges above, three oxidized LH1 variants were prepared. In variant A, LH1 was oxidized with 0.167 mM ferricyanide. The resulting fraction oxidized of LH1 was 2%, as detected by its bleached near-infrared spectra relative to the initial unoxidized optical density at 860 nm. In variant B, the LH1 system was oxidized with 5 mM ferricyanide. Hence the concentration of the ferricyanide of variant B is

approximately 30 times higher than variant A. The resulting fraction oxidized of LH1 was measured to be 15%. In the last variant, variant C, LH1 was oxidized using a mixture of 5 mM ferricyanide and 0.5 mM of ferrocyanide. The LH1 fraction oxidized was measured to be roughly 2%, essentially the same as variant A.

In the case of LH1, the basic redox reaction for the oxidation of BChl by ferricyanide ions is

$$Fe^{3+} + BChl \rightleftarrows Fe^{2+} + BChl^{+}$$

where BChl is any one member of the array of BChls in LH1. For discussion purposes it is useful to assume that the equilibrium condition can be described in the usual fashion by $[BChl^+][Fe^{2+}]/[BChl][Fe^{3+}] \equiv K_{EQ}$, where K_{EQ} is the equilibrium constant, $[Fe^{2+}]$ and $[Fe^{3+}]$ are the concentration approximations of the activities of the ferrocyanide and ferricyanide ions, respectively, and $[BChl^+]/[BChl]$ is the concentration ratio of oxidized BChl to unoxidized BChl in LH1. Whether the assumption of a simple equilibrium is valid will be discussed later. When the ferricyanide-to-ferrocyanide ratio is kept constant, the fraction oxidized is also expected to remain constant. Thus, when the total paramagnetic ferricyanide concentration is changed while the ferricyanide-to-ferrocyanide ratio is kept constant, the effect or lack of effect of ferricyanide concentration on the line width of oxidized LH1 can be accessed. Two equivalent solutions of LH1, A and B, are treated with different amounts of potassium ferricyanide. The third variant C is prepared with the same ferricyanide-to-ferrocyanide ratio as in variant A as judged by the fraction oxidized determined by the optical absorption spectra. The same fraction oxidized requires that $[BChl^+]_{A_{EQ}}/[BChl]_{A_{EQ}} = [BChl^+]_{C_{EQ}}/[BChl]_{C_{EQ}}$. From the equilibrium condition it follows that

$$\frac{[Fe^{2+}]_{A_{EQ}}}{[Fe^{3+}]_{A_{EQ}}} = \frac{[Fe^{2+}]_{C_{EQ}}}{[Fe^{3+}]_{C_{EQ}}}$$

For the small fraction oxidized where $\chi = 0.02$, only a small amount of ferricyanide ions reacts to form $BChl^+$ such that at equilibrium $[Fe^{2+}]$ almost maintains its initial value. Since for variant A, the initial ferrocyanide concentration before reaction with LH1 is essentially zero, then after reaction

$$[Fe^{2+}]_{A_{EQ}} = [BChl^+]_{A_{EQ}}$$

such that at equilibrium

$$\frac{[BChl^+]_{A_{EQ}}}{[Fe^{3+}]_{A_{INITIAL}} - [BChl^+]_{A_{EQ}}} = \frac{[Fe^{2+}]_{C_{INITIAL}} + [BChl^+]_{A_{EQ}}}{[Fe^{3+}]_{C_{INITIAL}} - [BChl^+]_{A_{EQ}}}$$

Consequently, for variants A and C

$$\frac{[BChl^+]_{A_{EQ}}}{0.167 \times 10^{-3} - [BChl^+]_{A_{EQ}}{}^{-3}} = \frac{0.5 \times 10^{-3} + [BChl^+]_{A_{EQ}}}{5.0 \times 10^{-3} - [BChl^+]_{A_{EQ}}}$$

Therefore, $[Fe^{2+}]_{A_{EQ}} = [BChl^+]_{A_{EQ}} = 1.57 \times 10^{-5}$ and the assumption that a small amount of ferricyanide reacted is reasonable. Since

$$\chi = \frac{[BChl^+]_{A_{EQ}}}{[BChl]_{A_{INITIAL}}} = 0.02$$

then

$$[BChl]_{A_{INITIAL}} = [BChl]_{B_{INITIAL}} = [BChl]_{C_{INITIAL}} = 50 \times [BChl^+]_{A_{EQ}} = 7.83 \times 10^{-4}$$

Therefore,

$$K_{EQ} = \frac{[BChl^+]_{C_{EQ}}([Fe^{2+}]_{C_{INITIAL}} + [BChl^+]_{C_{EQ}})}{([BChl]_{C_{INITIAL}} - [BChl^+]_{C_{EQ}})([Fe^{3+}]_{C_{INITIAL}} - [BChl^+]_{C_{EQ}})} = 2.111 \times 10^{-3}$$

To evaluate whether the assumption of an equilibrium situation in the oxidation of LH1 is reasonable, the fraction oxidized in variant B is now predicted from the equilibrium constant derived using variants A and C. For variant B, the initial concentration of ferrocyanide is essentially zero. Using K_{EQ} and $[BChl]_{B_{INITIAL}}$ from above along with $[Fe^{2+}]_{B_{INITIAL}} = 5.0 \times 10^{-3}$, the equilibrium equation can be solved for the fraction oxidized giving $\chi_B = 0.11$. The measured χ_B was 0.15. Considering the complexity of the system, the dependence of the ferricyanide/ferrocyanide couple on ionic strength and the lack of an additional redox mediator, the differences between calculated and measured fraction oxidized for variant B is rather good, supporting treating the chemistry as a simple equilibrium system. Regardless, the important result is that the concentration of ferricyanide ion is much larger in variant C than in variant A and essentially equal in variants B and C.

The EPR line widths of the three systems were measured as a function of temperature. The first two variants, A and B, served as benchmarks for upper and lower limits for the third variant C.

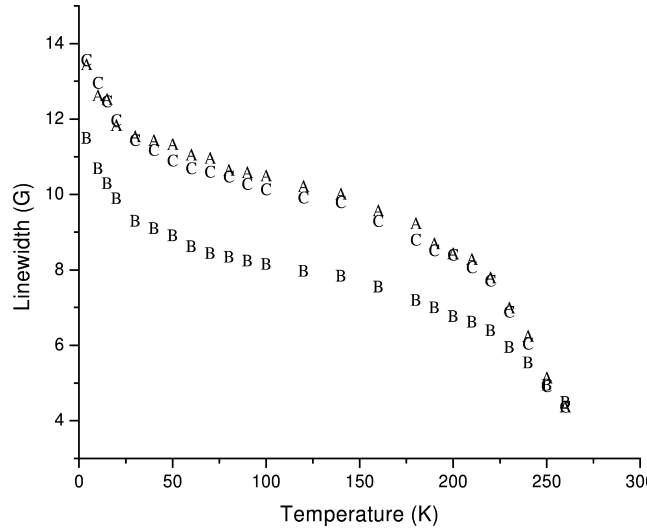

Figure 1. CWEPR line width of oxidized LH1 as a function of temperature for the three systems. (A) is variant A: LH1 oxidized by 0.167 mM ferricyanide yields 2% oxidized BChl. (B) is variant B: LH1 oxidized by 5 mM ferricyanide yield 15% oxidized BChl. (C) is variant C: LH1 oxidized by 5 mM ferricyanide and 0.5 mM ferrocyanide yield 2% oxidized BChl.

Figure 1 displays the dependence of measured CW EPR line widths for all three variants from temperature range 4−250 K. The three variants showed the same line-narrowing trend as the temperature increases. The line widths of variant A, which was the 2% oxidized LH1, are much wider than the line widths of variant B, which was the 15% oxidized LH1. This line width narrowing has been attributed to faster electron transfer in the system. The difference between variant A and B has been explained as follows: the more the LH1 is oxidized, the faster the electron transfer is and/or the greater the spin−spin exchange between $BChls^+$, so the line widths of the more highly oxidized LH1 are narrower. The CWEPR spectra of each of the three systems at four selected temperatures are shown in the Supporting Information. At 4, 40, and 180 K, the EPR line widths of system A are roughly the same as those of variant C, while variant B is significantly different. Only at 260 K are the line widths of the three systems the same as shown previously.

If a notable contribution from iron−BChl interactions affects the EPR line width in addition to electron/hole transfer between pigment molecules, then the line width series of the variant C should not agree with variant A. If the line widths in variant C are due to significant contributions from the direct or induced interactions between paramagnetic irons and oxidized LH1, then the line widths of variant C should be similar to those of variant B. However, variant C, which has a similar amount of ferricyanide left over after oxidation as variant B, exhibits a line width series that is broader than that of the second system. In fact, the line width changes for the entire C series coincide with variant A within experimental error. Therefore, one is forced to conclude that the line width is a function of the fraction of oxidized LH1 that is independent of the amount of ferricyanide used to oxidize BChl in LH1. These results support the view that the main physical process behind the line width change is due to electron dynamics within LH1. No noteworthy contribution to the line narrowing process or electron/hole hopping in the oxidized LH1 due to superexchange or spin−spin interactions between ferric ions and $BChl^+$s is observed.

With the influence of paramagnetic ferricyanide ruled out for EPR line width narrowing, the next task is to determine the role of $BChl^+$−$BChl^+$ spin exchange using the 2% oxidized LH1. The number of spins residing in a LH1 fragment can be approximated by a binomial distribution. The larger the number of BChls per LH1 unit, the greater is the probability of multiple oxidations per complex. The LH1 ring intactness after separation from the reaction center is estimated to range from 15 to 18 α/β polypeptide pairs.[27] Thus the corresponding effective aggregation size for the BChls would range from 30 to 36. Assuming a conservative aggregation size of 32 BChls for the LH1 preparation used in this study, in the case of 2% oxidized LH1, about 52% of the LH1 would remain unoxidized, 34% would have 1 unpaired spin, 11% would have 2 unpaired spins, and 2% would have 3 unpaired spins. The unoxidized LH1 does not give an EPR signal, so about 71% of the EPR signals have to come from the LH1 with 1 unpaired spin and about 23% from the LH1 with 2 unpaired spins and 4% from LH1 having 3 spins. In a system where there are 32 BChl*a*s in an LH1 ring, enough space exists to distribute 2 or 3 spins in an LH1. This distribution for 2% oxidized LH1 leaves the typical LH1 fragment with at most 3 spins. The spin−spin exchange rate, J, is an exponentially decreasing function with distance.[28] Considering electron/hole transfer, the likelihood of two radicals to be close enough for electron spin−spin exchange is small. The closest distance between two $BChl^+$'s in LH1 for which the spin−spin exchange rate would be significant is about 11 Å. Since 54% of the EPR signal originated from LH1, which has only 1 spin in it, the spin−spin exchange between two neighboring BChl cations does not exist. For the other 31% that have 2 spins and 11% that have 3 spins, the probability of two spins or more randomly placed next to each other without the ability to move within the large LH1 is very small. Therefore, the contribution of spin−spin exchange to EPR line width narrowing is expected to be very small. The EPR line widths of the 2% oxidized LH1 is narrowed significantly from the 13.5 G BChl radical monomer line width as the temperature increased from 4 K to about 9 G at 180 K, as illustrated in Figure 1 of the Supporting Information.

Conclusions

Two main experimental findings are evident. First, any interaction that exists between paramagnetic iron and oxidized BChl is not detectable from the EPR line width changes of $BChl^+$ unlike that reported in the case of the interaction between iron and nitroxide radicals. The EPR line width changes of oxidized LH1 are solely due to electron spin dynamics within the LH1 complex and paramagnetic iron is not involved. Second, in 2% oxidized LH1, spin−spin exchange between $BChl^+$s is negligible such that electron transfer dominates the spin dynamics. Clearly electron/hole transfer is the dominant source of the EPR line width narrowing for minimally oxidized LH1 in the frozen state and at cryogenic temperatures. Consequently, the energetic barrier to electron transfer within the BChl array in LH1 must remain low in the frozen state, indicating that freezing does not significantly alter the structure of the protein surroundings of the BChls and $BChl^+$s and that the electron transfer process on the average is characterized by $\Delta G = 0$. In other words, upon going from the liquid state to the solid state, the BChl cation is not energetically confined or trapped at a single site within the protein complex. However, the more dynamic and randomly oriented organic solvent that surrounds the covalently linked porphyrin arrays does energetically trap the cations upon freezing. In ordinary liquid solutions, a significant energetic barrier must be overcome for electron/hole migration to occur and therefore molecular-wire properties are severely diminished. In contrast, the existence of low temperature electron/hole transfer in LH1 suggests that the repetitive and relatively rigid nature of the protein provides for a fairly uniform and homogeneous environment where $\Delta G \cong 0$ for the electron/hole transfer between BChls. This uniformity and rigidity tends to prevent energetically trapping a $BChl^+$ at a single location, especially when going from the liquid state to the frozen state. In this view, the protein structure, which serves as the "solvent" for the BChl, provides the crucial factor for maintaining molecular-wire properties in oxidized LH1 in the frozen state and at cryogenic temperatures.

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Supporting Information Available: Figure comparing the CWEPR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Engelhardt, H.; Engel, A.; Baumeister, W. Stoichiometric Model of the Photosynthetic Unit of Ectothiorhodospira-Halochloris. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 8972.

(2) Gingras, G.; Picorel, R. Supramolecular Arrangement of Rhodospirillum-Rubrum B880 Holochromea as Studied by Radiation Inactivation and Electron-Paramagnetic Resonance. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 3405.

(3) Ikeda-Yamasaki, I.; Odahara, T.; Mitsuoka, K.; Fujiyoshi, Y.; Murata, K. Projection map of the reaction center-light harvesting 1 complex from Rhodopseudomonas viridis at 10 angstrom resolution. *FEBS Lett.* **1998**, *425*, 505.

(4) Jungas, C.; Ranck, J. L.; Rigaud, J. L.; Joliot, P.; Vermeglio, A. Supramolecular organization of the photosynthetic apparatus of Rhodobacter sphaeroides. *Embo J.* **1999**, *18*, 534.

(5) Miller, K. R. 3-Dimensional Structure of a Photosynthetic Membrane. *Nature* **1982**, *300*, 53.

(6) Qian, P.; Bullough, P. A.; Hunter, C. N. Three-dimensional reconstruction of a membrane-bending complex - The RC-LH1-PufX core dimer of Rhodobacter sphaeroides. *J. Biol. Chem.* **2008**, *283*, 14002.

(7) Stahlberg, H.; Dubochet, J.; Vogel, H.; Ghosh, R. Are the light-harvesting I complexes from Rhodospirillum rubrum arranged around the reaction centre in a square geometry. *J. Mol. Biol.* **1998**, *282*, 819.

(8) Stark, W.; Kuhlbrandt, W.; Wildhaber, I.; Wehrli, E.; Muhlethaler, K. The Structure of the Photoreceptor Unit of Rhodopseudomonas-Viridis. *Embo J.* **1984**, *3*, 777.

(9) Walz, T.; Jamieson, S. J.; Bowers, C. M.; Bullough, P. A.; Hunter, C. N. Projection structures of three photosynthetic complexes from Rhodobacter sphaeroides: LH2 at 6 angstrom LH1 and RC-LH1 at 25 angstrom. *J. Mol. Biol.* **1998**, *282*, 833.

(10) Warshel, A.; Papazyan, A.; Muegge, I. Microscopic and semimacroscopic redox calculations: What can and cannot be learned from continuum models. *J. Biol. Inorg. Chem.* **1997**, *2*, 143.

(11) Ratner, M. A.; Davis, B.; Kemp, M.; Mujica, V.; Roitberg, A.; Yaliraki, S. Molecular wires: Charge transport, mechanisms, and control. In *Molecular Electronics: Science and Technology*; New York Academy Sciences: New York, 1998; Vol. 852, p 22.

(12) Norris, J. R.; Uphaus, R. A.; Crespi, H. L.; Katz, J. J. Electron Spin Resonance of Chlorophyll and the Origin of Signal I in Photosynthesis. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 625.

(13) Srivatsan, N.; Kolbasov, D.; Ponomarenko, N.; Weber, S.; Ostafin, A. E.; Norris, J. R. Cryogenic charge transport in oxidized purple bacterial light-harvesting 1 complexes. *J. Phys. Chem. B* **2003**, *107*, 7867.

(14) Srivatsan, N.; Norris, J. R. Electron paramagnetic resonance study of oxidized B820 complexes. *J. Phys. Chem. B* **2001**, *105*, 12391.

(15) Srivatsan, N.; Weber, S.; Kolbasov, D.; Norris, J. R. Exploring charge migration in light-harvesting complexes using electron paramagnetic resonance line narrowing. *J. Phys. Chem. B* **2003**, *107*, 2127.

(16) Tang, J.; Dikshit, S. N.; Norris, J. R. Electron-Spin-Resonance Line-Shapes for One-Dimensional and 2-Dimensional Random-Walk Processes. *J. Chem. Phys.* **1995**, *103*, 2873.

(17) Kuroda, S.; Marumoto, K.; Ito, H.; Greenham, N. C.; Friend, R. H.; Shimoi, Y.; Abe, S. Spin distributions and excitation spectra of optically generated polarons in poly(phenylenevinylene) derivatives. *Chem. Phys. Lett.* **2000**, *325*, 183.

(18) Marumoto, K.; Takeuchi, N.; Kuroda, S. Nanoscale spatial extent of photogenerated polarons in regioregular poly(3-octylthiophene). *Chem. Phys. Lett.* **2003**, *382*, 541.

(19) Nowak, M. J.; Rughooputh, S.; Hotta, S.; Heeger, A. J. Polarons and Bipolarons on a Conducting Polymer in Solution. *Macromolecules* **1987**, *20*, 965.

(20) Susumu, K.; Frail, P. R.; Angiolillo, P. J.; Therien, M. J. Conjugated chromophore arrays with unusually large hole polaron delocalization lengths. *J. Am. Chem. Soc.* **2006**, *128*, 8380.

(21) Seth, J.; Bocian, D. F. Electron-Paramagnetic-Resonance Studies of Metalloporphyrin Anion-Radicals - Effects of Solvent, Counterion, Temperature, and Isotopic-Substitution on the Jahn-Teller Active(2)E(G) Ground-State. *J. Am. Chem. Soc.* **1994**, *116*, 143.

(22) Seth, J.; Palaniappan, V.; Johnson, T. E.; Prathapan, S.; Lindsey, J. S.; Bocian, D. F. Investigation of Electronic Communication in Multi-Porphyrin Light-Harvesting Arrays. *J. Am. Chem. Soc.* **1994**, *116*, 10578.

(23) Budker, V.; Du, J. L.; Seiter, M.; Eaton, G. R.; Eaton, S. S. Electron-Electron Spin-Spin Interaction in Spin-Labeled Low-Spin Methemoglobin. *Biophys. J.* **1995**, *68*, 2531.

(24) Rakowsky, M. H.; More, K. M.; Kulikov, A. V.; Eaton, G. R.; Eaton, S. S. Time-Domain Electron-Paramagnetic-Resonance as a Probe of Electron-Electron Spin-Spin Interaction in Spin-Labeled Low-Spin Iron Porphyrins. *J. Am. Chem. Soc.* **1995**, *117*, 2049.

(25) Rakowsky, M. H.; Zecevic, A.; Eaton, G. R.; Eaton, S. S. Determination of high-spin iron(III)-nitroxyl distances in spin-labeled porphyrins by time-domain EPR. *J. Magn. Reson.* **1998**, *131*, 97.

(26) Kolbasov, D.; Srivatsan, N.; Ponomarenko, N.; Jager, M.; Norris, J. R. Modeling charge transfer in oxidized bacterial antenna complexes. *J. Phys. Chem. B* **2003**, *107*, 2386.

(27) Beekman, L. M. P.; Steffen, M.; vanStokkum, I. H. M.; Olsen, J. D.; Hunter, C. N.; Boxer, S. G.; vanGrondelle, R. Characterization of the light-harvesting antennas of photosynthetic purple bacteria by stark spectroscopy. 1. LH1 antenna complex and the B820 subunit from Rhodospirillum rubrum. *J. Phys. Chem. B* **1997**, *101*, 7284.

(28) Haberkorn, R.; Michel-Beyerle, M. E.; Marcus, R. A. Spin-Exchange and Electron-Transfer Rates in Bacterial Photosynthesis. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 4185.

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