Metal Ion Modulated Electron Transfer in Photosynthetic Proteins

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ABSTRACT

Photosynthetic purple bacterial reaction center (RC) proteins are ideal native systems for addressing basic questions regarding the nature of biological electron transfer because both the protein structure and the electron-transfer reactions are well-characterized. Metal ion binding to the RC can affect primary photochemistry and provides a probe for understanding the involvement of local protein environments in electron transfer. The RC has two distinct transition metal ion binding sites, the well-known non-heme Fe^{2+} site buried in the protein interior and a recently discovered Zn^{2+} site located on the surface of the protein. Fe^{2+} removal and Zn^{2+} binding systematically affect different electron-transfer steps in the RC. Factors involved in the metal ion alteration of RC electron transfer may provide a paradigm for other biological systems involved in electron transfer.

Introduction

Many enzymes and proteins utilize the special properties of metal ions to perform a wide variety of specific functions associated with biological processes. Protein metal sites play key roles in enforcing protein structure, metal ion storage, dioxygen binding, electron transfer, and catalysis.[1] A critical process that requires metal ions is photosynthesis. Photosynthesis is the conversion of light to chemical energy. The initial energy conversion reactions take place in integral membrane proteins called reaction centers (RCs) and involve rapid, sequential electron transfers resulting in efficient charge separation across the membrane, establishing an electrochemical potential. In 1984, Huber, Michel, and Deisenhofer reported the first crystallization and structural determination of the RC protein from the purple bacterium *Rhodopseudemonas* (*Blastochloris*) *viridis.*[2] The related *Rhodobacter sphaeroides* structure (Figure 1) followed soon after.[3,4] Recently, structures of the RC complexes from higher plants, photosystem I (PSI)[5] and photosystem II (PSII),[6] have been resolved, providing evidence of their similarities and differences. The purple bacterial RC remains the classic "model" system for exploring basic structure and function relationships important for efficient photochemistry in both natural and artificial photosynthetic complexes. In the following, RC refers to the purple photosynthetic bacterial RC protein.

The RC consists of three 30−35 kDa protein subunits, L, M, and H, and nine cofactors: four bacteriochlorophyll (Bchl) molecules (two of which make up a "special pair", P), two bacteriopheophytin ($H_{A/B}$) molecules, two quinone ($Q_{A/B}$) molecules, and one non-heme iron atom (Figure 1).[7] Electron transfer occurs sequentially following photoexcitation of P. The radical pair $P^+H_A^-$ is formed within ∼3 ps of photoexcitation of P. The electron is transferred from H_A^- to the primary quinone acceptor (Q_A) within ∼200 ps, yielding the membrane-spanning charge-separated state $P^+Q_A^-$. Within about 200 μs, the electron reaches the final quinone acceptor, Q_B. After a two-electron, two-proton reduction, Q_BH_2 is released from the RC. The free hydroquinone is oxidized by cytochrome bc_1 complex, thereby releasing quinol protons into the periplasm. This creates a transmembrane proton gradient utilized for ATP synthesis.

The cofactors are embedded in the protein matrix of the L and M subunits, each composed of five transmembrane α-helices, and are arranged in two membrane spanning branches around an axis of 2-fold symmetry that is oriented perpendicular to the membrane plane. Electron transfer occurs almost exclusively via the L-branch from P to Q_A with an efficiency of ∼1. The hydrophilic H-subunit is located on the cytoplasmic side of the membrane and contains one intermembrane helix. The non-heme Fe (Figure 2) is buried in the protein interior between Q_A and Q_B.

Electron transfer occurs in many biological systems, wherein types of oxidation−reduction centers include protein side chains, small organic and inorganic molecules, or metalloproteins themselves.[8] Fundamental to understanding biological electron transfer is discerning the involvement of heterogeneous polypeptide environments surrounding the redox sites, particularly in electron transfer processes coupled to conformational changes or proton transfer. The RC is an ideal system to address these issues because both protein structure and electron transfer reactions are well-characterized. The basic principles derived from RC studies should be generally applicable to the role of metal ions in electron-transfer reactions in other biological systems.

The RC protein acts as a scaffold, holding cofactors at the appropriate distance and orientation for efficient electron and proton transfer. The specific role of localized anisotropic protein environments in influencing electron transfer is difficult to ascertain. The RC is a large protein composed of over 800 amino acids. Thus, determining how a small region of the protein's structure, electrostat-

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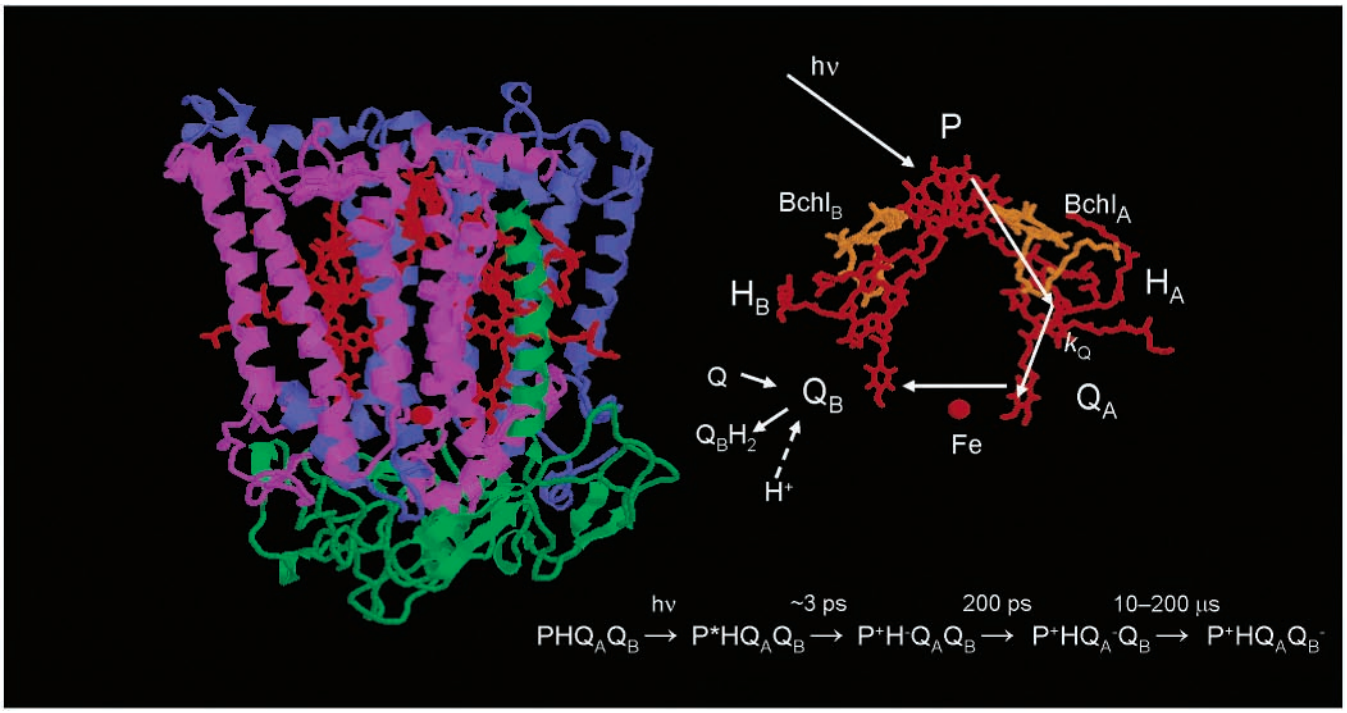

FIGURE 1. Reaction center from *Rb. sphaeroides* including three protein subunits, L (blue), M (purple), and H (green), the cofactors (red), and the electron-transfer pathway and proton uptake at Q_B.

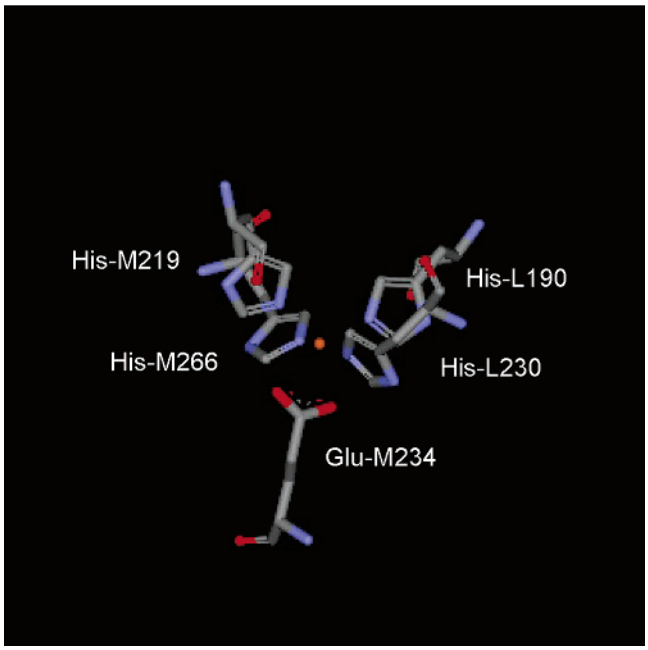

FIGURE 2. The environment of the Fe^{2+} is a distorted octahedron, the base plane of which is formed by three imidazole N_ϵ of histidines and one carbonyl oxygen; the apex is formed by a fourth histidine imidazole N_ϵ and a second carbonyl oxygen.[16]

ics, and dynamics influences the photosynthetic electron-transfer reactions is challenging. Site-directed mutagenesis is an important approach to determining the roles of specific amino acids.[9] Another approach is that of a bioinorganic chemist, utilization of a metal ion to provide a short-range local probe of the metal coordination sphere and surrounding protein environment.

The Fe Site

Thirty years ago, metal analysis of isolated RCs revealed the existence of one Fe atom per purified RC molecule from *Rb. sphaeroides*.[10] Electron paramagnetic resonance (EPR) experiments yielded the first evidence of the location of this Fe. At cryogenic temperatures, irradiation of the RC produced a narrow signal around $g = 2$ due to P^+ and a broad EPR signal centered at $g = 1.8$.[10,11] It was suggested that the broad signal might be due to a reduced state of Fe serving as the primary acceptor.[10] However, other experiments showed that the primary and secondary acceptors were bound quinone molecules.[12−14] (At this time, the H_A "primary" acceptor had not been identified.) Thus, it became evident that the broad EPR signal was due to the electron on the quinone acceptor interacting magnetically with the large magnetic moment of Fe. A similar broad EPR signal at $g = 1.8$ is observed when Q_B is reduced.[15] In the absence of paramagnetic Fe (removed or replaced with diamagnetic Zn^{2+}), the EPR signal narrows to that of a typical organic radical, shifting to the $g \approx 2$ region of the spectrum, which at conventional X-band EPR frequency overlaps with the P^+ signal.

A definitive role for the non-heme Fe has not been determined. Mossbauer, magnetization, and Fe extended X-ray absorption fine structure (EXAFS) measurements have shown that Fe exists as high-spin Fe^{2+} and does not change valence when the acceptor is reduced.[16] The location of Fe^{2+} between the quinone molecules suggested that the obvious role of the Fe^{2+} would be involvement in $Q_A^-Q_B \rightarrow Q_AQ_B^-$ electron transfer. To investigate this possibility, methods were developed to remove Fe^{2+} or replace it with other divalent metal ions, such as Zn^{2+},

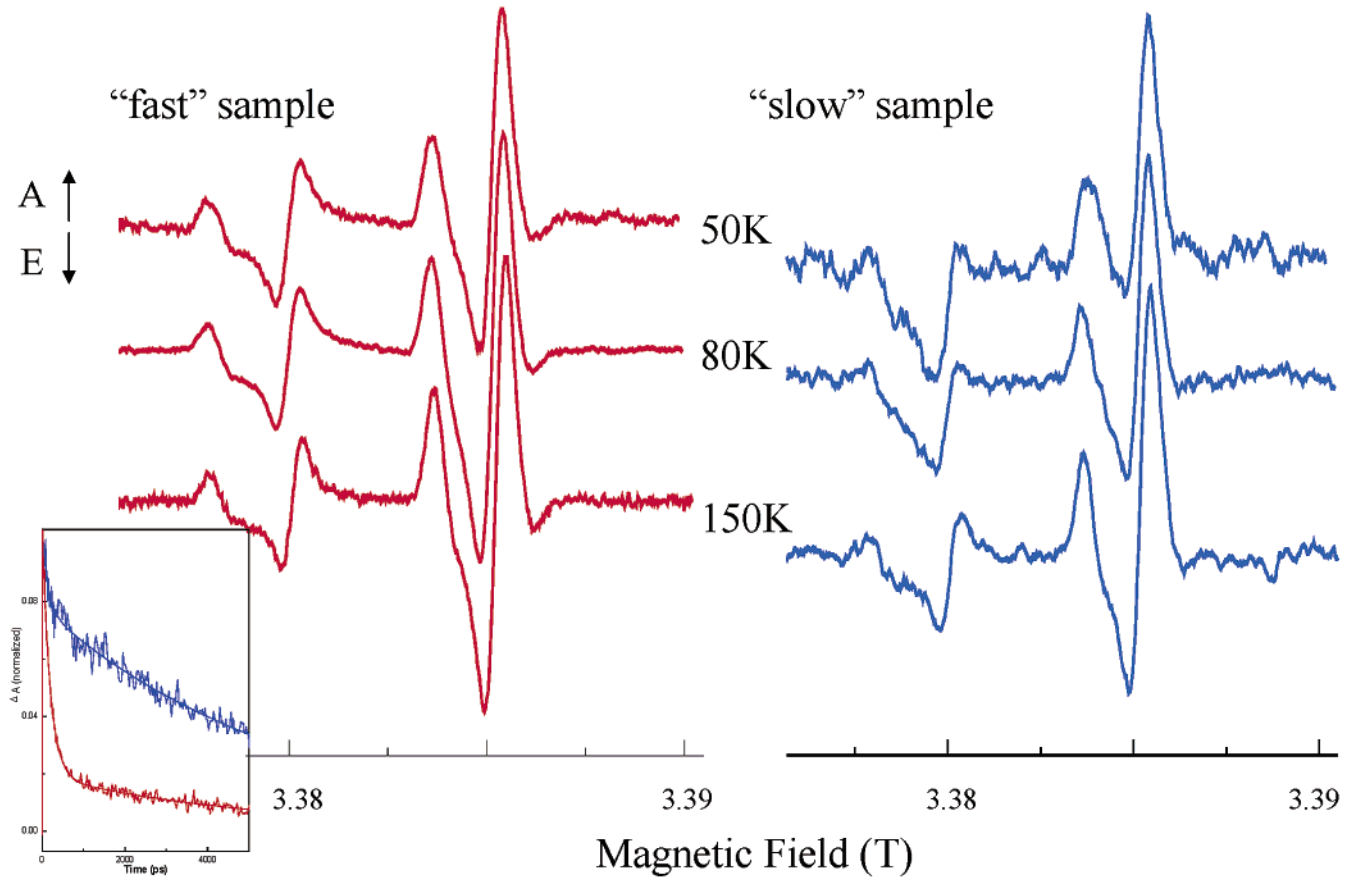

FIGURE 3. Temperature dependence of the W-band $P^+Q_A^-$ electron spin polarized signals of Zn-RCs with "fast" (red) and "slow" (blue) k_Q.[21] Examination of these data resulted in determining that the reorganization energy for the electron-transfer process between $P^+H_A^-Q_A$ and $P^+H_AQ_A^-$ is the dominant factor that is altered in "slow" k_Q Zn-RCs. The inset presents transient absorption measurements and fits of Fe-removed/Zn-replaced RCs showing the native "fast" $(200\ ps)^{-1}$ k_Q (red) and "slow" $(3-6\ ns)^{-1}$ k_Q (blue) rates measured at the H_A anion band at 670 nm.[19]

Mn^{2+}, Cu^{2+}, Ni^{2+}, and Co^{2+}.[17] Substitution of different metal ions into the Fe site did not significantly alter the electron-transfer characteristics.[17] Fe^{2+} apparently does not have an essential role in the $Q_A^-Q_B \rightarrow Q_AQ_B^-$ electron transfer. Later, it was discovered that a rate-limiting step precedes electron transfer, that is, electron transfer is conformationally gated.[18] Although different metal ions might actually influence the intrinsic electron-transfer rate, this could happen without any changes in the observed rate of $Q_A^-Q_B \rightarrow Q_AQ_B^-$.[16]

The apo-form of the RC, where Fe^{2+} is removed but not replaced by another metal ion, can be prepared.[17,19] For apo-RCs with the H-subunit intact, the observed $Q_A^-Q_B \rightarrow Q_AQ_B^-$ rate was approximately one-half the value of native RCs, becoming highly nonexponential.[17] Since electron transfer occurs in the absence of any metal ion in that site, a metal ion is not obligatory for electron transfer.

Although not critical for primary photochemical activity or interquinone electron transfer, removal of the non-heme Fe^{2+} from the RC protein does dramatically influence at least one electron-transfer reaction in the RC. The electron-transfer rate (k_Q) from H_A^- to Q_A proceeds within 200 ps in native RCs (Figure 1). In the case of apo-RCs, at least a 15-fold reduction in k_Q relative to this rate in Fe-containing RCs is observed at room temperature.[20] If Fe^{2+} is replaced by Zn^{2+}, either the native "fast", $(200\ ps)^{-1}$, k_Q or "slow", $(3-6\ ns)^{-1}$, k_Q can be obtained dependent on the Fe-removal procedure (Figure 3).[19] Thus, it is important to have kinetically defined Fe-removed/Zn-replaced samples for EPR spectroscopic experiments, because Zn^{2+} substitution does not ensure retention of the native k_Q[19] as previously thought.[20]

The origin of this dramatic decrease in rate has been examined using transient EPR spectroscopy.[19,21] Time-resolved spectra of the radical pair $P^+Q_A^-$ from Fe-removed/Zn-replaced RCs exhibits electron spin polarization (ESP), which provides structural information, such as the distance between and relative orientations of oxidized donor and reduced acceptor cofactors.[22,23] Also, insight into the interaction of radicals with the protein environment and kinetic information about the sequential charge separation process can be obtained.[19,24,25] Time-resolved high magnetic field EPR (95−130 GHz) on deuterated RC samples was important for these experiments.[21] High field and deuteration allow for resolution of the P^+ and Q_A^- radical signals; and high field generally emphasizes effects of sequential electron transfer that are manifest in the observed ESP.[26] Figure 3 shows the temperature dependence of the W-band (95 GHz) ESP EPR

spectra[21] of kinetically characterized Fe-removed/Zn-replaced deuterated RCs that had the native "fast" k_Q or "slow" k_Q.[19] The shape of the ESP EPR spectra for the "fast" k_Q sample does not show any significant temperature dependence. In contrast, the ESP EPR spectrum of the "slow" k_Q sample is temperature-dependent, exhibiting spectral changes that reflect the longer lifetimes of $P^+H_A^-$. Spectral fits allowed us to determine the temperature dependence of k_Q and structural details of the state $P^+Q_A^-$. No large structural change in $P^+Q_A^-$ geometry between "fast" and "slow" k_Q samples was found. However, a normal temperature trend was observed for "slow" k_Q samples with a decrease in rate coinciding with a decrease in temperature. Thus, in "slow" k_Q samples the $P^+H_A^-Q_A \rightarrow P^+H_AQ_A^-$ electron-transfer event, which is a quasi-activationless process in the native ("fast") RCs (exhibiting an unusual reverse temperature trend), has a small activation energy. Assuming no change in the free energy for the "fast" and "slow" samples, a 25% increase in the reorganization for the "slow" sample was determined. Apparently, increased reorganization energy for the electron-transfer process $P^+H_A^-Q_A \rightarrow P^+H_AQ_A^-$ is the dominant factor altered during Fe-removal procedures,[21] implicating an important role for protein reorganization energy in regulating electron-transfer processes.

A Suspicious Amount of Zn^{2+}

The existence of only one transition metal ion site, the Fe site, in the RC has been accepted for over 30 years. No other transition metal ions were observed in any of the early biochemical experiments or crystal structures. Thus, a truly unexpected finding resulted from our studies of Fe-removal/Zn-substitution procedures. Control experiments of isolated RCs from chromatophore membranes of *Rb. sphaeroides* R26 showed that RCs contained a small amount of Zn^{2+}, ~0.3 Zn/RC.[19] No additional Zn^{2+} was added during the purification procedure. The Zn^{2+} was retained by the RC after isolation and purification procedures, which included several ammonium sulfate precipitations and chromatography steps in the presence of EDTA; both unfavorable conditions for adventitious metal ion binding.

Suspicion set in; does the bacterial RC have a Zn^{2+} binding site? Stoichiometric Zn binding (Zn to RC mole ratio of 1) was obtained by incubating purified RCs with excess $ZnSO_4$ followed by gel filtration chromatography to remove unbound metal ions (Figure 4A).[27] Zn^{2+} binding by this method does not replace the non-heme Fe^{2+}. Stringent conditions, including chaotropic treatment, are necessary to remove the non-heme Fe^{2+} and replace it with Zn^{2+}.[19] Furthermore, metal analysis showed a total of 2 mol equiv of metal ion, with 1 mol equiv each of Fe^{2+} and Zn^{2+} bound to the RC. EPR analysis corroborates the metal analysis results: Zn^{2+} ligates to a site distinct from the non-heme Fe site. When Zn^{2+} is bound stoichiometrically to native Fe-containing RCs, only a light-induced P^+ signal, and not a Q_A^- signal, is observed in the $g = 2.0$ region (Figure 4B). Thus, Fe^{2+} is not displaced by Zn^{2+} and remains magnetically coupled to Q_A^-.[27]

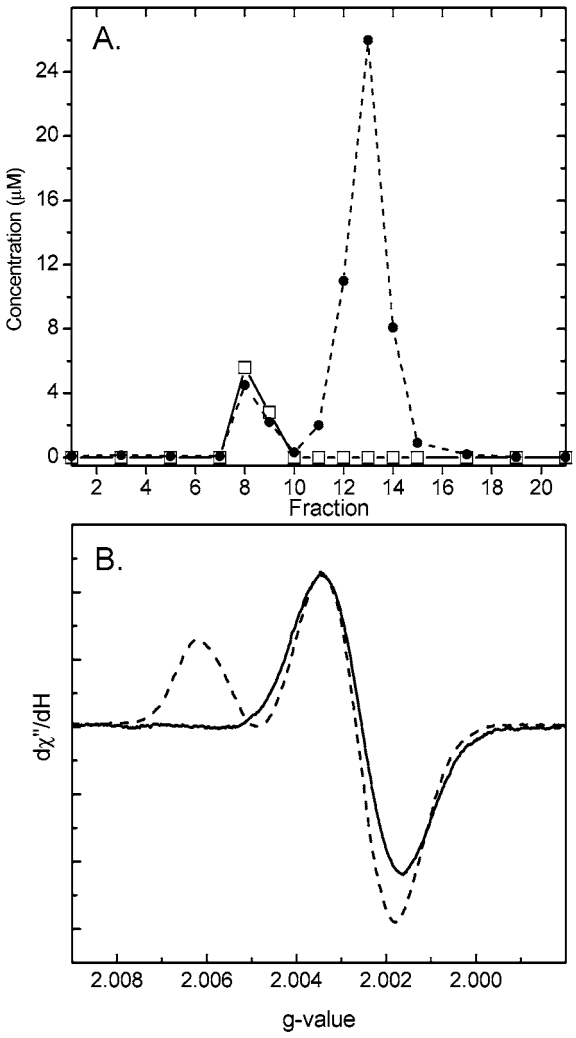

FIGURE 4. Panel A presents the size exclusion chromatography profile showing stoichiometric formation of Zn-RC complex.[27] Zn^{2+} (●) and protein concentrations (□) vs fraction collected are displayed. Panel B presents the light-induced EPR spectra of Zn-RCs at Q-band (34 GHz). The $P^+[Zn^{2+}Q_A]^-$ charge separated state (dashed line) with $g = 2.0026$ (P^+) and $g = 2.0049$ (Q_A^-) is observed for Fe-removed RCs with Zn^{2+} substituted into the Fe site. Only the P^+ signal is observed in the $g = 2$ region for RCs with Zn^{2+} bound to Fe-containing RCs (solid line).

Zn^{2+} can be removed by treatment with the metal-chelating resin Chelex 100. In contrast, treatment with Chelex does not remove metal ions (Fe, Mn, or Zn) bound to the Fe site. Chelex accessibility suggested that metal ion bound to the newly detected Zn^{2+} site is more easily exchanged than ions bound to the buried Fe site.[27] This provided the first clue about the Zn^{2+} site location, that is, near the RC surface. Surface accessibility could be the reason a second metal ion was not observed in the *Rb. sphaeroides* RC X-ray crystal structures. Typical crystallization conditions employ high concentrations of anions, which would be expected to precipitate surface-bound Zn^{2+}, or pHs unfavorable for metal ion binding. Thus, routine metal analysis, and not the protein crystal struc-

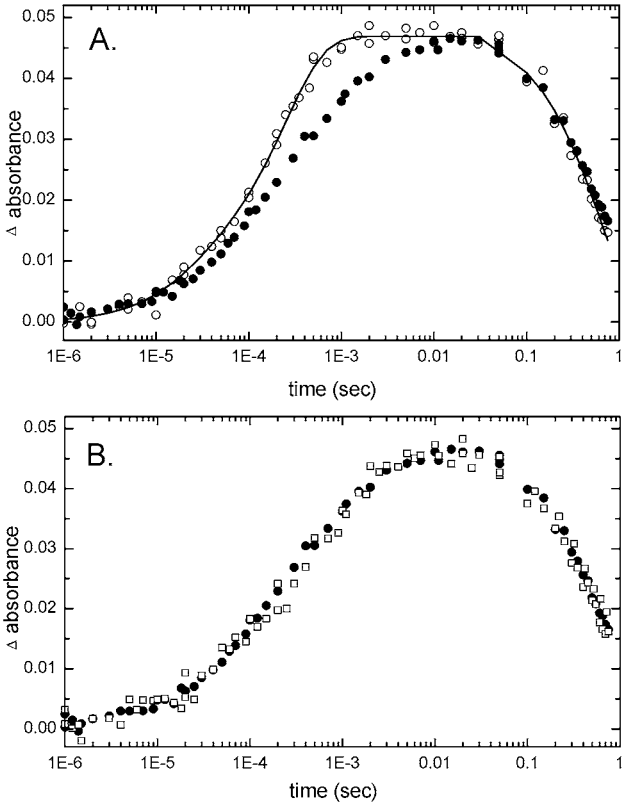

FIGURE 5. Time course for absorbance changes associated with the $Q_A^-Q_B \rightarrow Q_AQ_B^-$ electron-transfer process in *Rb. sphaeroides* RCs:[27] (A) RC samples with Zn^{2+} (1.2 Zn/RC, ●) or without Zn^{2+} bound (0.2 Zn/RC, ○) to the RC surface; (B) Comparison of the effect of Zn^{2+} vs temperature on kinetics. Data are shown for Zn-RCs (●) at 21 °C and RCs at 2 °C (□).

ture, was the starting point of this story. The important question is does Zn^{2+} have a functional role in the RC protein?

Zn^{2+} Binding Influences Electron Transfer

Transient optical measurements show that Zn^{2+} binding to the RC modulates $Q_A^-Q_B \rightarrow Q_AQ_B^-$ electron transfer. Absorbance changes at 757 nm (ΔA_{757}) reflect different electrochromic responses of the bacteriopheophytins to formation of the quinone anion states and have been assigned primarily to electron transfer.[28] At room temperature, in native RCs with no Zn^{2+} bound, heterogeneous kinetics composed of at least two distinct components are observed with a major (77%) slow component on the order of 200 μs. When Zn^{2+} is bound to the site distinct from the Fe site, the electron transfer between the quinones is slowed and the room-temperature kinetics become distributed across the microsecond to millisecond time domain (Figure 5A). ΔA_{757} continues to build in at 10 ms. Semiquantitative fits using three components yield an additional slowest phase on the order of ~3 ms, markedly longer than the 200 μs slowest component observed in non-Zn-containing RCs. This effect of metal binding on the kinetics is similar to the more global effect of cooling RCs to 2 °C in the absence of Zn^{2+} (Figure 5B). Thus, unlike the non-heme Fe, which has no apparent role in $Q_A^-Q_B \rightarrow Q_AQ_B^-$ electron transfer but is situated directly between Q_A and Q_B, Zn^{2+} binding modulates this reaction but does so from a remote position.

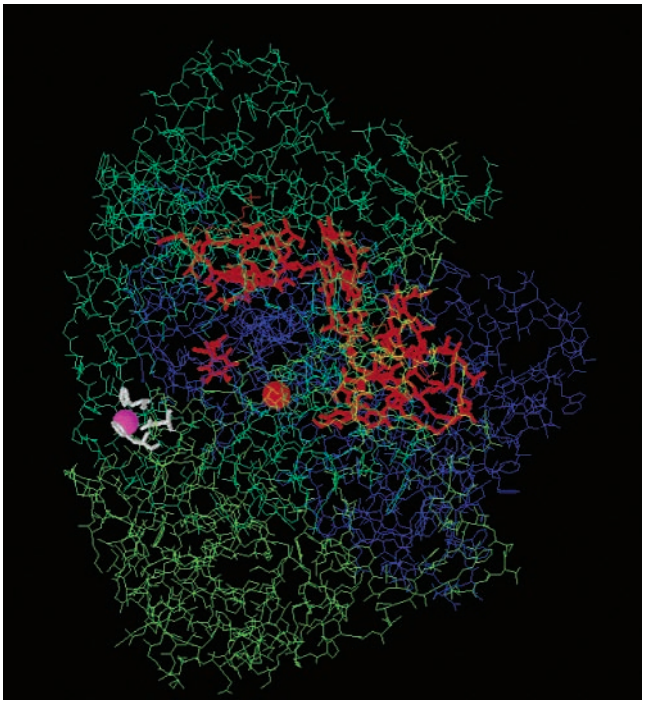

FIGURE 6. Structure of the RC from *Rb. sphaeroides*, showing the location of the Zn^{2+} binding site (pink).[30]

Where's the Zn^{2+}?

Interrogation of the *Rb. sphaeroides* crystal structure for typical Zn^{2+} ligands, such as histidine and cysteine, revealed a potential metal-binding site that includes a grouping of histidine residues on the surface of the H-subunit (H68, H126, and H128) and the L-subunit (L211).[27] Interestingly, these histidines are positioned beneath the Q_B binding pocket and surround a water channel proposed to be a proton pathway to Q_B. Subsequently, it was determined that Zn^{2+} binding to the RC influences proton uptake by Q_B, in addition to modulating electron transfer.[29] X-ray diffraction of RC crystals from *Rb. sphaeroides* soaked in solutions containing excess metal ion[30] revealed that Zn^{2+} binds to a site located near the protein surface on the H-subunit, 18 Å away from Q_B (Figure 6). Zn^{2+} is coordinated in a tetrahedral environment to two imidazole nitrogens from histidine-H126 and histidine-H128, one aspartate acid carboxylate from aspartate-H124, and one water molecule (Figure 7). The location of the metal site has been important for elucidating the proton entry point into the RC and understanding the proton pathway through the protein matrix.[29,31]

Mechanistic Aspects of Zn^{2+} Modulation of Electron Transfer

To understand how Zn^{2+} modulates electron transfer, one must understand important features of the process. The electron transfer between Q_A and Q_B is temperature-

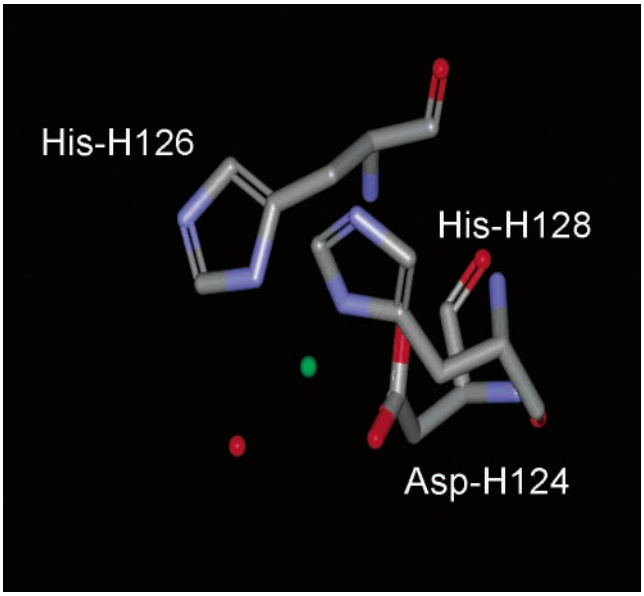

FIGURE 7. Coordination environment of the tetrahedral Zn^{2+} site.[30] Green ball represents Zn^{2+}; red ball represents a water molecule.

activated,[28,32] coupled to proton movement,[33] and believed to be rate-limited by protein motion.[18,34] Heterogeneous kinetics observed for $Q_A^-Q_B \rightarrow Q_AQ_B^-$ electron transfer at room temperature indicate that the RC structure is distributed around configurations that result in fast and slow electron transfer.[28] Distributed kinetics observed at low temperature suggest that the electron-transfer event reflects a thermal averaging of conformational substates, similar to that seen for reaction dynamics in other proteins.[28] Thus, electron transfer involves a complex interaction of protein and proton rearrangements that provide the electrostatic environment to accommodate efficient electron and proton transfers.

The distributed kinetics and similarity to low-temperature kinetics observed with Zn^{2+} binding (Figure 5) suggest that Zn^{2+} alters localized protein motions that are important for rapid electron transfer. Metal ion binding most likely does not directly influence the conformational gating step of $Q_A^-Q_B \rightarrow Q_AQ_B^-$ electron transfer[18] but rather acts by limiting the dynamic mobility of the localized Zn^{2+} binding site. Metal ions bound to a protein inherently enforce the structure of ligating amino acid side chains, and these perturbations could potentially extend through the surrounding protein, restricting a region of polypeptide.[35]

In addition to influencing protein structure, mobility, or both, Zn^{2+} binding could alter important electrostatic interactions. Zn^{2+} binding apparently does not have a significant effect on the electrostatic environment near Q_B.[27] However, electrostatic aspects of metal ion binding on proton pathways and pK_a shifts of protein residues have been investigated.[31,36−38] These studies suggest that metal binding causes an electrostatic perturbation of the coupled uptake, rearrangement, or both of protons. Although at physiological pH in isolated RCs $Q_A^-Q_B \rightarrow Q_AQ_B^-$ electron transfer does not involve direct protonation of quinones,[33] metal ion binding causes the coupled proton uptake of surrounding protein residues to become the rate-limiting process.[36−39]

A "Nonclassical" Zn^{2+} Site?

The structure of the photosynthetic RC Zn^{2+} site is not surprising, yet the action of the Zn^{2+} site in this system is unique compared to classic roles of Zn^{2+} in other proteins. Typically, when metalloproteins participate in electron-transfer reactions, the metal ion, for example, Cu^{2+}, Fe^{2+}, Mn^{2+}, or Co^{2+}, is one of the redox participants, either directly donating or directly accepting an electron.[8] In contrast, Zn^{2+} ($3d^{10}$), a nonredox active metal ion,[35] influences an electron-transfer event in the RC as a "passive" bystander from a distance ~18 Å away from the electron acceptor, Q_B.

Traditionally, Zn^{2+} sites in proteins have been classified as either catalytic or structural.[35,40,41] In most enzymes, Zn^{2+} is directly involved in catalysis, interacting with substrate molecules undergoing transformation. In others, the role is purely structural, stabilizing protein tertiary or quaternary structure. Although Zn^{2+} can exist in several coordination geometries, tetrahedral is the prevalent geometry for both catalytic and structural sites.[35] In catalytic sites, the Zn^{2+} is usually exposed and bound to a solvent molecule.[40,41] The most common ligand is histidine, followed by glutamate, aspartate, and cysteine. On the other hand, structural zinc sites have four protein ligands and no bound water molecule.[40,41] Cysteine and histidine are the preferred ligands; occasionally aspartate or glutamate provide additional ligation. Thus, the coordination environment of Zn^{2+} in the RC is typical of a catalytic Zn^{2+} site, yet the function is not obviously catalytic. Zn^{2+} slows both proton uptake and electron transfer. As far as a structural role, crystallography shows that no substantial changes in overall protein structure occur upon metal binding.[30]

Why would nature disrupt efficient electron and proton transfer by metal binding? Two results suggest that there might be a native functional role for the Zn^{2+} site. First, metal ions have been shown to influence proton reactions in intact RCs in chromatophore membranes of *Rb. sphaeroides*.[42] Second, different bacterial species have a structurally analogous surface metal site that modulates electron transfer.[43] In addition, it has been shown that surface histidines involved in metal ion binding play an important role in proton transfer, acting as proton donors to Q_B.[44] Perhaps nature has incorporated surface histidines as proton-transfer agents, and this is the native functional role of the site.[44] But then, why, in addition to the histidine, are the nonproton donors involved in metal ion binding, aspartate or glutamate residues, conserved in different species of RCs?[45]

Cu^{2+} Spectroscopic Studies of the RC Surface Metal Site

Although crystallography provides information about both the location and structure of the metal site, EPR spectroscopy can provide complementary information, includ-

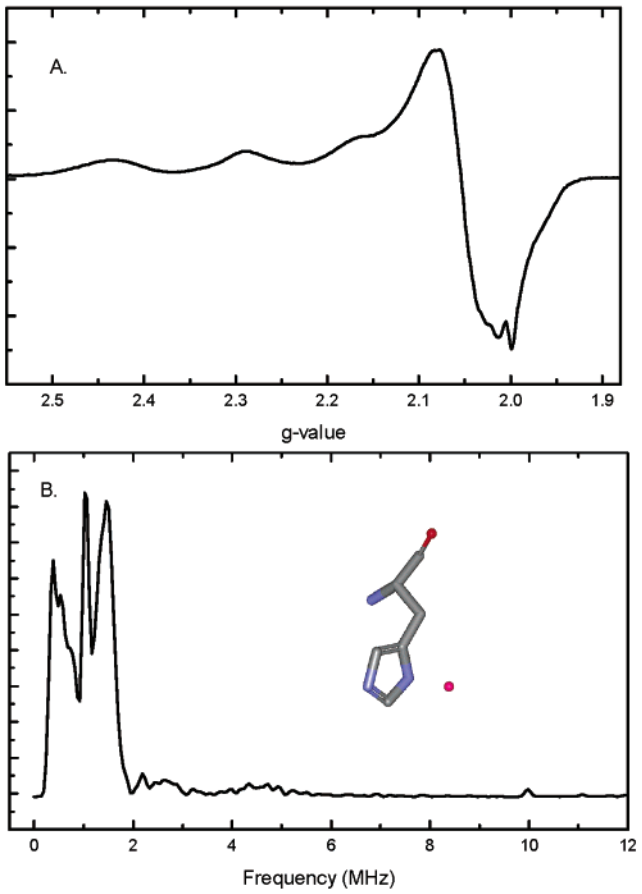

FIGURE 8. Cu^{2+} as a probe of the surface metal ion site:[47] (A) X-band (9 GHz) cw EPR spectrum of the type II Cu^{2+} center of RCs with Cu^{2+} bound to the surface site of *Rb. spheroides* RCs; (B) FT of time domain three-pulse ESEEM data indicating the presence of histidine ligation to the Cu^{2+} center.

ing metal site structure for noncrystalline samples, electronic coupling with remote amino acids, and magnetic interactions (exchange and dipole−dipole) between the metal center and other paramagnetic species in the protein. Thus, paramagnetic metal ions, such as Cu^{2+} ($3d^9$), are valuable spin probes of protein metal sites[1] and have been used to probe the RC Fe^{2+} site.[46−48] Likewise, EPR studies of Cu^{2+} bound to the RC surface metal ion site allow us to monitor the response of this metal site structure to light, magnetic interactions, and protein conformational changes. Like Zn^{2+}, Cu^{2+} ($3d^9$) binds specifically to a surface site on native RCs from *Rb. sphaeroides* and slows $Q_A^-Q_B \rightarrow Q_AQ_B^-$ electron transfer.[43,47] Cu^{2+} bound to this site exhibits an axially symmetric cw EPR spectrum (Figure 8A), typical of type 2 Cu^{2+} EPR signals, and light-induced spectral changes indicate that the surface Cu^{2+} is at least 23 Å removed from both the primary donor (P^+) and reduced quinone acceptor (Q_A^-).[43] Electron spin−echo envelope modulation (ESEEM) provided evidence for histidine ligation to the Cu^{2+} center (Figure 8B).[43]

Results from preliminary pulsed EPR and ENDOR studies of Cu^{2+}-RCs indicate a different ligation sphere for Cu^{2+} versus Zn^{2+}.[49] These structural differences might explain the different response of the RC to Cu^{2+} versus Zn^{2+} binding. Cu^{2+} binding slows $Q_A^-Q_B \rightarrow Q_AQ_B^-$ electron transfer to a lesser extent than Zn^{2+} binding, and distributed kinetics are not observed. Correlating different metal ion site structure to differences in the response of the RC electron-transfer events might help elucidate important mechanistic features. Different metal ions influence the two interquinone electron-transfer steps to varied extents,[27,29,36,38,43] and these differences may correlate to modified structures of the surface metal ion site observed in X-ray data for Zn, Cd, Co, and Ni[30] and EPR data of Cu^{2+}.[49] This concept is similar to metalloregulatory proteins wherein specific coordination environments are important components of the molecular basis of heavy metal recognition and transcriptional regulation.[50] A surface metal ion binding site might exhibit some flexibility and thus be able to accommodate different metal ions in their preferable coordination environments. Ultimately, a complete mechanism of metal ion modulation of electron and proton transfer must account for metal-specific functional differences, the distributed nature of electron-transfer kinetics, and the influence of the metal ion on protonation states of amino acids.

Concluding Remarks

RCs have two distinct transition metal ions, a Zn^{2+} site and an Fe^{2+} site. Whereas removal of the non-heme Fe^{2+} induces slow kinetics for electron transfer from the H_A^- to Q_A, Zn^{2+} binding slows interquinone electron transfer. The discovery of the surface Zn^{2+} site has both provided insights into and raised questions about electron-transfer coupled to proton transfer in the RC. Future experiments will help elucidate the mechanism of metal ion modulated electron and proton transfers, as well as address the question of a native functional role. Metal binding/removal provides a reversible way of altering both electron and proton transfer in the RC and a spectroscopic tool (e.g., Cu^{2+}) for investigating different species of RCs. These studies have been especially useful in elucidating the proton entry point and proton-transfer route to Q_B, thereby highlighting the general importance of surface histidines.[44] With regard to biological electron transfer, the influence of Zn^{2+} binding to a remote position of the RC and modulating electron transfer from a distance is unique. Reminiscent of allosteric metal ion regulation, the distributed kinetics indicate some local metal ion induced dynamic structural changes that influence electron transfer. These results highlight the importance of the protein matrix in modulating electron transfer. Perhaps additional systems will come to light wherein previously overlooked surface bound metal ion sites can be used to investigate electron transfer or other enzymatic reactions.

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