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Protein-Cofactor Interactions in Bacterial Reaction Centers from *Rhodobacter sphaeroides* R-26: II. Geometry of the Hydrogen Bonds to the Primary Quinone $Q_A^{\cdot-}$ by ^{1}H and ^{2}H ENDOR Spectroscopy

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ABSTRACT The geometry of the hydrogen bonds to the two carbonyl oxygens of the semiquinone $Q_A^{\cdot-}$ in the reaction center (RC) from the photosynthetic purple bacterium *Rhodobacter sphaeroides* R-26 were determined by fitting a spin Hamiltonian to the data derived from ^{1}H and ^{2}H ENDOR spectroscopies at 35 GHz and 80 K. The experiments were performed on RCs in which the native Fe^{2+} (high spin) was replaced by diamagnetic Zn^{2+} to prevent spectral line broadening of the $Q_A^{\cdot-}$ due to magnetic coupling with the iron. The principal components of the hyperfine coupling and nuclear quadrupolar coupling tensors of the hydrogen-bonded protons (deuterons) and their principal directions with respect to the quinone axes were obtained by spectral simulations of ENDOR spectra at different magnetic fields on frozen solutions of deuterated $Q_A^{\cdot-}$ in H_2O buffer and protonated $Q_A^{\cdot-}$ in D_2O buffer. Hydrogen-bond lengths were obtained from the nuclear quadrupolar couplings. The two hydrogen bonds were found to be nonequivalent, having different directions and different bond lengths. The H-bond lengths $r_{O\cdots H}$ are 1.73 ± 0.03 Å and 1.60 ± 0.04 Å, from the carbonyl oxygens O_1 and O_4 to the NH group of Ala M260 and the imidazole nitrogen N_δ of His M219, respectively. The asymmetric hydrogen bonds of $Q_A^{\cdot-}$ affect the spin density distribution in the quinone radical and its electronic structure. It is proposed that the H-bonds play an important role in defining the physical properties of the primary quinone, which affect the electron transfer processes in the RC.

INTRODUCTION

Two ubiquinones, Q_A and Q_B, are present in the reaction centers (RCs) of photosynthetic purple bacteria and are actively involved in the coupled electron-proton transfer processes that lead to the formation of the proton gradient across the photosynthetic membrane required for ATP synthesis (1). The two quinones have different redox potentials providing the driving force for the vectorial electron transfer (ET) from the primary to the secondary quinone. Since Q_A and Q_B are chemically identical molecules, their different properties in the ET chain must result from specific interactions with the protein environment (2–6). One of these interactions is hydrogen bonding between the carbonyl oxygens of the quinones and the RC protein. Q_A forms two hydrogen bonds to the RC protein (2,3,7). The bonds contribute to the binding and to the unusual chemical properties and function of the quinone. It is, therefore, important to characterize them in detail.

X-ray crystallographic structures of proteins are usually not obtained at high enough resolution to provide direct information on the positions of protons (e.g., the protons hydrogen-bonded to the quinones). Furthermore, specific intermediate states of the reaction cycle (e.g., the semiquinone radical anions $Q_A^{\cdot-}$ and $Q_B^{\cdot-}$) are only accessible in exceptional cases by x-ray crystallography (e.g., (3,8)). Consequently, other methods have been used, in particular electron paramagnetic resonance (EPR) and electron nuclear double resonance (ENDOR) spectroscopies, to study semiquinone radicals in bacterial RCs (4–6,9–19). These spectroscopies provide information about the electronic and spatial structure of the transient radicals $Q_A^{\cdot-}$ and $Q_B^{\cdot-}$ (reviewed in (4–6)).

In this work we focus on the primary quinone Q_A, which accepts one electron to form the ubisemiquinone radical anion, $Q_A^{\cdot-}$. The experiments were performed on RCs in which the Fe^{2+} was replaced by Zn^{2+}. However, we expect our results to be valid for native RCs since all spectral and kinetic properties of Zn-RCs are identical to Fe-RCs (20,21). Details on the spin-density distribution of this species have been obtained earlier by EPR and ENDOR spectroscopy (9–18) and showed pronounced shifts of the hyperfine couplings of $Q_A^{\cdot-}$ when compared with the free $UQ_{10}^{\cdot-}$ radical anion in organic solvents. This effect has been attributed to asymmetric hydrogen bonding to $Q_A^{\cdot-}$ in the RC proteins (9,10). The hydrogen-bonded protons are exchangeable with deuterons (in D_2O buffer) and could thus be identified via ^{1}H → ^{2}H exchange experiments in the spectra.

In a previous article (19), the ENDOR lines belonging to the exchangeable protons have been assigned to two protons hydrogen-bonded to the two carbonyl oxygens of $Q_A^{\cdot-}$. In this work, we used ^{1}H- and ^{2}H-ENDOR spectroscopies to determine the geometry of the two hydrogen bonds. The ENDOR experiments were performed at 80 K and at a microwave frequency of 35 GHz (Q-band), which provides improved spectral resolution compared to the commonly used frequency of 9 GHz (X-band). This enabled us to select molecules with

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particular sets of orientations with respect to the magnetic field (22). Since the ^{1}H and ^{2}H ENDOR spectra occur in different, nonoverlapping, frequency regions of the spectrum, we were able to simplify the ENDOR spectra (i.e., focus on the H-bonds) by using deuterated RCs in H_2O buffer and protonated RCs in D_2O buffer. Furthermore, the different ^{1}H → ^{2}H exchange rates of the two protons enabled us to preferentially deuterate (or protonate) one of the two carbonyl oxygens as described in (19). A standard spin Hamiltonian was fitted to the ENDOR spectra obtained at different magnetic field positions using the EasySpin package developed by Stoll and Schweiger (23). The fit yielded the principal components of the ^{1}H and ^{2}H hyperfine coupling (hfc) and the ^{2}H nuclear quadrupolar coupling (nqc) tensors and their principal directions with respect to the g-tensor axes of $Q_A^{\cdot-}$, which are related to the molecular axes of the quinone (13). The hydrogen-bond lengths were obtained from the ^{2}H nuclear quadrupolar couplings using the empirical relations of Soda and Chiba (24) and of Hunt and Mackay (25). By these procedures, the geometries of both hydrogen bonds were completely characterized.

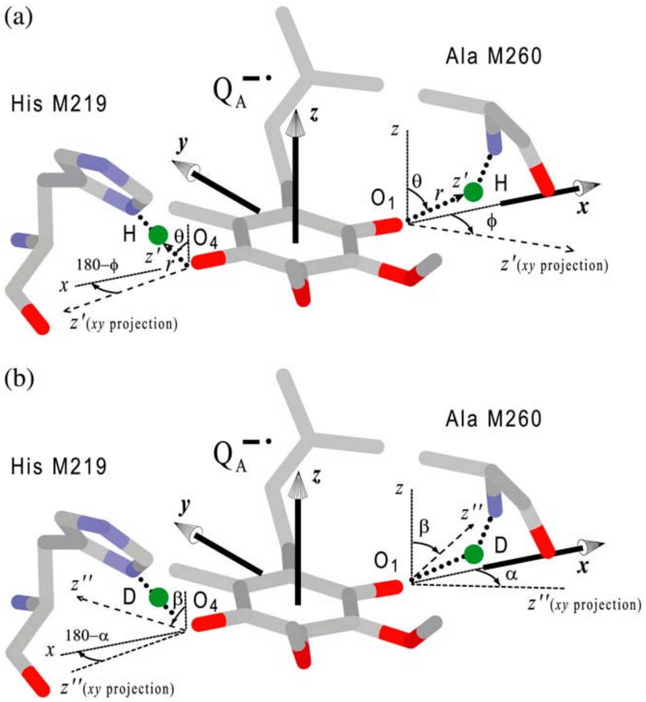

FIGURE 1 Structure in the vicinity of Q_A in the RC of *Rb. sphaeroides* (Brookhaven data bank entry 1AIG (3)) with the H-bonds (*a*), or the D-bonds (*b*) to the two carbonyl oxygens, O_1 and O_4. The principal axes of the g-tensor are along the molecular axes x, y, z (*a*). The principal axes of the hfc tensors of the protons in H-bonds are along the axes x', y', z' (for simplicity x' and y' are not shown, they lie in the plane perpendicular to z'), and are related to the g-tensor axes by the Euler angles ϕ and θ (31) (*a*). The proton in the hydrogen bond is characterized by the bond-length r and the angles θ and ϕ (*a*). The principal axes of the nqc tensor are along x'', y'', z'' (for simplicity x'' and y'' are not shown, they lie in the plane perpendicular to z''), and are related to the g-tensor axes by the Euler angles α, β and γ (31) (for simplicity γ is not shown) (*b*).

THEORY

Spin Hamiltonian and its relation to the structure

We focus on the interaction between the magnetic moment of the unpaired electron of $Q_A^{\cdot-}$ and the magnetic moments of protons or deuterons that form hydrogen bonds to the quinone oxygens O_1 and O_4 (see Fig. 1). The observed ENDOR spectra were interpreted using a spin Hamiltonian, $\mathcal{H}$, containing the electron and nuclear Zeeman interactions with the applied magnetic field $\mathbf{B_o}$, the hfc, and the nqc terms (e.g., (26)):

$$\mathcal{H} = \beta_e \mathbf{S}\cdot\mathbf{g}\cdot\mathbf{B_o} - \beta_N g_N \mathbf{I}\cdot\mathbf{B_o} + h\mathbf{S}\cdot\boldsymbol{A}\cdot\mathbf{I} + h\mathbf{I}\cdot\boldsymbol{P}\cdot\mathbf{I}, \quad (1)$$

where $\mathbf{S}$ is the electron spin operator, $\mathbf{I}$ is the nuclear spin operator of protons or deuterons in H-bonds to the carbonyl oxygens; $\boldsymbol{A}$ and $\boldsymbol{P}$ are the hfc and nqc tensors in frequency units, $\boldsymbol{g}$ is the electronic g-tensor, g_N is the g-factor of the corresponding magnetic nucleus (proton or deuteron) and β_e and β_N are the electron and nuclear magnetons, respectively, and h is Planck's constant. The first term in Eq. 1 represents the electronic Zeeman term that gives rise to the observed EPR spectra. The other three terms represent nuclear interactions, which are small and not resolved in the EPR spectrum but give rise to the ENDOR spectra. The hyperfine tensor component A_i is composed of anisotropic (dipolar) and isotropic contributions that can be written as

$$A_i = A'_i + A_{iso}. \quad (2)$$

For a proton in a typical hydrogen bond, the isotropic part A_{iso} is small compared to the anisotropic term A'_i, which arises from the dipolar interaction between the unpaired electron and the proton. In the simple point-dipole approximation (e.g., (26)) the anisotropic term can be written as

$$A'_i(\delta) = \frac{g_e\beta_e g_N\beta_N}{hr^3}\rho(3\cos^2\delta-1) = \frac{79.2}{r^3}\rho(3\cos^2\delta-1)\,[\mathrm{MHz}], \quad (3)$$

where g_e and g_N are the electron and proton g-values, ρ is the unpaired electron spin density at the quinone oxygen, δ is the angle between the applied magnetic field and the direction of the hydrogen bond, and r is the H-bond length in Å (for deuterons the numerical factor 79.2 is replaced by 12.16). The traceless tensor resulting from this approximation is axially symmetric, with principal components $A'_{\parallel}$ for $\delta = 0°$ (direction along the hydrogen bond) and $A'_{\perp}$ for $\delta = 90°$, with $A'_{\parallel} = -2\,A'_{\perp}$. The point-dipole model for the hyperfine interaction of H-bonds (see Eq. 3) has been shown to be valid in good approximation for quinone model compounds (27,28), in which the H-bonds lie in the quinone plane. In RCs, this is not the case leading to the difficulties discussed in the section on the H-bond lengths.