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On the role of tryptophan as a superexchange mediator for quinone reduction in photosynthetic reaction centers

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The rôle of tryptophan (W) in the quinone reduction in the reaction centers of *R. viridis* and *Rb. sphaeroides* is studied by a quantum chemical calculation of the electronic coupling matrix elements within the bacteriopheophytin-W-quinone subsystem, which is based on the X-ray structural data. The results favor the superexchange mechanism via W over the direct coupling. Uncertainties in the available X-ray coordinates and structural relaxation accompanying the formation of the P^+H^- state result in large changes in the relative contributions of the electronic couplings due to electron and hole superexchange. Aromatic amino acid residues may serve as essential functional components in electron transfer.

Reaction center; Quinone reduction; Electronic superexchange coupling; Tryptophan

1. INTRODUCTION

The reaction center (RC) of purple bacteria is a membrane-spanning protein within which the two primary electron transfer events of photosynthetic charge separation occur [1].

$$^1P^*HQ_A \longrightarrow P^+H^-Q_A \quad (1)$$

$$P^+H^-Q_A \longrightarrow P^+HQ_A^- \quad (2)$$

Reaction 1 involves electron transfer from the singlet excited state of the bacteriochlorophyll dimer (P) to the bacteriopheophytin (H), while reaction 2 corresponds to quinone (Q_A) reduction. The kinetic features of the second electron transfer step are similar in all bacterial RCs that have been isolated and studied so far [2–5]. For RCs of *Rhodobacter sphaeroides, Rhodopseudomonas viridis, Rhodobacter capsulatus* and *Chloroflexus aurantiacus*, the rate k_Q of reaction 2 at 300 K is reported to be 5×10^9 s^{-1} [2], 6×10^9 s^{-1} [3], 5×10^9 s^{-1} [4] and 3×10^9 s^{-1} [5], respectively. In all cases, the rate k_Q increases by a numerical factor of $\simeq 2$ between 300 K and 80 K [6–8]. This slight negative temperature dependence of k_Q has been interpreted [9,10] within the conventional non-adiabatic electron transfer theory to be indicative for an activationless electron transfer process with the rate given by

$$k_Q = \frac{2\pi |V|^2}{\hbar^2 \omega (2\pi p)^{1/2}} \left(\frac{\exp(\hbar\omega/k_B T) - 1}{\exp(\hbar\omega/k_B T) + 1} \right)^{1/2} \quad (3)$$

where V is the electronic coupling, ω is the characteristic protein medium vibrational frequency and $p = \Delta E/\hbar\omega$, with ΔE being the free energy gap for reaction 2. k_B is the Boltzmann constant and $\hbar$ is the Planck constant. Making use of the experimental rate $k_Q = 5 \times 10^9$ s^{-1} [2–5] together with the characteristic protein frequency $\hbar\omega = 100$ cm^{-1} [10], eqn 3 results in $V \simeq 4$ cm^{-1} for the matrix element of the electronic coupling between H^- and Q_A.

In the three-dimensional structure of the RCs of

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Abbreviations: RC, reaction center; P, bacteriochlorophyll dimer; Q_A, quinone; W, tryptophan

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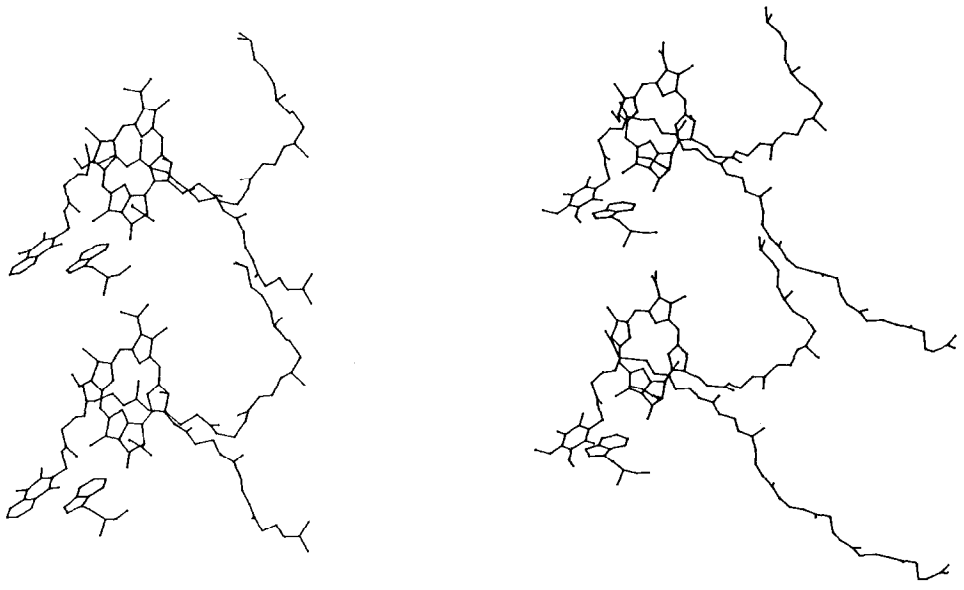

Fig.1. Stereo plots of the cofactors bacteriopheophytin H and quinone Q together with tryptophan W across the A branch of the RC. (a) *Rb. sphaeroides* [11,12]; (b) *R. viridis* [13].

both *Rb. sphaeroides* [11,12] and *R. viridis* [13], tryptophan M252 and M250, respectively, is the only amino acid residue close to both H and Q_A. This tryptophan (W) is in van-der-Waals contact with both H and Q_A, however, in the two species there are differences in its exact positioning relative to the π-system of the quinone (fig.1). The same tryptophan is conserved in all purple bacterial RCs that have been sequenced so far as well as in the RC of the green bacterium *Chloroflexus aurantiacus* and in photosystem II of plants [14].

It is the goal of this paper to explore the role of the tryptophan as a functional component for electron transfer between H and Q. A direct participation of tryptophan as a kinetic intermediate can be excluded because of its high reduction potential. Thus, it can only participate as a superexchange mediator [15,16].

The intermolecular superexchange system H-W-Q_A in *R. viridis* and *Rb. sphaeroides* is especially transparent since there is only a single amino acid residue (AAR) mediating between donor and acceptor. In the following, we estimate the relative contribution of the superexchange coupling to the effective coupling between H^- and Q_A through W. This estimate rests on the intermolecular overlap approximation [15] and is based on the structural data of the RCs of *R. viridis* [13] and *Rb. sphaeroides* [11,12].

2. METHOD AND RESULTS

The superexchange interaction enhances the direct electron coupling between the electron donor H^- and acceptor Q_A which are separated by a large distance (center-to-center distance $\simeq$ 14 Å, edge-to-edge distance $\simeq$ 10 Å [13]). This effect originates from mixing the donor electronic state with a state HW^-Q_A (or $H^-W^+Q_A^-$) involving the amino acid residue W, located between H and Q_A. Two possible superexchange interactions can exist in parallel: (i) electron superexchange through HW^-Q_A; (ii) hole superexchange through $H^-W^+Q_A^-$.

The effective coupling V is the sum of the direct coupling V_d and the superexchange coupling V_{super}, being given by

$$V = V_d + V_{super} \tag{4}$$

where the superexchange coupling, which consists of the sum of interactions (i) and (ii), is

$$V_{super} = V_{super}^- + V_{super}^+ \tag{5}$$

with the electron superexchange contribution being

$$V_{super}^- = \frac{V_{HW}^- \cdot V_{WQ}^-}{\delta E_{HW}^-} \tag{6a}$$

and the hole superexchange contribution being

$$V_{super}^+ = \frac{V_{WQ}^+ \cdot V_{WQ}^+}{\delta E_{HW}^+} \tag{6b}$$

V_{HW}^- is the electronic coupling between H^-WQ_A and HW^-Q_A, and V_{HW}^+ is the coupling between H^-WQ_A and $H^-W^+Q_A^-$. V_{WQ}^-

is the electronic coupling between HW^-Q_A and HWQ_A^-, while V_{WQ}^+ is the coupling between $H^-W^+Q_A$ or HWQ_A^-. δE_{HW}^- is the vertical energy difference between the states H^-WQ_A and HW^-Q_A, while δE_{HW}^+ is the vertical energy difference between the states H^-WQ_A and $H^-W^+Q_A^-$. Both vertical energy differences are taken at the nuclear configuration of the intersection of the potential energy surfaces of the states H^-WQ_A and HWQ_A^- [17]. The effectiveness of the superexchange interaction term depends on (1) the values of the electronic couplings with the intermediate states, and (2) the vertical energy difference. Both factors are more favorable for aromatic than for aliphatic AARs as intermediates because aromatic groups are characterized by a larger intermolecular overlap of π-orbitals which enhances the electronic couplings, and by lower reduction potentials which decrease the vertical energy difference.

Some semiempirical computational methods were recently advanced for the estimate of intermolecular electron couplings which induce electron transfer processes in the RC [18–21]. In this paper we shall use the intermolecular overlap approximation recently advanced by us [15,21] to obtain information regarding the relative magnitudes of the electronic transfer integrals. The calculation of the intermolecular overlap integrals requires the molecular orbital (MO) coefficients of the active orbitals and the intermolecular atomic overlap integrals. The MO coefficients were taken from the SCF-MO-INDO calculations [22,23] using the X-ray structural data for the relevant cofactors and for the tryptophan. The intermolecular atomic overlap integrals S_{IJ} between the molecular entities I and J were calculated using Slater atomic orbitals. More details of the method can be found elsewhere [21]. The electronic couplings are assumed to be proportional to the intermolecular overlaps S_{IJ}, i.e.,

$$V_{IJ} = K \cdot S_{IJ} \qquad (7)$$

where K is a proportionality coefficient. For intermolecular electron transfer integrals in aromatic crystals of naphthalene and anthracene, $K = 22$ eV [24]. For electron transfer integrals between cofactors in the photosynthetic RC the proportionality coefficient may be larger, i.e. for the (superexchange) interaction between the bacteriochlorophyll dimer and the accessory bacteriochlorophyll $S_{IJ} = 1.12 \times 10^{-4}$ [21] and $V_{IJ} = 60$ cm^{-1} [21] so that $K = 66$ eV. In what follows, we shall use the coefficient $K = 22$ eV bearing in mind that the individual electronic couplings may be underestimated by a numerical factor of $\simeq 3$ while the superexchange interaction (eqn 6), which involves the product of two electronic couplings, may be underestimated by about an order of magnitude.

Both possible superexchange interactions, which involve electron superexchange (i) and hole superexchange (ii) are characterized by large values of S_{WQ}, originating from the large overlap of the π-system of Q_A and W with center-to-center distances of $\simeq 3$ Å. An experimental indication of this large overlap can be inferred from the appearance of ^{14}N and ^{15}N hyperfine couplings in the ENDOR spectra of Q_A^- in *Rb. sphaeroides* [25].

For the electron superexchange (i) the vertical energy difference is given by $\delta E_{HW}^- = \Delta E_{HW} + \lambda$, where ΔE_{HW} is the equilibrium energy gap between H^-W and HW^-, and λ is the medium reorganization energy. ΔE_{HW} can be estimated from the difference in the reduction potentials $\Delta E_{HW} = E_{RED}(H) - E_{RED}(W)$. The electrochemical reduction potential of tryptophan is not available, but from the information on similar organic molecules in polar solution one may conclude that it is $\simeq -2$ V when measured against a saturated calomel electrode (SCE) [26]. The reduction potential of H is $E_{RED}(H) = -0.8$ V (SCE) [27]. Thus, $\Delta E_{HW} = 1.2$ eV. Regarding λ, it is known that the reorganization energies in the RC are in the range between 0.2 and 0.5 eV [10,15,21]. The vertical energy difference can be estimated therefore as 1.7 eV, and in view of the uncertainties in the estimate we shall take $\delta E_{HW}^- \simeq 2$ eV.

The hole superexchange (ii) involves the intermediate $H^-W^+Q^-$. The vertical energy difference can be roughly estimated as $\delta E_{HW}^+ = I - EA - P - C$ where $I = 7$ eV [28] is the ionization potential of tryptophan, $EA \simeq 1$ eV is the electron affinity of menaquinone, $P \simeq 1$ eV is the polarization energy of the medium by $H^-W^+Q^-$, and $C \simeq 3$ eV is the Coulomb interaction within $H^-W^+Q^-$. Thus, $\delta E^+ \simeq 2$ eV, being close to the value of δE_{HW}^-.

The calculations of the intermolecular overlap integrals for electron and hole superexchange were done using the ultimately refined X-ray coordinates for *R. viridis* [29] at 2.3 Å resolution and for *Rb. sphaeroides* at 2.8 Å resolution [11,12]. The results for the molecular overlaps are given in table 1. Values in paren-

Table 1

Intermolecular electronic overlaps (in units of 10^{-4}) between active orbitals on bacteriopheophytin H, tryptophan W and quinone Q in RCs of *R. viridis* and *Rb. sphaeroides*

Species	Electron transfer		Hole transfer		Direct electron transfer
IJ:	HW	WQ	HW	WQ	HQ
R. viridis	0.6	45	0.1	105	8×10^{-4}
Rb. sphaeroides	0.3 (0.3)	24 (92)	0.16 (0.14)	91 (58)	55×10^{-4}

Values are given for electron transfer involving only the LUMOS of H, W and Q and for hole transfer through the intermediate $H^-W^+Q^-$ involving the HOMO of W. Numbers in parentheses refer to a translational displacement of W by $\Delta x_i = \Delta y_i = \Delta z_i + 0.2$ Å, for all atomic coordinates which is within the error of the X-ray structure analysis

Table 2

Electronic couplings in units of cm^{-1} for the superexchange processes and the direct process

Species	Coupling		
	V_{super}^-	V_{super}^+	V_d
R. viridis	0.5	0.2	0.015
Rb. sphaeroides	0.15 (0.6)	0.3 (0.16)	0.12

A value of $K = 22$ eV is used (eqn 6). Numbers in parentheses refer to a translational displacement of W by $\Delta x_i = \Delta y_i = \Delta z_i = 0.2$ Å in all atomic coordinates

theses refer to a calculation with the structural data involving a translational shift of W by 0.2 Å in all cartesian atomic coordinates x_i, y_i and z_i, which is well within the estimated experimental uncertainty of the coordinates, i.e., ± 0.4 Å for *Rb. sphaeroides* [11]. This calculation was performed to assess the error in calculated matrix elements due to errors in the X-ray structure analysis and/or changes of the matrix elements due to configurational relaxation [30].

From the overlap values in table 1 we calculated the electronic couplings V_{IJ} using eqn 6 with $K = 22$ eV. The superexchange couplings for the two interactions computed according to eqn 6 together with the direct electronic coupling are presented in table 2.

3. DISCUSSION

Our analysis of the mechanism of quinone reduction in RCs of *R. viridis* and *Rb. sphaeroides* shows that the superexchange interaction is of comparable magnitude in both species and for both electron and hole superexchange. The direct coupling in *Rb. sphaeroides* is different from the one in *R. viridis* mainly due to the different quinones, ubiquinone and menaquinone, present in the two RCs. In the case of *R. viridis* the direct coupling is negligibly small compared to the two superexchange couplings. In *Rb. sphaeroides* the direct coupling is smaller than V_{super} but still of comparable magnitude with the superexchange couplings. The total coupling, $V = V_d + V^+_{\text{super}} + V^-_{\text{super}}$, calculated for the two RCs, is smaller than the observed value of 4 cm^{-1} by about an order of magnitude. As was discussed above, the scaling factor $K = 22$ eV may not be appropriate for the present case. With the scaling factor $K = 66$ eV (as used for the primary charge separation step) we can reproduce the observed coupling. With this scaling factor of $K = 66$ eV the relative contribution of the direct coupling becomes smaller. A rough assessment of the numerical results for *Rb. sphaeroides* (table 2) reveals that for this scaling V_d (proportional to K) is lower than $V^+_{\text{super}} + V^-_{\text{super}}$ by a numerical factor of 5–10, as compared to the contribution of the superexchange coupling (proportional to K^2). Although this argument favors the predominance of the superexchange mechanism, the direct exchange cannot be neglected, especially since over distances of more than 10 Å the numerical results for electronic couplings are particularly sensitive to the tails of the electronic wavefunctions of H and Q [31].

The computations are based on the coordinates obtained from the X-ray analysis on the RC crystal in its equilibrium state. Estimated errors in the crystallographic analysis of *Rb. sphaeroides* RC are of the order of ± 0.4 Å. Moreover, possible structural relaxation of the precursor state P^+H^- is another source for uncertainties in the values of the atomic coordinates. In order to obtain some idea about the possible effects due to the coordinates' uncertainties we have performed a calculation (results appear in parentheses in tables 1 and 2) for *Rb. sphaeroides*, in which the coordinates of the tryptophan were shifted by 0.2 Å. As can be seen from table 2 the effects are large. The superexchange coupling via the electron mechanism is increased by a numerical factor of 4, whereas the coupling via the hole superexchange becomes smaller by a factor of 2. The qualitative result regarding the importance of superexchange remains intact, but we must conclude that even the present accuracy of the atomic coordinates is still insufficient for a quantitative estimate of the electronic coupling elements.

Substitution of tryptophan against valine in site-specific mutagenesis has been reported to reduce both the photosynthetic growth factor of bacteria and the cytochrome oxidation activity of the isolated RC in the case of *Rb. capsulatus* [32]. The drastic change can be due to a deleterious structural effect brought about by mutagenesis or, in a later stage, by the isolation procedure of the RC. Alternatively, the observed malfunction can reflect a significant decrease of the electronic coupling and thereby of the electron transfer rate between H^- and Q_A. Any interpretation of such effects requires information on the 3D structure together with the electron transfer rate k_Q and its temperature dependence for both the specific mutant and the native species. Neglecting configurational changes in the protein, the mutagenesis results are consistent with a significant superexchange contribution to the coupling between H^- and Q_A by tryptophan. Upon exchange of the aromatic tryptophan against the aliphatic valine the contribution of superexchange to the electronic coupling is expected to be reduced, resulting in a considerable decrease of k_Q.

In conclusion, aromatic amino acid residues may serve, in addition to their structural role, as *essential functional components* in electron transfer processes as superexchange mediators.

They may even function as genuine redox components as the tyrosine residue, which acts as electron donor to $P680^+$ in photosystem II [33]. The contribution of superexchange to the overall coupling between H^- and Q seems to be similar for different species as concluded from the almost identical electron transfer pattern between H^- and Q_A throughout purple and green bacteria in conjunction with the universal conservation of this tryptophan in the primary sequences. However, individual superexchange parameters may well depend on the organism which may differ in the nature of the cofactors and the detailed structure.

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Note added in proof: After completion of our calculations on reaction centers of *Rps. viridis* it was brought to our attention that S.O.J. Scherer and S.F. Fischer were also performing theoretical studies on the tryptophan problem and had come to similar results.