Charge Separation in Photosynthetic Reaction Centers

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Structural influences on the direction of electron transfer in the charge separation process of the photosynthetic reaction centers of *Rhodopseudomonas viridis* and *Rhodobacter sphaeroides* are studied using quantum chemical models to calculate the electronic factor. Our results support the sequential mechanism for the primary charge separation. Using crystallographic coordinates refined in 1994, we find a larger coupling between the special pair D and the accessory bacteriochlorophyll B_A of the A branch than between D and B_B of the inactive B branch. We have been able to localize the coupling to the acetyl group of ring I of D_B and the methyl group of ring III of B_A. The corresponding contact between D_A and B_B has less coupling, apparently due to a distorting hydrogen bond between the acetyl group on D_A and the imidazole side group of HisL168. The coupling between B_A and bacteriopheophytin Φ_A is accomplished by two methyl groups, directly connected to the conjugated π system of the chromophore.

1. Introduction

The geometrical structures of two bacterial reaction centers have been known for some time,[1−3] and the field is open for studies on how the arrangement of cofactors and protein influences the mechanisms of charge separation and subsequent transfer of the separated electron. In *Rhodopseudomonas* (*Rps.*) *viridis* (Figure 1) and *Rhodobacter* (*Rb.*) *sphaeroides* the charge separation occurs in a reaction center (RC) consisting of four bacteriochlorophylls (BChl *b* and BChl *a*, in *Rps. viridis* and *Rb. sphaeroides*, respectively [Figure 2]) called D_A, D_B, B_A, and B_B and two bacteriopheophytins (BPh *b* and BPh *a*, respectively) called Φ_A and Φ_B. D_A and D_B form a dimer D, also called the special pair. D, B_A, and Φ_A are organized in a chain and similarly with D, B_B, and Φ_B. The symmetry connection between the A and B subsystems is approximately C_2.[1−4] The primary charge separation and electron-transfer (ET) step is asymmetrical and occurs from D via B_A to Φ_A[5] in the natural system and almost all mutants studied. Extensive experimental work has been carried out to determine the factors that cause this asymmetry. For example McDowell et al. have shown[6] that if an axial ligand of any of the BChl *a*'s is mutated in such a way that the Mg^{2+} ion is lost, there is no change in the direction of the charge flow toward the A side.

The chromophores are attached to the protein by hydrogen bonds and van der Waals forces: the cofactors of the A branch mainly to the L subunit and those of the B branch mainly to the M subunit. In particular there is a conserved hydrogen bond between the acetyl group of ring I of D_A and HisL168 of the L subunit.[3] In the symmetric position there is no hydrogen bond in *Rb. sphaeroides* but a rather close contact between D_B and B_A.[3] In *Rps. viridis* there is a hydrogen bond between TyrM195 and the acetyl group of ring I of D_B.

Progress in computer technology in recent years has made calculation of semiempirical[7−16] and even ab initio[17] wave functions for these extensive systems possible. Wave functions for the chromophores obtained using semiempirical methods[18] appear to be very similar to wave functions calculated by ab initio methods[19] and show qualitatively the correct spectrum compared to the experiments. We have designed a scheme within a well-tested semiempirical approach whereby the electronic factor for ET reactions between chromophores or other groups can be obtained.[15,20,21] We will here adapt that scheme to the HyperChem programs[22] where the semiempirical method used for the spectrum is ZINDO/S.[23]

In the absorption spectrum of the RC of *Rps. viridis* there is a large red shift of the S_1 state compared to the S_1 state of the monomer of BChl, from 790 to 960 nm. The excited state may be thought of as partly excitonic and partly of charge-transfer nature.[16,24] Possibly there are some oscillations of charge within D immediately after excitation. Important for us is that all evidence suggests that the charge is taken from both monomers of the special pair at charge transfer. Calculations also show that the lowest excited states correspond to one-electron excitations without large configuration interaction contributions to the wave function.[8]

Originally some experiments seemed to suggest that the photoexcited electron jumps directly from the special pair to Φ_A without occupying B_A. However, more recent experiments by Zinth et al. show clearly[25] that there is sequential electron transfer via an intermediate state of the type $D^+B_A^-\Phi_A$, which is occupied for a short time as originally suggested by Shuvalov et al.[26] and Haberkorn et al.[27] In our previous work we found a negligibly small probability for direct transfer,[15] consistent with the results of others[28] and in general agreement with the sequential model.[25−27] The direct ET model, on the other hand, was justified theoretically on the basis of a superexchange model, where a single virtual state ($D^+B_A^-\Phi$ in this case) mediates the ET.[29] Further discussion of theoretical models will be given below.

To understand the photosynthetic ET processes in detail, it is important to know which structural factors are essential for directed ET. The high forward ET rate of the primary charge

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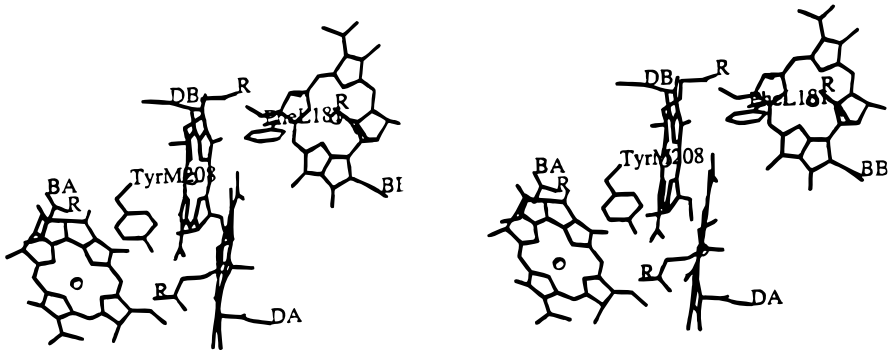

Figure 1. Stereoview of the special pair and accessory BChl's of *Rps. viridis* including TyrM208 and PheL181, using the crystallographic coordinates of ref 2 (1prc.pdb). Phytyl chains (R) have been omitted from the figure.

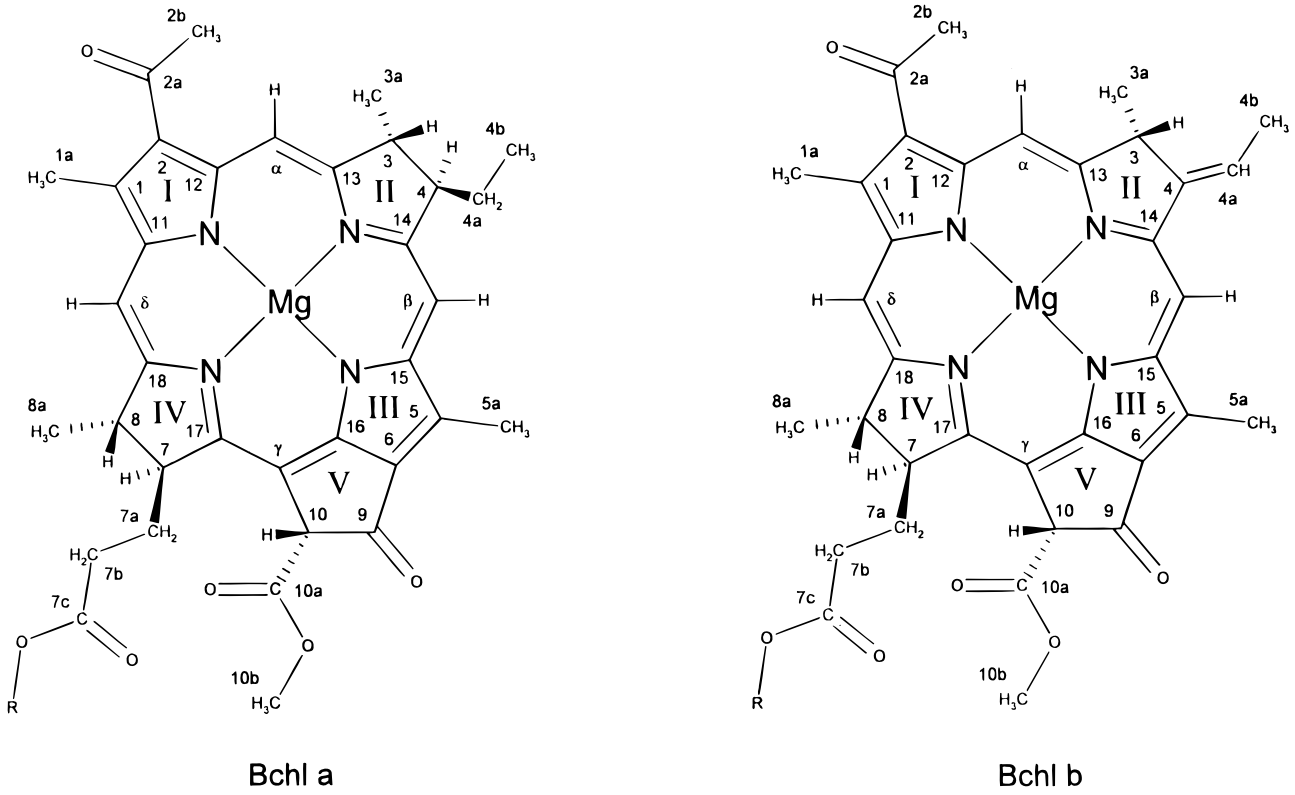

Figure 2. BChl *a* and BChl *b* monomers.

separation step is explained by the Marcus model as due to equal reorganization energy and free energy change.[30] These energies depend on the structure of the chromophores (being π systems, etc.) and the surrounding protein structure. Energetic factors of this type appear to be important in directing the electron selectively along the A pathway,[28] but there is hardly any solid proof for that. The slow return rate to the ground state may also be explained in the Marcus model. If the reorganization energy from the ground state to the excited charge-transfer state is small, the energy surfaces for the two states are almost parallel and have no crossings at low energy.

Directional influences may also occur due to the electronic coupling between the donor and acceptor parts of the protein. The magnitude of the coupling determines the rate via the Landau−Zener approximation.[31] In proteins where the coupling is small, the rate is proportional to the electronic coupling squared. The same dependence appears in methods where the nuclear motion is treated quantum mechanically.[32] The structural dependencies of the electronic coupling have been discussed in the literature. Moser et al. treated this problem on the basis of measured rate constants for a number of ET steps in the RCs of *Rps. viridis* and *Rb. sphaeroides*.[33] When the rate dependence associated with the reaction barrier was eliminated, they found that the remaining structural influence due to the electronic factor appears to be a uniform exponential distance dependence, without apparent directional properties.[33] Gray et al., on the other hand, have emphasized the opposite point of view on the basis of data from other protein systems, that directional differences in electronic factor are generally of great importance for the rate of ET reactions.[34] Theoretically the latter result is clearly expected, since the electronic factor is sensitive to changes in the structure.[20,35,38] The possibility that evolution has used this possibility to achieve directional ET cannot be excluded.

In the present case the donor and acceptor orbitals are π orbitals extended over the conjugated parts of the chromophores. In such a case the electronic factor is a product of the coefficient squared of the π orbital at the contact atomic orbital, multiplied by an exponential decrease factor over empty space.[36] The uniform exponential decrease as a function of center-to-center distance of the chromophores, as suggested by Moser et al.,[33] can hardly be justified, although a limited set of data shows an

exponential trend. The reason is that in a conjugated system the coefficients of the atomic orbitals are of the order of the inverse of the square root of the number of atomic orbitals of the conjugated system. The total decrease of the electronic factor will then be less.

In the present paper a model for the calculation of the electronic factor based on molecular orbitals will be applied to the primary charge separation in the reaction center of *Rps. viridis* and *Rb. sphaeroides*. This theoretical model has been extensively tested on small systems by comparison to results from ab initio calculations and found to give a qualitatively correct description.[35,36] We find that the reason for rate differences between the A and B branches as well as differences in the rate between the two primary fast ET steps to a large extent can be ascribed to differences in electronic factors. We also conclude that the same type of "ET pathway" is used for *Rps. viridis* and *Rb. sphaeroides*.

2. Theoretical Model

For small electronic couplings the rate for a single ET step may be obtained[21] as

$$k = \frac{2\pi}{\hbar} H_{12}{}^2 F \qquad (1)$$

where F is the Franck−Condon factor, which depends only on the nuclear motions. Alternative forms of the rate equation have been used.[37−39] The electronic coupling H_{12} is best defined as half of the energy splitting $|\Delta|$ at the seam of two crossing energy surfaces, whose electronic states have a different localization of the electronic density, corresponding to the electron being at the donor (Ψ_D) or acceptor (Ψ_A). If $|\Delta|$ is small, as is the case in proteins, the rate is proportional to Δ^2 by eq 1.

At the seam the two wave functions are of the type $\Psi_D + \Psi_A$ and $\Psi_D - \Psi_A$. In the case of proteins it is unlikely to hit a zero crossing due to symmetry or by chance. The consistency of the results may be tested in calculations of the electronic factors in different points on the seam. In our experience the electronic factor shows a small relative variation along the seam.

In ET reactions in proteins the transition state is very sharp since $|\Delta|$ is small compared to the reorganization energy. Very small changes in the nuclear coordinates at the seam lead to large changes in one singly occupied orbital along the reaction path. Changes in other orbitals are small. It is then possible to rewrite the many-electron problem to a one-electron problem. We use semiempirical methods[23,40] and consider the energy difference between the LUMO and the LUMO+1 rather than the corresponding total energy difference.

We have made use of the HyperChem programs,[22,23] by which it is very easy to get an overview of the system. The reaction field necessary to reach the avoided crossing between the energy curves was accomplished with the help of a positive and a negative ion, H_3O^+ and Cl^-, respectively. The two electronic states that meet at the avoided crossing are different in the two orbitals, LUMO and LUMO+1, of the ground state. One of these orbitals is localized on the donor and the other one on the acceptor. At the avoided crossing they are as close as possible in energy, and the character is 50% of each of donor and acceptor with different sign combinations. Since the excited states are of one-electron nature,[12,15] we may obtain the electronic factor as the orbital energy difference between LUMO and LUMO+1 for the ground state in the external field when this orbital energy difference is a minimum, corresponding to the avoided crossing. This is easily done with the help of HyperChem, which also provides an intersection plot of the orbitals (Figure 3).

Our previous results showed that the calculated $|\Delta|$ at the avoided crossing between the states $D^*B_A\Phi_A$ and $D^+B_A\Phi_A^-$ is very small (8×10^{-7} eV), obviously too small to explain the high ET rate. In subsequent calculations we included other parts of the protein of *Rps. viridis* which are located between the special pair and Φ_A and which may provide a larger coupling than via B_A.[15] In the first of these calculations the phytyl chain of D_A was included and in the second the phytyl chain and the phenol side group of TyrM208 (M210 in *Rb. sphaeroides*), located in between D and B_A. The value of $|\Delta|$ increased by a factor of 4 in the first calculation and another factor of 4 in the second calculation. $|\Delta|^2$ (and the rate k) is thus increased by a factor of $(4^2)^2 = 256$ compared to the case when only coupling via B_A is taken into account. On the other hand, removing B_A with phytyl and TyrM208 present has no effect on the value of Δ. We conclude that B_A is much less important than M208 for the coupling between the states $D^*B_A\Phi_A$ and $D^+B_A\Phi_A^-$. The coupling via M208 (M210) is still too small to explain the high ET rate, however.

In the calculations of the coupling between D and B_A (the sequential model) using the old unrefined coordinates we obtained a value of $|\Delta|$ of 5 cm^{-1}, which is close to other estimates (10 cm^{-1};[17] 5.9 cm^{-1};[41] 5.4 cm^{-1};[42] or somewhat smaller[8]).

3. Electronic Coupling in the Sequential Model

In the calculations on the primary ET step using the refined coordinates[2,3] we include the special pair D and one accessory BChl, either B_A or B_B. Another set of calculations is done on the second step from B_A to Φ_A. Examination of the structure reveals three possible contacts between D and B_A: (1) close to the attachment of the phytyl chain of D_A and the ethyl (BChl *a*) or vinyl (BChl *b*) group at ring II of B_A; (2) via a water molecule in contact with the axial imidazole group of D_A and the carbonyl group at ring V of B_A; (3) between the acetyl group of ring I of D_B and the methyl group on ring III of B_A. There are corresponding contacts with the accessory B_B of the M branch. The influence on the electronic factor $|\Delta|$ was tested by intercepting overlap one at a time at these contacts. In the first case the phytyl chain and the CO_2R group between this group and ring IV were removed and replaced by hydrogen. The second contact was broken by removing the water molecule. We found no decrease of $|\Delta|$ after these modifications (Table 1). The third contact was broken by replacing the methyl group of ring III of B_A by hydrogen. There was a substantial decrease of $|\Delta|$ (Table 1). We therefore conclude that the third contact is the important one. In the subsequent calculations all atoms at the contact points mentioned were included, but the atoms at the third contact were manipulated. In all cases we had strong indications that $|\Delta|$ was dependent on the nature of the third contact.

The calculations with all groups retained were repeated for the system $D+B_B$. The value of $|\Delta|$ was smaller by a factor of 3 than in the corresponding calculation involving B_A, which leads to a factor of 9 in the rate. We conclude that the contact (3) is more important in the case $D+B_A$ than in the case $D+B_B$ on account of a larger electronic factor.

Figure 3 shows LUMO and LUMO+1 at the important contact points between D_B and B_A for *Rps. viridis*. Notice that the MOs have approximately the same shape, but are in-phase and out-of-phase, respectively, in the region between the dimer

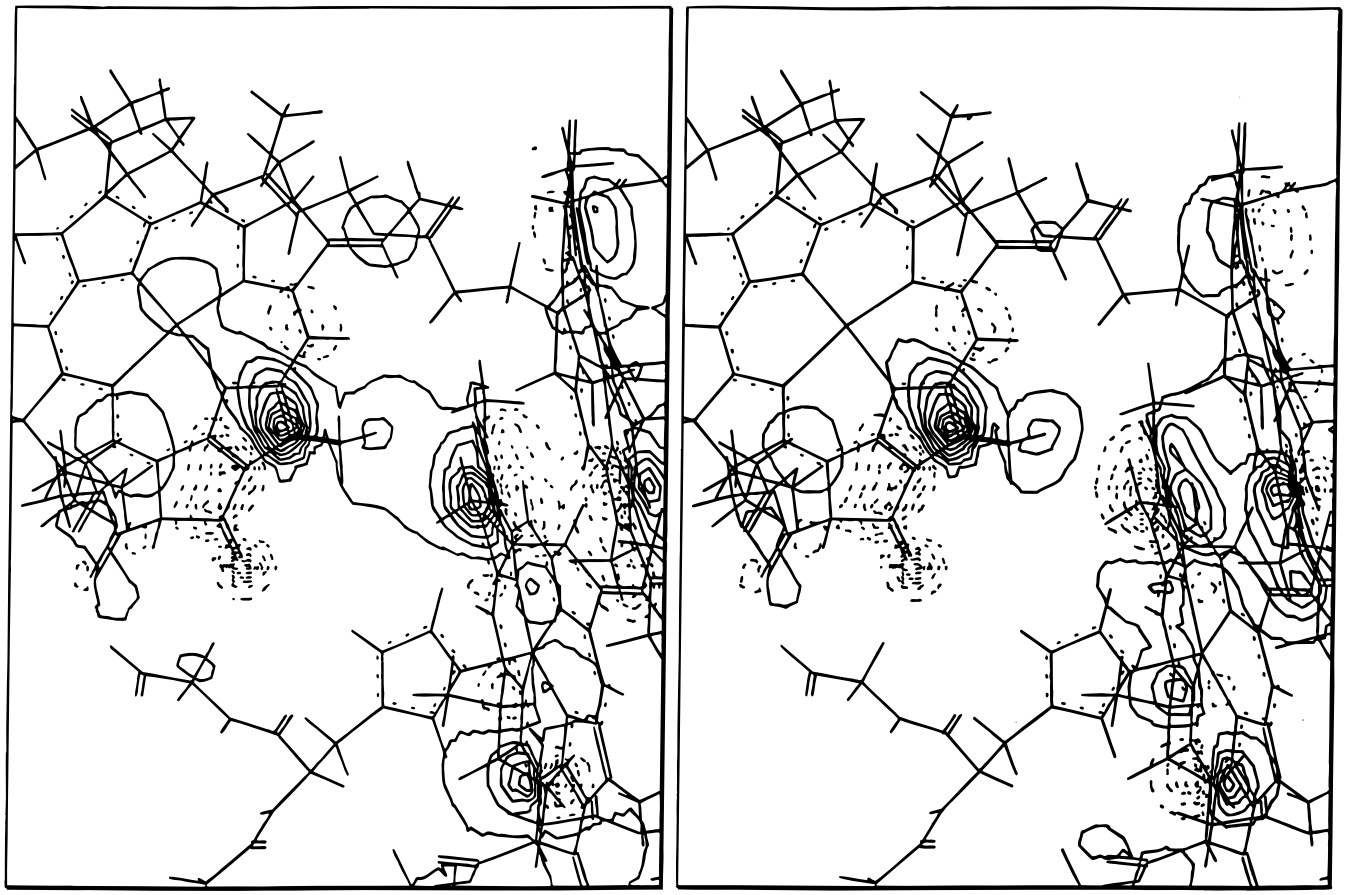

Figure 3. LUMO (left) and LUMO+1 (right) at the connection point D−B_A for *Rps, viridis*. The wave function is given in a plane roughly perpendicular to the D planes and parallel but above the plane of B_A.

TABLE 1: Electronic Factor $|\Delta|$ ($=2H_{12}$) Calculated with the Help of HyperChem

ET step	groups included	Δ (cm^{-1})
Rps. viridis		
$D \rightarrow B_A$	all	19
	(1) all but phytyl	26
	(2) all but H_2O	19
	(3) all but $CH_3 \rightarrow H$	7.3
$B_A \rightarrow \Phi_A$	all	207
	$2\times CH_3 \rightarrow 2\times H$	11
Rb. sphaeroides		
$D \rightarrow B_A$	all	21
	(1) all but phytyl	21
	(2) all but H_2O	21
	(3) all but $CH_3 \rightarrow H$	1.5
	(4) acetyl→H	7.7
$B_A \rightarrow \Phi_A$	all	14
	all but $2CH_3 \rightarrow 2H$	0.27

and the monomer. Also notice that LUMO and LUMO+1 are bonding between D_B and D_A, contrary to the HOMO (not shown). This means that we can expect a shorter equilibrium distance between these two chromophores in the excited state, which may in fact be the cause of the broadening of the absorption spectrum.

In *Rb. sphaeroides* the groups corresponding to (3) on the dimer and monomer also give a large coupling. In both cases contact (3) is conserved in BChl *a* and BChl *b* and involves side groups connected to the two pyrrole rings which belong to the conjugated system. In contact (1) on the other hand, the side groups are different in BChl *a* and BChl *b*, and both side groups are attached to the saturated pyrrole groups which do not belong to the conjugated system. In both cases there is a tunneling pathway from the π system on D directly to the π system on the accessory B_A via the methyl group on ring III.

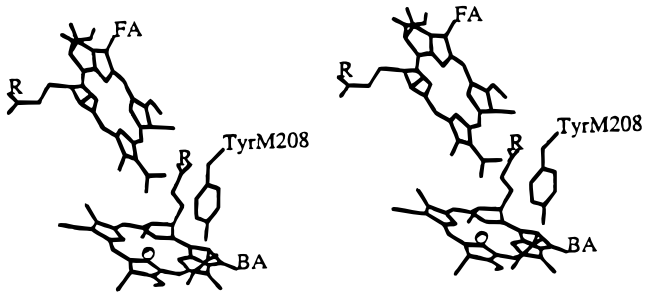

Figure 4. Interaction between B_A and Φ_A for *Rps. viridis.*

The coupling between B_A and Φ_A was also calculated. For *Rps. viridis* we found a larger $|\Delta|$ than in the case of D−B_A (Table 1). The contact is via two methyl groups (Figure 4), both from ring I. We notice, however, that the coupling is much larger in the case of *Rps. viridis* than in the case of *Rb. sphaeroides* (Table 1). Examination of the distances between the chromophores in the two cases reveals the reason for this. The distance between the carbon atoms is 3.28 Å in the former case and 3.86 Å in the latter case. In previous work the electronic factor has been calculated for the approach of two CH_4 molecules both by semiempirical and ab initio methods.[42] We found good agreement between the two methods and a decrease of the coupling with distance which corresponds roughly to the values calculated here for the methyl−methyl contact between B_A and Φ_A. The reason for the great difference in distance between the crystallographic coordinates is not clear to us. There appears to be no reason for such a large difference.

4. Discussion

The calculated electronic factor for direct ET from the special pair D to Φ_A ($D^*B_A\Phi_A \rightarrow D^+B_A\Phi_A^-$) is very small. This is inconsistent with the measured high ET rate from D to Φ_A, but consistent with experimental and theoretical results in other proteins for the same donor−acceptor distance.[33] We may conclude, in agreement with our earlier paper,[15] that the high rate can only be explained by sequential ET, which is consistent with new experimental data.[25]

In the superexchange model[29] only one state of type $D^+B_A\Phi_A$ is included in a perturbation theory:

$$V_{13} = \frac{V_{12}V_{23}}{E_2 - E_1} \tag{2}$$

$E_2 - E_1$ is the excitation energy to a hypothetical charge-transfer state of type $D^+B_A\Phi_A$ but without atomic polarization (since the geometry of the direct ET system applies) and thus cannot be obtained experimentally.

Equation 2 has been applied to the problem of ET in RC to calculate the relative magnitude of the direct electronic coupling between the special pair and Φ of the M and L subunits. Equation 2 is derived on the assumption $E_2 - E_1 \gg V_{12}$ and $E_2 - E_1 \gg V_{23}$. However, in the case V_{12} and V_{23} tend to zero, $E_2 - E_1$ also needs to do so to have large enough V_{13}. A closer analysis shows that there will then be a significant population of the intermediate state with the electron on B_A ($D^+B_A^-\Phi$), which means sequential ET.

There is another problem with the superexchange method. Although the derivation is similar to the derivation of the Green function method,[20] closely related to the method used here, it differs from the latter method in that only one term is included in the final eq 2. The Green function method may be written as a sum over bridge states[20] of the same type as in eq 2. In our experience a great number of such terms is necessary for a converged result. The mixing of the donor and acceptor orbitals which gives a probability for ET from donor to acceptor appears as an interaction with a great number of excited states rather than just one. The conclusion is that the superexchange method is less useful in the present application.

In the present case it is assumed in the application of the superexchange model that the coupling is provided by the accessory B_A. However, we have found that B_A may be left out in the calculation of Δ corresponding to direct transfer, whereas leaving out other bridging groups such as TyrM208 (210) and the D_A phytyl chain leads to smaller values of $|\Delta|$. There are good reasons for that. M208 but not the accessory B_A is placed near the connection line between D and Φ_A. In the sequential model, however, this is not important since there are two distinct electron leaps.

A remarkable thing with biological reaction centers is that they are capable of using singlet states for charge separation, thereby maintaining a higher chemical potential for the photosynthetic process. Obviously back reactions to the lower triplet state must be avoided. However, the hypothetical direct mechanism involves the same MO for forward ET as for the back reaction to the triplet. It is hard to avoid the conclusion that a fast back reaction to the triplet state would occur, had $|\Delta|$ for direct transfer been sufficiently large to explain the very fast forward reaction. The sequential mechanism solves this problem, since the intermediate triplet state $D^+B_A^-\Phi_A$ has almost the same energy as its corresponding singlet state. There is hence a large barrier for the reverse ET via this triplet state.

We have examained in detail the coupling between the special pair D and the accessory BChl's and between B_A and BPh (Φ_A). The most significant coupling is between the conjugated system of D_B, close to the acetyl group of ring I, and a methyl group at ring III of B_A, which is close to the conjugated system of this chromophore, a possibility that has been suggested in earlier work.[43] The corresponding coupling from D_A to B_B was considerably smaller. This suggests that ET is favored along the A branch not only because of energy but also because of electronic coupling. The coupling between the two conjugated systems are through-space couplings, possibly helped by the orbitals in the vicinity of the contact point. However, it is the π orbital of the carbon atom to which the carbonyl group is attached rather than the π orbital of carbonyl itself that is important in the coupling.

Replacing the TyrM210 residue by the larger tryptophan leads to a much slower rate of the primary charge separation.[44,45] In the past this has usually been ascribed to a higher energy of the $D^+B_A^-\Phi$ state. In our mind an entirely different reason may also be important. The M210 residue (M208 in *Rps. viridis*) is pointing into the crevice between D_B and B_A. A larger residue is likely to lead to an increased distance between D_B and B_A and hence a smaller electronic factor for the primary charge separation. Temperature fluctuations of M210 may also explain the decrease of rate at increasing temperatures.[37]

5. Conclusions

It is an interesting fact that the photosynthetic apparatus of two different species have evolved with slightly different chromophores to the bacterial RCs of the present, which are very similar in their geometrical structure and mechanism. In previous work the characteric features of this geometry have been discussed almost exclusively on the basis of free energy. The interpretation of rate data has lead to the conclusion that the electronic coupling is not much affected by the protein and decreases uniformly and exponentially with distance. Theoretically there is no reason why the electronic factor should not react to structural changes. It is more reasonable that evolutional pressure leads to similar optimum conditions for biological ET reactions. There are strong indications in the work of Gray and co-workers[34] that artificial systems behave more erratically than natural ones and with smaller electronic factors. For the same reason the mutated systems usually have slower charge separation rates.

Although the accuracy in the theoretical predictions may still be low, there is no reason to doubt that the variations in $|\Delta|$, calculated for different geometries, should also take place in reality to about the same extent. It is unlikely that improved calculations (basis set, calculated integrals, extended configuration interaction) should decrease the sensitivity to structural factors. In the present case the primary charge separation is directly from a π^* orbital to a π^* orbital of another chromophore across an empty gap, and one expects, at least, a strong dependence on the width of this gap.

The connections between ring I of D_A and D_B and the protein are particularly interesting. One of the two connections consists of a hydrogen bonded[2,3,46] to the conserved L168 residue. By site-directed mutagenesis one has concluded[47] that this hydrogen bond is essential to modify the chemical potential. However, also the electronic factor may change. The hydrogen bond causes a distortion of the ET pathway between D_A and B_B, which helps to direct ET along the A branch. In the corresponding contact between D_B and B_A there is overlap between the π systems, which is crucial to perform charge separation.

We see hints of evidence that structural modifications have evolved to optimize hydrogen bonding and the electronic factor for the A branch at the same time. This same evolutional strategy has been used for *Rps. viridis* and *Rb. sphaeroides*.

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