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MODEL PHOTO REACTION CENTERS  
VIA GENETIC ENGINEERING


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Abstract

A series of reaction centers of *Rhodobacter capsulatus* isolated from a set of mutated organisms modified by site-directed mutagenesis at residues M208 and L181 are described. Changes in the amino acid sequence at these sites affect both the energetics of the systems as well as the chemical kinetics for the initial ET event. Two empirical relations among the different mutants for the reduction potential and the ET rate are presented.
I. Introduction

The photosynthetic process generates chemical energy by separation of charge across the membrane-bound reaction center (RC) through several ultrafast electron transfer (ET) reactions. The determinations of the RC protein structure [1-3] and the ability to carry out site-specific mutagenesis in the purple photosynthetic bacteria have allowed detailed investigations of structure-function relationships in these electron transfer systems. The x-ray structure (Fig. 1) shows two possible electron transfer pathways along either branch forming the pseudo C₂ symmetry in the reaction center complex, yet only one branch is photochemically active. On examining the structure an obvious question immediately arises: What significant features break the C₂ symmetry and result in unidirectional electron transfer? Although overall the L- and M-subunits of the protein follow C₂ symmetry amazingly well, many pairs of C₂ sites break the C₂ symmetry. One of the most obvious breaks in the C₂ symmetry occurs in the amino acids at sites M208 and L181 (Fig. 1). The M208 and L181 positions are two locations in the protein that are related by the C₂ symmetry axis. Since M208 is a tyrosine (Tyr) and L181 is a phenylalanine (Phe), the symmetry is broken. Moreover, M208 is conserved as a tyrosine residue near the chromophores of the photoactive pathway in Rb. capsulatus, Rb. sphaeroides and Rp. viridis. Likewise, L181 on the inactive branch is a phenylalanine residue in all three species. Because the tyrosine and phenylalanine are conserved in three species, all of which have fast electron transfer times of approximately 3 ps [4], these residues have been suspected to play a dominant role in influencing the ET rate and directionality.

In this paper, we discuss a series of reaction centers of Rb. capsulatus isolated from a set of mutated organisms modified by site-specific mutagenesis at residues tyrosine M208 and phenylalanine L181. Amino acid substitutions at these sites alter both the energetics of the systems and the chemical
kinetics for the initial ET event. The internal relations among the different mutants for the reduction potential and the ET rate are discussed.

II. Experimental Approach

The construction and initial characterization of the mutants will be published in detail elsewhere [5]. Briefly, the *Rb. capsulatus* mutagenesis system was used as described previously [6, 7]. Genes for the L and M subunits were separated from each other prior to mutagenesis by subcloning the appropriate segment into a bifunctional plasmid (pBS, Stratagene). Mutants were constructed by oligonucleotide-directed synthesis as described in ref. [8]. Mutant reaction center genes were returned to the *puf* operon carried on a broad host range vector pU2922 [7], and were then transferred to *Rb. capsulatus* by conjugation with *E. coli* mobilizing strain S17-1 [9]. Reaction centers were purified as described by Bylina et. al. [10].

Since the amino acid sequence of the reaction center protein of *Rb. capsulatus* is so similar to *Rb. sphaeroides* R-26, we have assumed that the wild type *Rb. sphaeroides* R-26 reaction center structure is an accurate model for the mutated, reaction center proteins of *Rb. capsulatus*. We are also assuming that the mutations induce no structure changes large enough to change the ET rates. In order to understand the origin of the kinetic differences induced by the mutations at sites L181 and M208, we have attempted to correlate the ET rate with the free energy of the initial chemistry. To perform the correlation we have measured the reduction potential of the primary donor in the dark. The $P/P^+$ midpoint potentials $E_{1/2}$ were determined by a chemical titration of the reaction center in tris-LDAO buffer at pH 7.8 with a potassium ferricyanide/ferrocyanide redox couple. The state of oxidation of the special pair was monitored by optical absorption spectroscopy.

A standard procedure for determining the rate of photoinduced charge separation is to measure the lifetime of the excited, singlet state of the primary donor, $P^*$. Both stimulated emission and
spontaneous emission have been used to obtain the ET rate constants. Details of the fluorescence measurements [11] and the stimulated emission measurements [12] have been presented in separate publications. In most of the cases that have been studied by both methods, the fast component of the spontaneous emission agrees with the single rate constant obtained earlier by stimulated emission.

III. Results and Discussions

Fig. 2 shows the correlation between the ET rates and the reduction potentials for the ten mutants studied here. The log of the rate constant plotted versus \( E_{1/2} \) is well described by a parabola shown as a solid line in Fig. 2. However, in this paper instead of emphasizing the parabolic relationship between the ET rate and reduction potential [13], we will discuss empirical relations for the ET rates and redox potential among the mutants. The two empirical equations that have been found from the experimental data are as follows:

\[
\frac{1}{\tau_{\text{obs}}} = \frac{1}{\tau_{L181}} + \frac{a_1}{\tau_{M208}} + \frac{1}{\tau_x} \quad (1)
\]

\[
E_{1/2} = E_{rL181} + a_2 E_{rM208} + E_{rx} \quad (2)
\]

where \( \tau_{\text{obs}} \) is the experimentally determined fast lifetime, \( \tau_{L181} \) and \( \tau_{M208} \) are the effective lifetimes of the amino acid at the respective position, \( \tau_x \) is the effective lifetime from the remaining amino acids; \( E_{1/2} \) is the experimentally measured redox potential, \( E_{rL181} \) and \( E_{rM208} \) are the contributions to the redox potential from the amino acids at respective position, \( E_{rx} \) is the contribution to the redox potential from the remaining amino acids; and finally \( a_1 \) and \( a_2 \) are the asymmetric factors for the two different locations. There are four amino acids involved in nine of our mutants. So for each equation above we have nine pieces of information and six unknowns such that each set of equations is over determined. For example, in Eq. (1) the six unknowns are the four effective lifetimes for four amino acids and \( \tau_x \) and \( a_1 \). Even with the experimental error of our experiments we get converged solutions for both Eq. (1) and (2) by doing a non-linear least square minimization. The values of the effective lifetimes and the redox
contributions for different amino acids and their interpretations will be given in a separate publication [14].

It is interesting to note that the asymmetric factor for both physical variables are somewhat different, that is: $a_1 = 1.5$, and $a_2 = 1$. The difference in the asymmetric factors $a_1$ and $a_2$ implies that the active side M208 residue and the inactive side L181 residue have roughly the same contribution in altering the reduction potential of special pair donor, but the M208 residue on the active side plays a more important role than that of the L181 residue on the inactive side in modifying the chemical kinetics. One reasonable interpretation for above result is based on the distances between the prosthetic molecules of RC and the amino acid residues M208 and L181. The x-ray crystal structure from *Rb. sphaeroides* shows that the distances from special pair donor to the M208 and the L181 positions are very similar (about 4 Å). In contrast, the M208 residue is much closer to the electron acceptor, the accessory bacteriochlorophyll (4 Å), than the L181 residue is (12 Å). Because of the similar distance to the special pair, one would expect that the M208 residue and L181 residue make approximately equal contributions to the energy level or redox properties of special pair state ($P^+$). On the other hand, this model would allow the M208 residue to interact with the positive charge on the special pair ($P^+$) and the negative charge on the electron acceptor of the active branch (such as $B_L^-$), whereas the L181 residue could only interact with the oxidized special pair because this residue is more than 12 Å from the active electron acceptor. The stabilization (or destabilization) from the two residues could come from the polarizability or the coulombic interactions with the charged chromophores, thus lowering (or raising) the energy of the charge separated state and correspondingly affect the electron transfer rates. As a result, the M208 residue would have a larger influence in altering the ET rate than the L181 residue. Interpreted in this manner the experimental results indicate that the M208 site is about 1.5 times more effective in altering the ET rate than the L181 site for the this series of RC mutants of *Rb. capsulatus*.
IV. Conclusion

We have examined several symmetry related site-specific mutants of *Rb. capsulatus* and presented data of ET rate and redox properties relevant to the amino acid modifications. Several new implications are embedded in our experimental data. In this paper, we discussed only one aspect of these, namely the internal relations for the ET rates and reduction potentials among different mutants. The two empirical relations presented here agree qualitatively with the structure based on the x-ray crystal data.

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References


Prosthetic groups used in the electron transfer steps of bacterial photosynthesis based on the structure of the reaction center protein from *Rb. sphaeroides*. The only two amino acid residues that are shown are at L181 and M208. The C$_2$ axis divides the two amino acid groups and the active branch from the inactive branch.

The parabolic relationship between $E_{1/2}$ and the inverse of the average lifetime. This data is for nine mutants and wild type of *Rp. capsulatus*. The two letter abbreviations are based on the single letter abbreviations of the protein amino acids. Y = tyrosine, F = phenylalanine, T = threonine, K = lysine, Q = glutamine. Wild type = WT = FY. To correspond with Figure 1 the left hand letter of the pair of letters is the inactive side, i.e., the L181 site. The right hand letter of the pair of letters is the active side, i.e., the M208 site.
Figure 2