THE PROTEIN'S ROLE IN TRIPLET ENERGY TRANSFER IN BACTERIAL REACTION CENTERS

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1. Introduction

When photosynthetic organisms are subjected to high-light conditions in nature, electron transfer becomes blocked as the rate of conversion of light into charge-separated states in the reaction center (RC) exceeds the capacity of the soluble carriers involved in cyclic electron transfer. In that event, a well-characterized T_0-polarized triplet state ^TP, is formed on the primary donor, P, from the $P^+H_A^-$ state (reviewed in [1]). In an aerobic or semi-aerobic environment, the major role of the carotenoid (C), also bound by the RC, is to quench ^TP prior to its sensitization of the $^1\Delta_g$ singlet state of oxygen -- a potentially damaging biological oxidant. The carotenoid performs this function efficiently in most bacterial RCs by rapidly accepting the triplet state from P and dissipating this excited-state energy into heat through internal conversion. The lowest-lying triplet states of P and the carotenoid are sufficiently different that ^TP can promote oxygen to its excited singlet state whereas ^TC can quench the ^TP state (reviewed in [2]).

In the RC structure, the carotenoid, either 1,2 dihydroneurosporene in *Rhodopseudomonas (Rps.) viridis* [3] or spheroidene in *Rhodobacter (Rb.) sphaeroides* [4,5,6], is located near bacteriochlorophyll monomer B_B about 15 Å from the periplasmic surface of the complex. It is bound in a high-energy 15-15′-cis configuration that is responsible for many of its spectroscopic properties [5]; the *cis* bond is within van der Waals distance of the edge of the macrocycle of B_B (3.7 Å) and is 10 Å from the nearest atoms of P. The carotenoid interacts with amino acids of the A, B, and C helices as well as those of a short interhelix connecting loop (*cd* segment). Its environment is relatively hydrophobic; six tryptophan residues and eight phenylalanine residues are located within 5 Å of the carotenoid [5].

The triplet energy transfer reaction is presumably controlled by the exchange mechanism described by Dexter [7] which requires near orbital overlap between the donor and acceptor species. Because the distance between P and the carotenoid is too large to allow the orbital overlap required for efficient exchange, the transfer involves B_B as a bridging molecule [8]. The transfer process is thermally activated; the observation of the ^TC signal (indicative of excited state transfer) at temperatures between 95 and 150 K is dependent upon the triplet energy level of the molecule bound at the B_B site [8].

The carotenoid is "protective", i.e., able to quench triplet states from P, in RCs of *Rb. sphaeroides* and *Rb. capsulatus* and *Rhodospirillum (Rsp.) rubrum.* We have determined the rate constants for triplet energy transfer in these three species and show in this paper that they vary widely. In light of the overall sequence, structural, and functional homology between the RC complexes of these species, it is likely that small

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changes in the protein environment surrounding the P, B_B, and C cofactors are responsible for the observed differences in the rates of reactions associated with the carotenoid. We also show that site-specific mutants of the *Rb. capsulatus* RC can begin to mimic species-specific differences in the rates of the triplet energy transfer reaction.

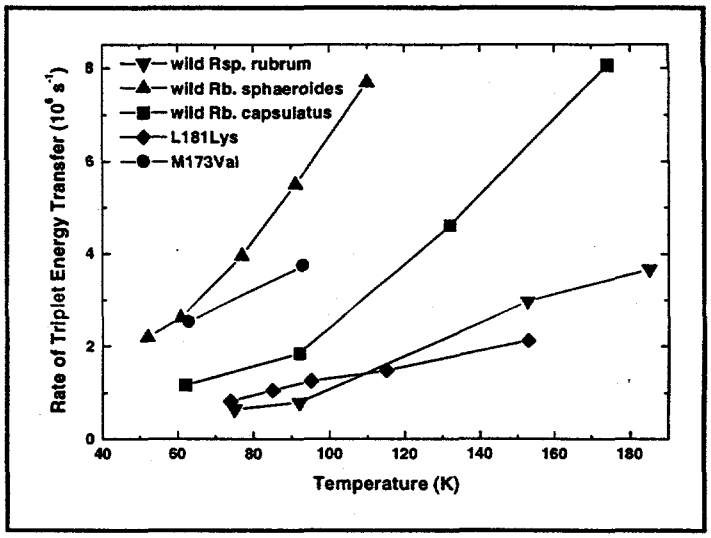

Figure 1. Temperature dependence of rates of triplet energy transfer to the carotenoid in selected RCs.

2. Procedure

Mutants were constructed and cultures were grown as described previously [9]. Chromatophore samples were prepared as detailed in [10]. Direct-detection, continuous-wave time-resolved electron paramagnetic resonance spectroscopy (TR-EPR) was conducted using a Bruker ESP300E spectrometer and E046XK-T bridge equipped with an Oxford cryostat, low Q "split-ring" cavity, and broad band amplification resulting in electronically-limited EPR response times of < 50 ns. Laser excitation was achieved through the second harmonic output of a YAG laser (Quantel). Transient spectra were recorded as described earlier [11]. Kinetics were directly recorded on a 175 MHz digital oscilloscope (LeCroy).

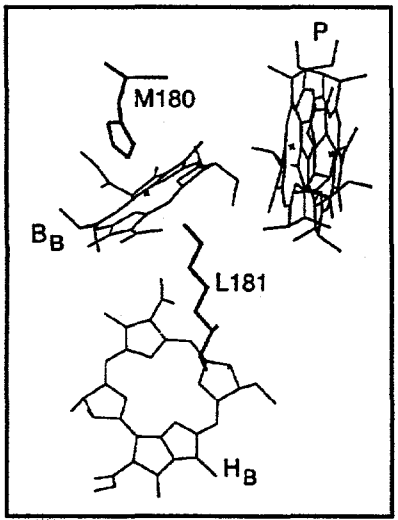

Figure 2. Molecular model of hexacoordination of the Mg of B_B by L181Lys.

3. Results and Discussion

We have used TR-EPR to measure the rates of triplet energy transfer to the carotenoid in RCs from three different species of purple photosynthetic bacteria -- *Rb. sphaeroides*, *Rb. capsulatus*, and *Rsp. rubrum* (Fig. 1). RCs of *Rb. sphaeroides* are most efficient in performing this quenching reaction; ^{T}C can be observed at 40 K in the *Rb. sphaeroides* RCs, but cannot be seen in *Rb. capsulatus* RCs until the temperature is raised to 70 K. RCs of *Rsp. rubrum* are even less efficient. At 100 K, rates of triplet energy transfer in spheroidene-containing wild-type RCs are 6.8 x 10^6 s^{-1} in *Rb. sphaeroides* and 2.3 x 10^6 s^{-1} in *Rb. capsulatus*; the rate in spirilloxanthin-containing wild-type *Rsp. rubrum* RCs is 1.0 x 10^6 s^{-1}.

We had previously constructed a family of strains that carry site-specific mutations at residues L181Phe and M208Tyr, located near P, B_A, and B_B. We determined the efficiency of triplet energy transfer from P to C for the members of the mutant family using light-modulated EPR spectroscopy [10]. At temperatures ≥70 K, we observed reduced signals from the carotenoid in most of the RCs with L181 substitutions. In particular, triplet transfer efficiency was reduced in all RCs in which a lysine at L181 donates a sixth ligand to B_B (Fig. 2). We speculated that the change in the transfer rate is caused by the change in the planarity or position of B_B

that is caused by the addition of this sixth ligand [10], which would decrease the electronic overlap between P and B_B. Determination of the temperature dependence of the rate of triplet transfer shows that the L181Lys mutation causes a decrease in the efficiency of this reaction (KY strain; Fig. 1) such that it more closely resembles that of the *Rsp. rubrum* RC.

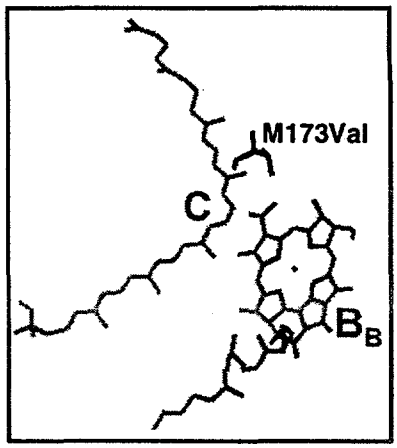

Figure 3. Position of M173Val relative to C and B_B in the structure of the native *Rb. sphaeroides* RC [5].

3.1 *Molecular modeling and rational design of mutant.*

To determine the sequence variations that might contribute to the threefold increase in triplet transfer efficiency in RCs of *Rb. sphaeroides* versus those of *Rb. capsulatus* (Fig. 1), we aligned the sequences of RC genes from 22 species of photosynthetic bacteria and analyzed the variability of residues that were located within 5 Å of either B_B or C in the *Rb. sphaeroides* RC structure [5]. The sequence alignments and structure analysis suggested residue M173 as a candidate for mutation. M173 is either a proline or a valine in the 22 sequences that were compared. M173Pro is an obvious difference that distinguishes the RCs of *Rb. capsulatus* from those of several other species of purple non-sulfur bacteria, including *Rb. sphaeroides*, that have Val at this site. This residue is positioned such that it could influence the electronic properties of B_B and/or C; the position of M173Val is shown in Figure 3. In the *Rb. sphaeroides* RC structure [5], M173Val is located within ~ 4 Å of the 15,15'-cis bond of C, and it is about 4.2 Å from the closest substituent of B_B.

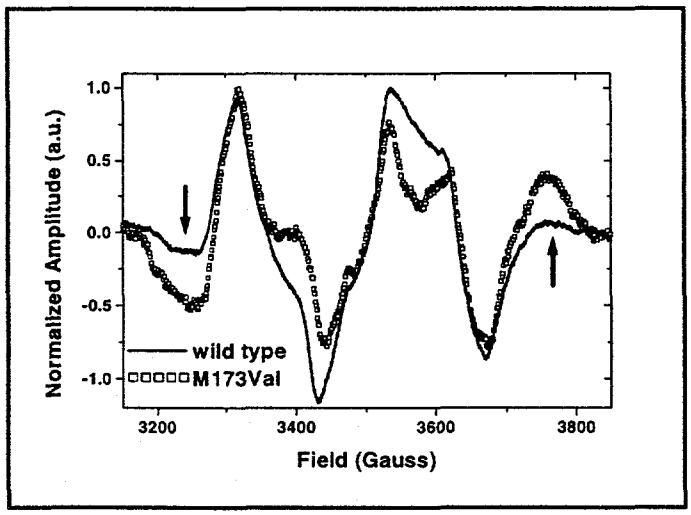

Figure 4. Time-resolved EPR of the triplet state of wild-type and M173V RCs of *Rb. capsulatus* at 100 K, 200 ns following a 532 nm laser flash. Differences in the spectra are due to a greater proportion of ^TC in the M173V RCs, indicating increased transfer efficiency. Although ^TC and ^TP overlap substantially, the Z features of ^TC (indicated by arrows at 3250 G and 3750 G) are uniquely resolved and used to monitor arrival of the excited state on C. The signals in the center of the spectra are overlapping triplet features of ^TP and ^TC.

We constructed the site-specific M173Pro→Val mutant of *Rb. capsulatus* and have subjected it to preliminary TR-EPR analysis (Figures 1 and 4). These data show that at 100 K triplet transfer is >2.5-fold faster in this mutant than the same triplet transfer reaction in the wild-type RC of *Rb. capsulatus*. Therefore, for the first time, rational design has been used to produce an RC that is significantly more efficient in the photo-protection process.

Prolines add rigidity to the polypeptide, therefore the substitution of proline by a valine might be expected to add flexibility to the chain in this region of the mutant RC. That flexibility might lead to changes in the relative positions of the cofactors involved in the triplet transfer reaction, which would alter its efficiency

[7,12]. Modeling of the opposite Val→Pro mutation in the *Rb. sphaeroides* RC structure [5] shows that it would be easily tolerated, requiring no change in the dihedral angles. Thus, it is not expected that the Pro→Val switch in *Rb. capsulatus* would be problematic. Instead of an increase in flexibility, it is therefore likely that the size of the side chain at this position may be more important in influencing the rate of the transfer reaction because it may modify the position of B_B, thus determining the extent of its orbital overlap with P. Additional mutants -- to Ala, Ile, and Thr -- will test this hypothesis. If this hypothesis is valid, we would not expect to see a change in the transfer rate with the substitution of Ala for Pro. Thr is similar to Val in that it also has a branched side chain, but it is more polar. The Ile substitution, one CH_2 unit longer than Val, might be too large and could reduce the rate (as seen previously with the L181Lys mutation) by causing a shift in the position of B_B, thus altering its orbital overlap with P. These constructions are in progress.

In summary, site-specific changes within the RC protein of *Rb. capsulatus* can be designed to increase or decrease the efficiency of transfer of the triplet state from P to the carotenoid. These changes within one species can begin to mimic interspecies differences in the rates of this energy transfer reaction.

4. Addendum

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