Triplet state EPR of reaction centers from the His\textsuperscript{L173} $\rightarrow$ Leu\textsuperscript{L173} mutant of \textit{Rhodobacter sphaeroides} which contains a heterodimer primary donor

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Abstract

Electron paramagnetic resonance (EPR) spectroscopy has been used to examine the triplet states in reaction centers of \textit{Rhodobacter sphaeroides} which have undergone a genetic modification affecting the primary donor. Reaction centers containing the His\textsuperscript{L173} $\rightarrow$ Leu\textsuperscript{L173} substitution in the amino acid sequence have a primary donor which consists of a BChl-BPh heterodimer. The triplets formed in this heterodimer reaction center were compared with those formed in the wild-type reaction center which contains the BChl-BChl homodimer. Both reaction centers transfer triplet energy to the carotenoid under illumination at liquid nitrogen temperatures (~90 K). However, the intensity of the carotenoid triplet signal is significantly decreased in the Leu\textsuperscript{L173} mutant compared with the wild-type reaction center. At 12 K, in wild-type reaction centers only the primary donor triplet is observed. The Leu\textsuperscript{L173} mutant exhibits a signal similar to that observed by Bylina et al. (1990) in His\textsuperscript{M200} $\rightarrow$ Leu\textsuperscript{M200} mutant reaction centers from \textit{Rb. capsulatus}. The values of the zero-field splitting parameters of this triplet are discussed within the context of various models for the primary donor triplet state. No alteration in the ability of the carotenoid to quench the primary donor triplet state results from mutations at these sites.

Abbreviations: BChl – bacteriochlorophyll; BPh – bacteriopheophytin; EPR – electron paramagnetic resonance; LDAO – lauryl-dimethylamine N-oxide

Introduction

The primary electron donor (P) in the bacterial photosynthetic reaction center is a dimer of bacteriochlorophyll molecules (BChl\textsubscript{Lp} and BChl\textsubscript{Mp}) (Norris et al. 1974, Feher et al. 1975). Upon absorption of a photon, P is promoted to an excited singlet state, P\textsuperscript{*} (Holten et al. 1978, Kirmaier and Holten 1987) and electron donation from P\textsuperscript{*} to a bacteriopheophytin acceptor (BPh\textsubscript{h}) occurs within a few ps (Holten et al. 1978) over a distance of about 17 Å (Deisenhofer et al. 1985, Michel et al. 1986, Allen et al. 1988, Deisenhofer and Michel 1988, Yeates et al. 1988, Chang et al. 1991) with essentially 100% yield (Wraight and Clayton 1973). When electron transfer from BPh\textsubscript{h} to the subsequent quinone acceptor is blocked, charge recombination can result in the formation of the triplet state, 3P, directly on the primary donor (Parson et al. 1975, Budil and Thurnauer 1991). In photosynthetic bacterial reaction centers isolated from wild type strains (e. g. \textit{Rhodobacter sphaeroides} 2.4.1) one specifically-bound carotenoid exists per reaction center complex (Cogdell et al. 1976, Cogdell and Frank 1987).
At temperatures above ~35 K the carotenoid in the bacteriochlorophyll-a containing strains rapidly quenches $3P$ and a triplet state electron paramagnetic resonance (EPR) signal associated with the carotenoid is observed (Frank et al. 1983). Below 35 K, transfer to the carotenoid is inhibited and only the $3P$ signal is observed (Parson and Monger 1978, Frank et al. 1983). Recent reports have described mutations which alter the properties of the primary donor in *Rb. capsulatus* (Kirmaier et al. 1988, Breton et al. 1989, and Bylina et al. 1988 and 1990) and in *Rb. sphaeroides* (Hammes et al. 1990, Schenck et al. 1990, McDowell et al. 1991, Laporte et al. 1993). In *Rb. sphaeroides*, converting the conserved histidines at L173 or M202 to leucine results in replacement of one of the bacteriochlorophylls (BChl$_L$ or BChl$_M$, respectively) comprising the primary donor with a bacteriopheophytin (BPh). These BChl-BPh heterodimer mutants are able to perform the primary charge separation, although the quantum yield of the charge separated state $P^+\cdot BPh_-$ is reduced to ~50% in these mutants compared to ~100% in the *Rb. sphaeroides* wild-type (McDowell et al. 1991). The reduction in yield is caused by an enhanced decay of the intradimer charge transfer state (BChl$^+_L\cdot$BPh$_L$ or BChl$^+_M\cdot$BPh$_M$) to the ground state and a decreased rate of formation of $P^+\cdot BPh_-$ EPR studies on $P^+$ in the Leu$^{M202}$ heterodimer of *Rb. capsulatus* indicate that the linewidth of the radical is broadened from 10.6 G to 12.6 G compared to the wild-type homodimer $P^+$ signal (Bylina et al., 1990). In the *Rb. sphaeroides* heterodimer mutants the $P^+$ signal is broadened from 9.8 G to 12.6 G in reaction centers from the Leu$^{M202}$ mutant and to 12.4 G in reaction center from the Leu$^{173}$ mutant (Huber et al., 1990). This has been taken as evidence that the cation radical is primarily localized on the BChl half of the heterodimer primary donor (Bylina et al. 1990). Electron nuclear double resonance (ENDOR) experiments have supported this assignment of asymmetry in the spin distribution of $P^+$ (Huber et al. 1990). This contrasts previous proton ENDOR studies on the homodimer reaction centers which indicate that the cation radical is delocalized symmetrically over both BChl$_L$ and BChl$_M$ molecules (Norris et al. 1971, Okamura et al. 1982). Recent $^{15}$N ENDOR experiments on $P^+$ in crystalline reaction centers of *Rb. sphaeroides* R-26 indicate a less symmetric spin distribution than indicated by the proton ENDOR experiments (Lendzian et al. 1992).

The primary donor triplet state and its mechanism of formation, the radical pair mechanism, have been studied extensively by EPR spectroscopy. For reviews see Hoff (1979) and Budil and Thurnauer (1991). The triplet state spin sublevels of $\pi$-electron conjugated molecules are split at zero magnetic field owing primarily to dipolar interaction. The zero-field splitting is described by two independent spin Hamiltonian parameters, denoted $|D|$ and $|E|$. From EPR studies of several photosynthetic bacterial systems, notably *Rb. sphaeroides*, the zero-field splitting values for $3P$ (for *Rb. sphaeroides* wild type strain 2.4.1 $|D|$=0.0188 cm$^{-1}$, $|E|$=0.0032 cm$^{-1}$ (Clarke et al. 1977, Thurnauer 1979)) have been found to be significantly smaller than those observed for monomeric BChl $a$ in solution (Thurnauer 1979, Den Blanken and Hoff 1983). The values of the zero-field splitting parameters for BChl $a$ vary from $|D|$=0.0224 to 0.0238 cm$^{-1}$ and $|E|$=0.0053 to 0.0069 cm$^{-1}$ depending on the solvent and presumably the state of ligation of the BChl (Thurnauer 1979, Den Blanken and Hoff 1983). Values frequently cited as 'monomeric' BChl $a$ parameters are those obtained in toluene containing 10% pyridine by high-field EPR ($|D|$=0.0224 cm$^{-1}$, $|E|$=0.0053 cm$^{-1}$, Thurnauer 1979). Using these values one finds that the $|D|$ value for the primary donor triplet in vivo is ~16% smaller than the $|D|$ value of BChl $a$ measured in vitro. This observation led to a model for the primary donor triplet that invoked exciton coupling between the two BChl $a$ molecules of the dimer to account for the reduction in $|D|$ (Clarke et al. 1977). In this model, the molecular interactions are assumed larger than the molecular triplet state zero-field splittings so that in this 'strong spin-coupling limit', the spin Hamiltonian describing the spin interaction becomes the average of the spin Hamiltonians of the isolated molecules. Norris et al., (1989) pointed out that because the structure of the primary donor deduced from X-ray crystallographic studies shows essentially coplanar halves of the dimer, a pure exciton model can account for only a ~4% reduction in $|D|$ versus the in vitro value. Consequently, Norris et al., (1989) introduced an additional charge-transfer contributions to the overall triplet state wavefunction for $3P$ to rationalize the observation of a smaller $|D|$