S07-005

Conserved electronic structure of the primary donor in reaction centres of sulphur and non-sulphur purple bacteria

F Müh[1], AT Gardiner[2], H Witt[3], C Schulz[3], JF Imhoff[4], RJ Cogdell[2], W Lubitz[3]

[1]*Inst. f. Experimentalphysik, Freie Universität Berlin, Arnimallee 14, D-14195 Berlin, Germany, Fax (+49)03083856081, e-mail: mueh@physik.fu-berlin.de.*

[2]*IBLS-Division of Biochemistry & Mol. Biol., University of Glasgow, Glasgow G12 8QQ, Scotland.*

[3]*Max-Volmer-Laboratorium. f. Biophysikalische Chemie, Technische Universität Berlin, Str. d. 17. Juni 135, D-10623 Berlin, Germany.*

[4]*Inst. f. Meereskunde, Universität Kiel, Duesternbrooker Weg 20, D-24105 Kiel, Germany.*

Keywords: purple bacteria, reaction centre, primary donor, spin density distribution

Introduction

In all purple bacteria the photosynthetic apparatus is located in an intracytoplasmic membrane, which can be isolated and investigated by spectroscopic techniques. The light-induced charge separation process catalysed by the RC starts from the excited singlet state $^1P^*$ of the primary electron donor, which at least for some species is known to be a bacteriochlorophyll (BChl) dimer (Fig. 1). Information about the electronic structure of P is crucial for an understanding of its functional properties. In the $P^{\bullet+}$ state, which can be generated by illumination of membranes or RC, the un-paired electron is distributed over the dimer and interacts magnetically with protons of both BChl moieties, P_L and P_M. Therefore, the so-called highest occupied molecular orbital (HOMO) of P can be studied by using ENDOR spectroscopy and related

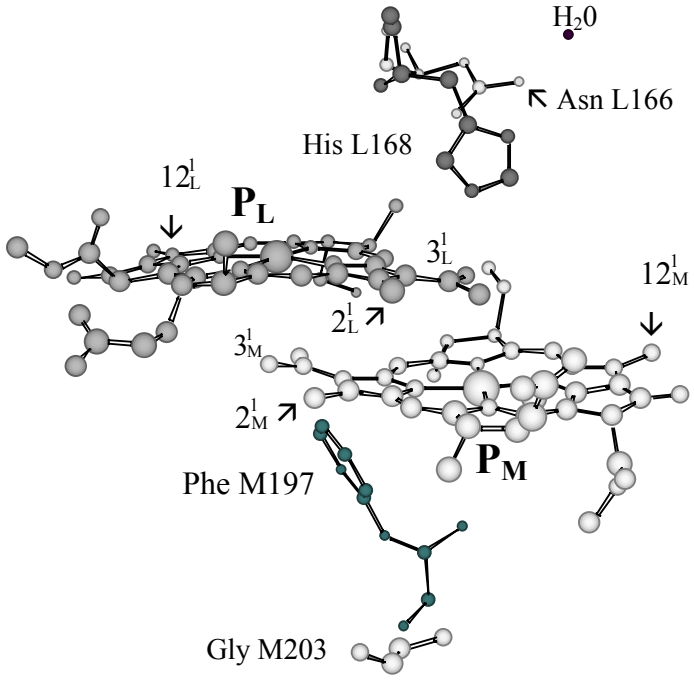

Fig. 1. X-ray structure of P in *Rba. sphaeroides* (Stowell *et al.* 1997) with nearby residues of the wild type sequence.

techniques. Experiments performed on RC single crystals revealed an unequal distribution of the electron with 68% of the spin density on P_L (Lendzian *et al*. 1993). This has important implications for the optical properties of P (see Discussion). The same asymmetry of $P^{\bullet+}$ has been found in membranes from various species of non-sulphur purple bacteria (Rautter *et al*. 1994), whereas for isolated RC an influence of the detergent used to solubilise the membrane protein has been recognized (Rautter *et al*. 1994, Müh *et al*. 1997). In order to elucidate the significance of this asymmetry, we extended the earlier ENDOR studies of membranes to a number of sulphur and non-sulphur purple bacteria that contain BChl *a*. The spectra are analysed by comparison with spectra of isolated mutant RC of *Rhodobacter* (*Rba.*) *sphaeroides* that were designed on the basis of sequence alignments (Komiya *et al*. 1988, Ivancich *et al*. 1997).

Materials and methods

Mutagenesis was performed according to the altered sites mutagenesis procedure (Promega, Heidelberg, Germany). The mutated *Rba. sphaeroides puf* operon was cloned into the broad host range vector pRKSCH and expressed in the deletion strain ΔLM1.1 (Ivancich *et al*. 1997). Mutant strains were grown semi-anaerobically in the dark and wild type species photosynthetically. Membranes and RC were isolated by well-known procedures (see, e.g., Wang *et al*. 1994). Detergent exchange experiments and ENDOR/TRIPLE spectroscopy were performed as described earlier (Rautter *et al*. 1994, Müh *et al*. 1997).

Results

At 160 K the ENDOR spectrum of $P^{\bullet+}$ in membranes shows a characteristic pattern of hyperfine couplings (hfc). Information about the distribution of the unpaired electron is obtained from hfc assigned to the methyl protons at positions 2^1 and 12^1 of each dimer half (Fig. 1), since these are less sensitive to structural heterogeneity of the BChl macrocycles in the frozen state (Rautter *et al*. 1994). However, due to spectral overlap, it is often not possible to accurately determine the methyl proton hfc from frozen solution spectra. Solubilisation of the RC is

Table 1: Methyl proton hfc constants A (in MHz) of light-induced $P_{866}^{\bullet+}$ in solubilised RC of wild types and mutants.

	Rba. sphaeroides				*Rsp. rubrum* [c]
	WT	NH(L166)	GM(M203)	FY(M197)	
$A(12^1_L)$	5.70	5.83	5.77	6.36	5.15
$A(2^1_L)$	3.96	3.77	4.03	4.46	3.55
$A(12^1_M)$	3.20	3.15	2.92	2.23	3.90
$A(2^1_M)$	1.37	1.45	1.32	0.93	1.60
R_L [a]	1.44	1.55	1.43	1.43	1.45
R_M [a]	2.34	2.17	2.21	2.40	2.43
ρ_L [b]	0.68	0.68	0.70	0.77	0.61

[a] $R_i = A(12^1_i)/A(2^1_i) \quad (i = \mathrm{L}, \mathrm{M})$.

[b] $\rho_L = \left(A(2^1_L) + A(12^1_L)\right)/\left(A(2^1_L) + A(12^1_L) + A(2^1_M) + A(12^1_M)\right)$.

[c] from Rautter *et al*. 1994.

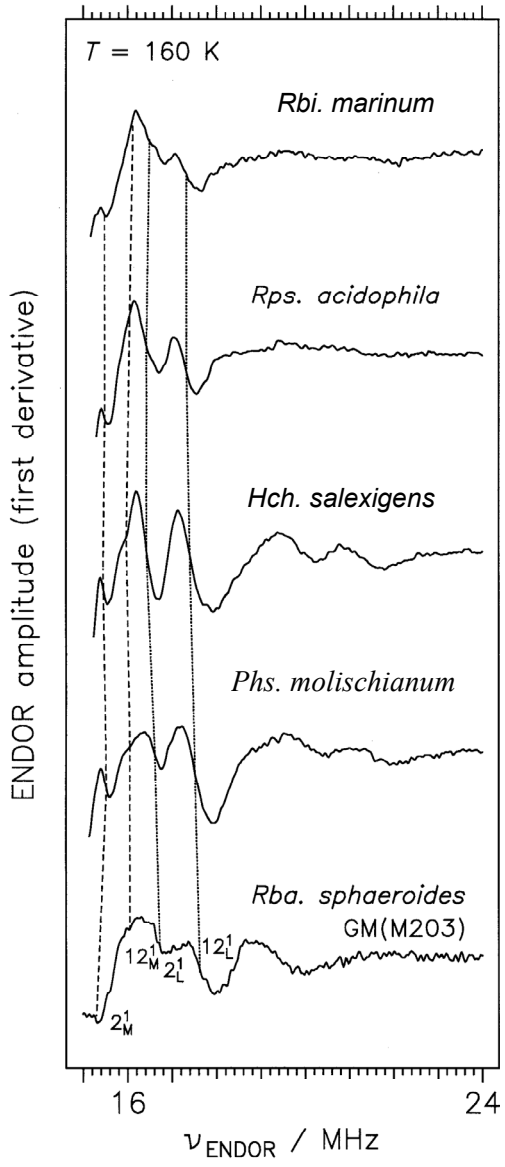

Fig. 2. Representative ^{1}H-ENDOR spectra (high frequency part) of light-induced $P^{\bullet+}$ in membranes of different bacterial species as compared with the *Rba. sphaeroides* mutant GM(M203). The assignment of signals to molecular positions of P is based on a comparison with ^{1}H-Special TRIPLE resonance spectra of isolated mutant RC in liquid solution (Fig. 3).

required to perform high resolution Special TRIPLE resonance (ST) spectroscopy at elevated temperatures, but then detergent effects must be taken into account (Müh *et al*. 1997).

ENDOR of $P^{\bullet+}$ was performed on membranes of *Rba. sphaeroides*, *Rhodospirillum* (*Rsp*.) *rubrum*, *Rubrivivax gelatinosus*, *Rhodocista centenaria*, *Rhodopseudomonas* (*Rps*.) *acidophila*, *Rps. cryptolactis*, *Rps. palustris*, *Rba. blasticus*, *Rhodothalassium salexigens*, *Rhodopila globiformis*, *Rhodobium* (*Rbi*.) *marinum*, *Rhodocyclus tenuis*, *Phaeospirillum* (*Phs*.) *molischianum*, *Ectothiorhodospira* (*Ect*.) *vacuolata*, *Ect. haloalkaliphila*, *Halorhodospira halophila*, *Marichromatium purpuratum*, *Allochromatium vinosum* and *Halochromatium* (*Hch*.) *salexigens* (for reclassification of species, see Imhoff et al. 1998). The hfc pattern is quite similar in all cases, but slight shifts of intense signals are observed (Fig. 2). Only in some cases the pattern can be simulated by using the hfc tensor components of *Rba. sphaeroides* (Lendzian et al. 1993).

Since RC isolation protocols are not available for most species, we constructed site-directed mutants of *Rba. sphaeroides* on the basis of sequence alignments in order to mimic the protein environments of P occurring in these species. The mutants are FY(M197), which introduces a hydrogen bond to the 3^1_M–acetyl group (Kuglstatter *et al*. 1999), NH(L166), which modifies the H-bond between His L168 and the 3^1_L–acetyl group (Ivancich *et al*. 1997), and GM(M203), which removes a difference between the L- and M-sequence (Komiya *et al*. 1988). In addition, FY(M197) was combined with the other two mutations (Fig. 1). In all mutant RC the two conformations $P^{\bullet+}_{866}$ and $P^{\bullet+}_{850}$ of the $P^{\bullet+}$ state were observed depending on solubilisation conditions, but *only* $P^{\bullet+}_{866}$ corresponds to $P^{\bullet+}$ in the membranes (Rautter *et al*. 1994, Müh *et al*. 1997). An increase of the fraction ρ_L of spin density on P_L is only caused by the replacement of Phe M197 with Tyr. The other mutations merely change the intramolecular spin density distribution (represented by R_L and R_M, Tab.1) with no significant net effect on the fraction ρ_L of spin density on P_L (Table 1, Fig. 3). A comparison of the membrane spectra of the *Rba. sphaeroides* mutants with those of the different species shows that in most cases the observed differences can be understood as resulting from small changes similar to NH(L166) and GM(M203), whereas a larger asymmetry like in FY(M197) is not observed (Fig. 2). In the remaining cases, the pattern is more similar to *Rsp. rubrum*, for which isolated RC have been investigated earlier and a somewhat more symmetric spin density distribution has been found

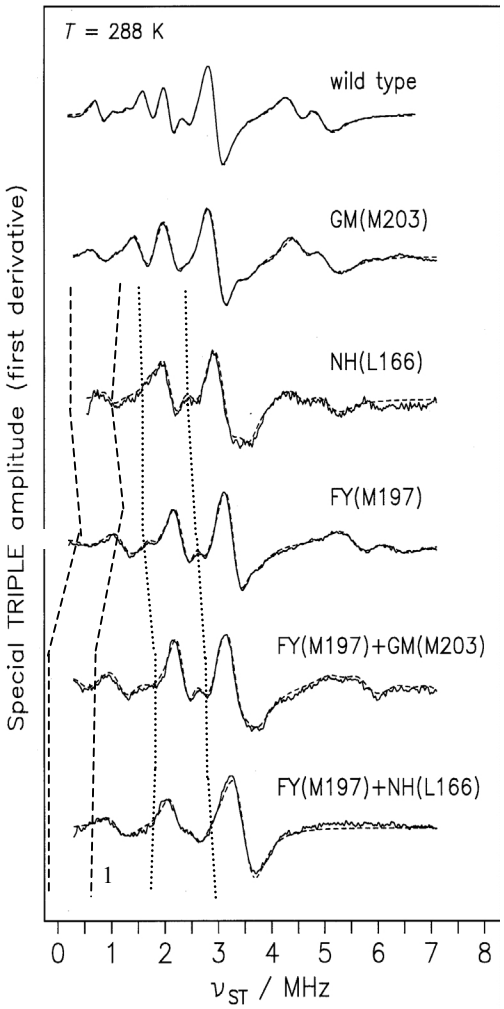

Fig. 3: ^{1}H-Special TRIPLE resonance spectra of $P_{866}^{\bullet+}$ in isolated mutant RC of *Rba. sphaeroides*. The mutated residues are shown in Fig. 1. Signals assigned to the methyl protons are indicated by dotted (P_L) and dashed (P_M) lines. Note that $\nu_{ST} = \nu_{ENDOR} - \nu_H$ with ν_H = 14.6 MHz.

(Table 1). In summary, we conclude that in all studied species $0.6 < \rho_L < 0.7$. We can, however, not exclude that other species exist with $\rho_L \approx 0.8$, in which a Tyr is present at position M197.

Discussion

An asymmetric spin density distribution implies an asymmetric distribution of charge, although recent semi-empirical calculations suggest that ρ_L does not agree quantitatively with the fraction of positive charge on P_L in the $P^{\bullet+}$ state (Reimers *et al*. 2000). The unequal sharing of the unpaired electron by the two dimer halves is a consequence of an energy difference between the two HOMO of P_L and P_M (Lendzian *et al*. 1993), which also indicates a possible energy difference of the two charge transfer (CT) states $P_L^+P_M^-$ and $P_L^-P_M^+$. This splitting in turn determines the CT character of the transition from the ground state of P to ^{1}P* (Scherer & Fischer 1997). Our finding of a conserved asymmetry of the HOMO of P suggests that a fine-tuning of the energy differences between P_L and P_M might be important for a proper function of the primary donor in RC of purple bacteria.

Acknowledgments

We thank Drs. F. Lendzian (TU Berlin, Germany) and J. Rautter (formerly TU Berlin) for experimental support and Profs. J.C. Williams and J.P. Allen (ASU Tempe, USA) for providing DNA of the mutant NH(L166). This work was supported by Alexander-von-Humboldt-Stiftung, EU (FMRX-CT98-0214) and DFG (Sfb 312 and 498).

References

Imhoff JF, Petri R, Süling J (1998) *Int. J. Syst. Bacteriol.* **48**, 793–798, 1129–1143.

Ivancich A, Mattioli TA, Artz K, Wang S, Allen JP, Williams JC (1997) *Biochemistry* **36**, 3027–3036.

Komiya H, Yeates TO, Rees DC, Allen JP, Feher G (1988) *Proc. Natl. Acad. Sci. USA* **85**, 9012–9016.

Kuglstatter A, Hellwig P, Fritzsch G, Wachtveitl J, Oesterhelt D, Mäntele W, Michel H (1999) *FEBS Lett.* **463**, 169–174.

Lendzian F, Huber M, Isaacson RA, Endeward B, Plato M, Bönigk B, Möbius K, Lubitz W, Feher G (1993) *Biochim. Biophys. Acta* **1183**, 139–160.

Müh F, Rautter J, Lubitz W (1997) *Biochemistry* **36**, 4155–4162.

Rautter J, Lendzian F, Lubitz W, Wang S, Allen JP (1994) *Biochemistry* **33**, 12077–12084.

Reimers JR, Hutter MC, Hughes JM, Hush NS (2000) *Int. J. Quantum Chem.* **80**, 1224–1243.

Scherer POJ, Fischer SF (1997) *Chem. Phys. Lett.* **268**, 133–142.
Stowell MHB, McPhillips TM, Rees DC, Soltis SM, Abresch E, Feher G (1997) *Science* **276**, 812–817.
Wang S, Lin S, Lin X, Woodbury NW, Allen JP (1994) *Photosynth. Res.* **42**, 203–215.