Tuning of the redox potential of the primary electron donor in reaction centres of purple bacteria: effects of amino acid polarity and position

Diane Spiedel[a], Michael R. Jones[b,*], Bruno Robert[c]

[a] *Department of Molecular Biology and Biotechnology, University of Sheffield, Western Bank, Sheffield S10 2UH, UK*
[b] *Department of Biochemistry, School of Medical Sciences, University of Bristol, University Walk, Bristol BS8 1TD, UK*
[c] *Section de Biophysique des Fonctions Membranaires, DBJC/CEA and URA 2096 CNRS, C.E.A. Saclay, 91191 Gif/Yvette, France*

Received 24 June 2002; revised 27 July 2002; accepted 30 July 2002

First published online 14 August 2002

Edited by Gianni Cesareni

Abstract **Mutation of residues His L168 and Phe M197 in the reaction centre from *Rhodobacter sphaeroides* has an unusually strong effect on the mid-point redox potential (E_m) of the pair of bacteriochlorophylls that form the primary donor of electrons, tuning E_m over a range of nearly 250 mV. This effect is correlated to the accompanying change in the permanent dipole of the L168 or M197 residue, suggesting it is mediated by changes in charge–dipole interactions. Comparisons with mutations made at a variety of other positions show that this correlation is particular to this residue pair, perhaps reflecting their proximity to the ring I regions of the dimer bacteriochlorophylls that form the overlap region between these molecules.**

Key words: Reaction centre; Photosynthesis; Redox potential; Bacteriochlorophyll; Permanent dipole; Charge–dipole interaction

1. Introduction

Reaction centres are integral membrane protein complexes that convert light energy into a biologically useful form. In photosynthetic bacteria such as *Rhodobacter sphaeroides* the reaction centre consists of a protein scaffold and ten cofactors. The initial event of energy transduction consists of a light-induced, transmembrane electron transfer from the primary electron donor (P), a pair of excitonically coupled bacteriochlorophyll (BChl) molecules (P_A and P_B) located near the periplasmic side of the membrane, to a molecule of ubiquinone located near the cytoplasmic side of the membrane. Each of the constituent steps in this transmembrane electron transfer occurs with a yield close to unity.

A large number of experimental and theoretical studies have been conducted with a view to explaining the remarkable efficiency of this transmembrane electron transfer. Understanding the energetics of individual electron transfer steps requires the characterisation of a number of parameters, including the redox potentials of the electron carriers (see [1] for a review). A particular feature of the primary electron donor is that its redox properties can be investigated in a relatively straightforward way by chemical titration. The structure of the reaction centre has evolved in such a way that the P BChl dimer is more easily oxidised than isolated, monomeric BChl. The mid-point redox potential of the P/P^+ redox couple (E_m P/P^+) has a value of around 500 mV, whereas E_m BChl/$BChl^+$ has a value of approximately 800 mV.

A large number of mutations have been engineered in the vicinity of P to investigate how the redox properties of this BChl pair are influenced by the surrounding protein (for reviews of this topic see [2–6]). It has been shown that changes of this protein environment have a significant influence on the redox potential of P, but the physico-chemical mechanisms underlying this modulation are not yet clear. It has been proposed that an important aspect of this modulation is the formation of strong hydrogen bonds between the conjugated carbonyl groups of the P BChls and the surrounding amino acid side-chains [3,7]. However, it is becoming apparent that hydrogen bond formation cannot be the sole explanation for the effects observed, and that the modulation of the redox potential of P is complex and multifactorial [8].

In the present report, we have analysed the effects of a series of mutations at positions His L168 [9] and Phe M197 [10]. These symmetry-related residues are located on either side of the ring I region where the two BChls of the dimer overlap (see Fig. 1). We show that there is a correlation between the effect of a mutation on E_m P/P^+ and the change in the permanent dipole of the mutated L168 or M197 residue (ΔSPD), and that this strong effect is a particular feature of this pair of residues.

2. Results and discussion

2.1. Changes in E_m P/P^+ in the L168/M197 series

Residue His L168 was mutated to Phe, Leu, Arg, Lys or Asp [9], and residue Phe M197 was mutated to His, Arg, Lys, Asp or Tyr [10]. The reaction centre in which His L168 was mutated to Phe is denoted HL168F, and an equivalent nomenclature is used for all other mutations. Values of E_m P/P^+ were determined by chemical titration, and had a maximum error of ±10 mV [9,10]. The effect of each mutation is expressed in Table 1 as the change in E_m P/P^+ relative to the control titration on the wild-type reaction centre (ΔE_m P/P^+). The values of ΔE_m P/P^+ obtained for the HL168F, FM197Y and FM197H reaction centres were similar to those reported previously [3,11–13]. Also listed in Table 1 is a value of ΔE_m P/P^+ reported recently for a HL168E mutant [14]. For the

*Corresponding author. Fax: (44)-117-928 8274.
E-mail address: m.r.jones@bristol.ac.uk (M.R. Jones).

Abbreviations: BChl, bacteriochlorophyll; ΔSPD, change in side-chain permanent dipole; P, primary donor of electrons; P^+, primary donor cation

0014-5793/02/$22.00
PII: S0014-5793(02)03203-9

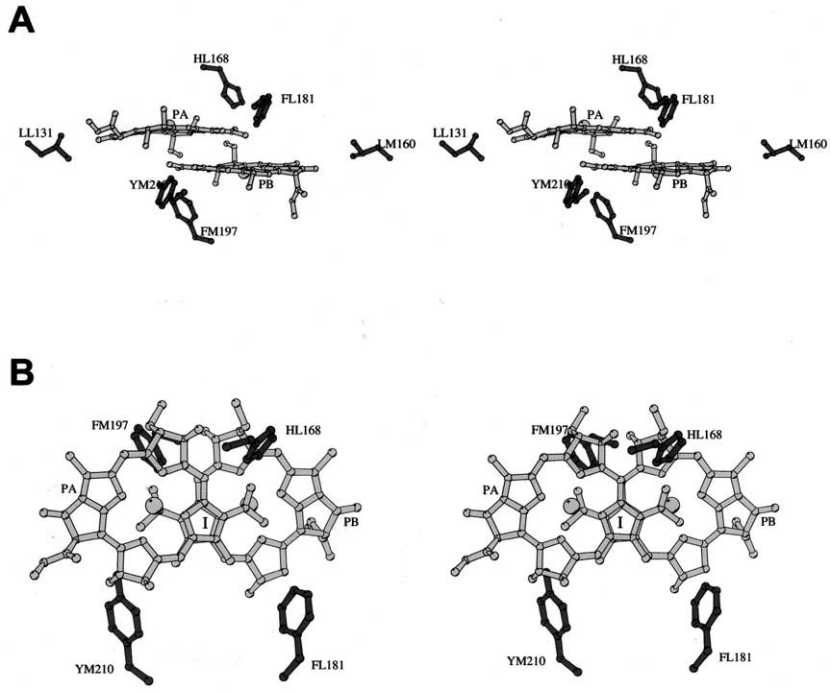

Fig. 1. Stereo views of the primary donor of electrons in the *Rb. sphaeroides* reaction centre. A: Edge-on view of the BChl dimer, showing the positions of residues His L168, Phe M197, Leu L131, Leu M160, Tyr M210 and Phe L181. B: Face-on view of the dimer, showing how the BChls overlap at ring I of the macrocycle, and the positions of His L168 and Phe M197 relative to this overlap region. The figure was prepared using the coordinates of the wild-type reaction centre and the program MOLSCRIPT [34].

L168 residue, E_m P/P$^+$ decreased in the order H > R > E > K > D > F > L. Similarly, E_m P/P$^+$ decreased in the order H > R > K > Y > D > F for the M197 residue.

The best known feature of His L168 is that it donates a hydrogen bond to the acetyl carbonyl group of the P_A BChl. Fourier transform (FT)-Raman spectroscopy has shown that mutation of this His to Phe or Leu removes this hydrogen bond [15]. The corresponding shift in the position of the band in the FT-Raman spectra, attributed to the stretching frequency of the C=O bond, provides an estimate of 4.8–4.9 kcal mol^{-1} for the strength of the bond donated by His L168 [7,9,15]. Conversely, mutation of Phe M197 to His introduces a new hydrogen bond interaction with the acetyl carbonyl of the P_B BChl with an estimated strength of 3.3–3.5 kcal mol^{-1} [7,10]. Table 1 shows the strengths of hydrogen bonds donated by other residues at the L168 and M197 positions when it was possible to estimate them from FT-Raman data (see footnote to Table 1). The data argues against a simple correlation between the strength of the hydrogen bond and the value of E_m P/P$^+$. For example, Tyr and His residues at position M197 donate hydrogen bonds of similar strengths (3.0 and 3.5 kcal mol^{-1}, respectively), but

Table 1
Effects of mutation on the primary electron donor

	ΔE_m P/P$^+$ (mV)	ΔSPD (Debye)	Residue-BChl H-bond (kcal mol^{-1})	Source
His L168	0	0.00	4.90	[15]
HL168R	−60	−2.27	ambiguous[a]	[9]
HL168E	−75	−3.63	n.d.[b]	[14]
HL168K	−86	−2.36	0.00	[9]
HL168D	−91	−3.63	?[c]	[9]
HL168F	−95	−3.97	0.00	[7]
HL168F	−115	−3.97	0.00	[9]
HL168L	−123	−4.66	0.00	[9]
Phe M197	0	0.00	0.00	
FM197H	125	3.97	3.30	[7]
FM197H	119	3.97	3.50	[10]
FM197R	78	1.70	?[c]	[10]
FM197K	69	1.61	?[c]	[10]
FM197Y	46	1.14	3.00	[10]
FM197Y	31	1.14	n.d.[a]	[12]
FM197D	20	0.34	2.30	[10]

[a]FT-Raman spectroscopy yielded ambiguous results, possibly due to sample heterogeneity.
[b]n.d.: not determined by FT-Raman spectroscopy.
[c]The band corresponding to the stretching frequency of the acetyl carbonyl of the relevant BChl is absent from the FT-Raman spectrum, possibly as a result of an out-of-plane rotation of the group [10].

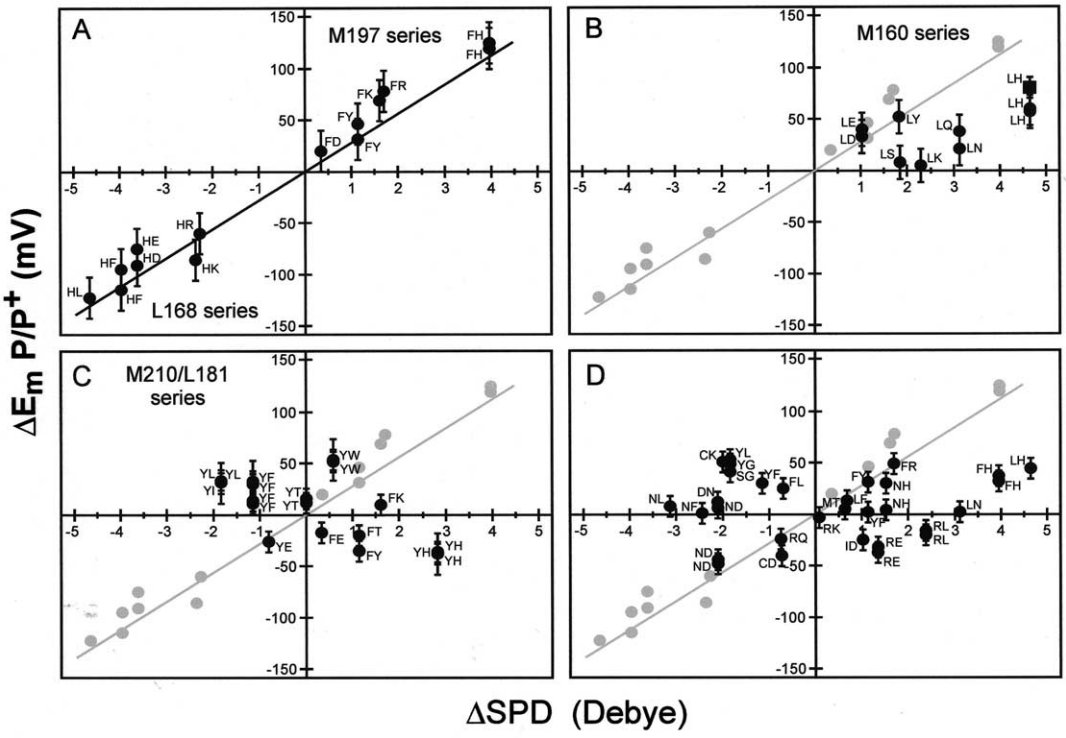

Fig. 2. ΔE_m P/P$^+$ for mutant reaction centres plotted as a function of the associated ΔSPD. In all cases, the nature of each mutation is given as a two letter code (e.g. HF = His to Phe) A: Mutations at the L168/M197 position. A best-fit line was drawn through the data by linear regression, constraining the line to pass through the zero point on both axes (that represents the wild-type complex). B: Mutations at the M160 position (circles) or Leu L131 to His (square). For comparison, the data is superimposed on data for the L168/M197 series from panel A, shown in grey. C: Mutations at the M210 or L181 position. D: Mutations at other positions in the protein environment of the P BChls. In all panels, error bars are calculated from the reported error in the original study of the particular mutant complex.

bring about quite different increases in E_m P/P$^+$ (+46 and +119 mV, respectively) [10]. In the case of the L168 position, a Lys residue produces a value of E_m P/P$^+$ that is 37 mV greater than when Leu is present at this position, but the FT-Raman spectrum indicates that the Lys residue also does not donate a hydrogen bond to the acetyl carbonyl of the P_A BChl [9].

In both series of mutants, presence of a His residue has a particularly strong effect on E_m P/P$^+$. In the M197 series, the change in E_m P/P$^+$ in the presence of a His residue is 50% greater than in the presence of any other amino acid. At the L168 position, when considered relative to a Leu residue, presence of a His residue had twice the effect on E_m P/P$^+$ of any other amino acid. A feature of the His side-chain is that the imidazole ring possesses a large permanent dipole (4.66 D [16]) that is almost 50% greater than the next most polar residue (Asn/Gln, 3.13 D [16]). Given this, we have examined whether there is a correlation between the change in the value of the side-chain permanent dipole (ΔSPD) on mutation of the L168 or M197 residue (Table 1), and the change in the value of E_m P/P$^+$. Values of the side-chain permanent dipole for amino acids were taken from a recent paper by Sandberg and Edholm [16]. These values were (in Debye): Arg, 2.39; Asn, 3.13; Asp, 1.03; Cys, 0.31; Gln, 3.13; Glu, 1.03; His, 4.66; Lys, 2.30; Met, 1.2; Phe, 0.69; Ser, 1.84; Thr, 1.84; Trp, 2.42; Tyr, 1.83. All other amino acids were assigned a value of 0.0 D. The analysis assumes that, where relevant, all of these buried side-chains are in the uncharged state. We know that this is the case for Asp M197, as this residue donates a hydrogen bond to the acetyl carbonyl group of the P_L BChl [10].

As can be seen from Fig. 2A, ΔE_m P/P$^+$ exhibits an approximately linear dependence on the polarity of the L168/M197 residue pair over a range of almost 250 mV, with the potential increasing with the sum of the dipole strength of this pair of residues. The scatter in the plot presumably reflects variations in precise position and orientation of the dipole relative to the P BChls in the different mutants, as the residues in the two series vary considerably in their size and detailed structure.

2.2. Comparisons with the effect of mutating Leu M160

Does this correlation also apply to other positions in the P binding pocket? Residue Leu M160 is located close to the keto carbonyl group of the P_B BChl (Fig. 1), and replacing this Leu by a more polar residue also increases E_m P/P$^+$ [17,18]. Ivancich and coworkers have introduced eight different polar residues at the M160 position, and reported that a His residue brings about the strongest increase in E_m P/P$^+$ (57 ± 16 mV) [18]. However, in this case the introduction of a His residue did not have a markedly stronger effect than any other mutation (the next most effective residue, Tyr, increased E_m P/P$^+$ by 52 ± 16 mV). With the exception of Lys, all of the mutations at the M160 position resulted in the formation of a hydrogen bond with the keto carbonyl of the P_B BChl, as inferred from the FT-Raman spectrum [18]. However, as discussed by Ivancich and coworkers, there was no straightforward correlation between the strength of this bond and the

increase in E_m P/P$^+$. In particular, Asp, Gln, Glu, Tyr and His mutations introduced hydrogen bonds of the same strength (3.3 kcal mol^{-1}), but ΔE_m P/P$^+$ was 24 mV (± 16 mV) higher in the His mutant than in the Asp mutant, with Gln, Glu and Tyr having intermediate values. In their report, Ivancich and coworkers commented on the possible influence of the electrostatic properties of the introduced residue [18], but they did not carry out a detailed analysis of this point.

In Fig. 2B, values of ΔE_m P/P$^+$ reported for the M160 series of mutants [18] are plotted against calculated values of ΔSPD, and the data is superimposed on the equivalent data for the L168/M197 mutants. As can be seen, the M160 mutants did not show any obvious correlation between the two parameters. Mutations of the M160 residue that produced similar changes in the value of ΔSPD, such as Ser and Tyr, had markedly different effects on E_m P/P$^+$. Conversely, mutations that produced similar changes in E_m P/P$^+$, such as Tyr and His, were associated with rather different values of ΔSPD. Also included in the plot is a Leu to His mutation at residue L131 (square). This is the symmetry-related residue to Leu M160, and so is adjacent to the keto carbonyl group of the P_A BChl. Introducing a His residue at this position has a similar effect on E_m P/P$^+$ as a Leu to His mutation at position M160 [7].

2.3. Mutations of Tyr M210 and Phe L181

A series of mutations has also been made at the symmetry-related pair Tyr M210/Phe L181 [19–22]. As can be seen from Fig. 2C, replacement of Tyr M210 with a less polar residue (Phe, Ile, Leu) produces an increase in E_m P/P$^+$, whilst replacement of either Tyr M210 or Phe L181 with a more polar residue produces a decrease in E_m P/P$^+$. In the case of the former mutations, FT-Raman spectroscopy has shown that the mutations have no effect on the hydrogen bond interactions between the P BChls and the surrounding protein [23].

The two mutations that did not follow this trend were Tyr M210 to Glu, and Tyr M210 to Trp. In the latter case, the published X-ray crystal structure of the Tyr M210 to Trp mutant shows that the new Trp is positioned such that a six carbon ring of the Trp side-chain is closer to the P BChls than the polar part of the side-chain [24]. This structural detail may explain why a Trp residue at the M210 position has a similar effect on ΔE_m P/P$^+$ as a Phe, Leu or Ile residue, despite the fact that a Tyr to Trp mutation involves an increase in permanent dipole.

Taking these observations on the Tyr M210 to Trp mutant into account therefore, there is a reasonable correlation between ΔE_m P/P$^+$ and ΔSPD for the YM210/FL181 series. However, the dependence of ΔE_m P/P$^+$ on ΔSPD is not as steep as for the L168/M197 series, and an increase in side-chain polarity produces opposite effects in the two classes of mutants, namely, increases in ΔE_m P/P$^+$ in the L168/M197 series and decreases in the M210/L181 series. It is worth noting that the distances between the L181/M210 and L168/M197 pairs and the macrocycles of P BChls are similar, but they have rather different orientations relative to the P BChls. The L168/M197 residues are located on opposite sides of the overlapping rings I of the P BChls, nearly perpendicular to the BChl macrocycles, whilst the L181/M210 residues are located in the same planes as these macrocycles, approximately equidistant from the edge of both BChls (Fig. 1).

2.4. Other mutations in the vicinity of the P BChls

To complete this analysis, Fig. 2D shows data for all other mutations that have been constructed in the environment of the P BChls and where ΔE_m P/P$^+$ has been reported [11,12,18,25–30]. Taken together with the rest of the data in Fig. 2, the general picture that emerges is one in which, apart from at the L168 and M197 positions and a couple of other exceptions, single point mutations have the capacity to modulate E_m P/P$^+$ over a range of no more than ± 50 mV. Fig. 2d shows that, regardless of whether they increase or decrease the polarity of the environment of P, most mutations increase E_m P/P$^+$. Examining the data in detail it becomes obvious that the position of the mutation has an important bearing on its effects. As an illustration, a Y→F mutation at residue L162 produced the same ΔE_m P/P$^+$ as an F→Y mutation at residue L167, despite that fact that these mutations are associated with equal, but opposite, values of ΔSPD.

2.5. Tuning of E_m P/P$^+$ in the bacterial reaction centre

It is generally accepted that the optical, electronic and electrochemical properties of the P BChls are tuned by the protein environment. What is not clear is the extent to which this tuning is achieved by the surrounding protein acting as an inert scaffold, holding the two P BChls in a very specific dimeric arrangement, and the extent to which tuning is achieved by specific BChl–amino acid interactions.

This report collates available data on the effects of mutagenesis on the redox properties of the P BChls. Most of the mutations made in the vicinity of the P BChls either have no effect on E_m P/P$^+$, or produce an increase of ≤ 50 mV (Fig. 2), regardless of whether they involve a decrease or increase in the polarity of the mutated side-chain. No clear pattern emerges from the data presented in Fig. 2 when taken as a whole. A possible explanation for this is that E_m P/P$^+$ is principally sensitive to disruptions of the overall structure of the protein environment. If the reaction centre is engineered such that the redox potential of P is close to the minimum achievable (taking into account the interaction with His L168), then bringing about relaxations in this protein scaffold through mutation might reasonably be expected to result in an increase in E_m P/P$^+$. It would follow that randomly perturbing the structure of P should result, in most cases, in an increase in E_m P/P$^+$.

A subset of the mutations surveyed in Fig. 2D produced a decrease in E_m P/P$^+$. Notably, these involve a change in acid/base character from basic to neutral (R→L, R→Q), basic to acidic (R→E) or neutral to acidic (C→D, I→D, N→D). These decreases in E_m P/P$^+$ have been assigned to charge–charge interactions, the removal of a positive charge and/or the introduction of a negative charge stabilising the P$^+$ state [29,30]. The relatively modest change in E_m P/P$^+$ produced by these charge–charge interactions has been attributed to strong dielectric screening by the surrounding protein/solvent matrix, perhaps involving counterions that to a large extent alter the charge–charge interaction with P$^+$ into a charge–dipole interaction [30].

2.6. The strong influence of the L168/M197 residue pair

The strongest effects on E_m P/P$^+$ are seen following mutations of His L168 and Phe M197. The strong correlation depicted in Fig. 2A suggests that this ∼250 mV modulation is largely a response to the change in the electrostatic inter-

action between the BChl molecule and the adjacent L168 or M197 residue. X-ray crystallography of some of these mutant complexes has shown that the mutations do not induce large-scale change in the structure of the P BChls and the surrounding protein matrix [9,10].

Modulation of redox potential through charge–dipole interactions has been extensively studied in proteins involved in electron transfer such as cytochromes (see [31] and other articles in the same issue). In cytochrome b_{562}, for example, it was recently shown that such interactions can tune the redox potential of heme *b* over a range of nearly 100 mV [32]. In bacterial reaction centres, similar charge–dipole interactions were recently suggested to be at the origin of large redox potential shifts of the Q_A quinone molecule observed in some mutant complexes [33].

As discussed above, the presence of a dipole-bearing residue at the L168 and M197 positions may, or may not, be accompanied by the formation of a hydrogen bond interaction with the adjacent acetyl carbonyl. However, there is no obvious correlation between ΔE_m P/P^+ and the change in hydrogen bond strength. On the contrary, the correlation found between the ΔSPD and ΔE_m P/P^+ appears to be valid whether a hydrogen bond is formed or not. This suggests that, in itself, the formation of this hydrogen bond has at best a secondary effect on the redox potential of P.

Another point to note is that there is evidence that the FM197R, FM197K and HL168D mutations induce a rotation of the acetyl carbonyl group out of the plane of the dihydrophorbin ring. This proposal is based on the absence of the band attributable to the stretching mode of the relevant carbonyl group from the FT-Raman spectrum, indicating that this carbonyl is no longer part of the conjugated system of the P BChls [9,10]. In the case of the FM197R mutant this proposal is supported by the results of X-ray crystallography that show a 20° out-of-plane rotation of the acetyl carbonyl group of the P_B BChl [10]. These three mutations have no particular effect on E_m P/P^+ relative to those that do not appear to affect conjugation of the acetyl carbonyl group. This indicates that the particularly strong effect of the electrostatic changes at the L168/M197 positions is not mediated by the acetyl carbonyl group. Given this, it is possible that the crucial factor is the proximity of the L168/M197 residue pair to the ring I regions of the P BChls, with the primary donor being particularly sensitive to the dipole strength of residues immediately adjacent to this overlap region.

Acknowledgements: This work was supported by the Biotechnology and Biological Sciences Research Council of the United Kingdom. The authors wish to thank Dr. Paul Fyfe for his help with Figure 1.

References

[1] Moser, C.C., Keske, J.M., Warncke, K., Farid, R.S. and Dutton, P.L. (1992) Nature 355, 796–802.
[2] Woodbury, N.W. and Allen, J.P. (1995) in: Anoxygenic Photosynthetic Bacteria (Blankenship, R.E., Madigan, M.T. and Bauer, C., Eds.) pp. 527–557, Kluwer Academic Publishers, The Netherlands.
[3] Allen, J.P. and Williams, J.C. (1995) J. Bioenerg. Biomembr. 27, 275–283.
[4] Parson, W.W. (1996) in: Protein Electron Transfer (Bendall, D.S., Ed.), pp. 125–160, BIOS Scientific Publishers, Oxford.
[5] Hoff, A.J. and Deisenhofer, J. (1997) Phys. Rep. 287, 1–247.
[6] Van Brederode, M.E. and Jones, M.R. (2000) in: Enzyme-Catalysed Electron and Radical Transfer (Scrutton, N.S. and Holzenburg, A., Eds.), pp. 621–676, Kluwer Academic/Plenum Publishers, New York.
[7] Mattioli, T.A., Lin, X., Allen, J.P. and Williams, J.C. (1995) Biochemistry 34, 6142–6152.
[8] Ivancich, A., Artz, K., Williams, J.C., Allen, J.P. and Mattioli, T.A. (1998) Biochemistry 37, 11812–11820.
[9] Spiedel, D., Roszak, A.W., McKendrick, K., McAuley, K.E., Fyfe, P.K., Nabedryk, E., Breton, J., Robert, B., Cogdell, R.J., Isaacs, N.W. and Jones, M.R. (2002) Biochim. Biophys. Acta 1554, 75–93.
[10] Ridge, J.P., Fyfe, P.K., McAuley, K.E., van Brederode, M.E., Robert, B., van Grondelle, R., Isaacs, N.W., Cogdell, R.J. and Jones, M.R. (2000) Biochem. J. 351, 567–578.
[11] Murchison, H.A., Alden, R.A., Allen, J.P., Peloquin, J.M., Taguchi, A.K.W., Woodbury, N.W. and Williams, J.C. (1993) Biochemistry 32, 3498–3505.
[12] Wachtveitl, J., Farchaus, J.W., Das, R., Lutz, M., Robert, B. and Mattioli, T.A. (1993) Biochemistry 32, 12875–12886.
[13] Lin, X., Murchison, H.A., Nagarajan, V., Parson, W.W., Allen, J.P. and Williams, J.C. (1994) Proc. Natl. Acad. Sci. USA 91, 10265–10269.
[14] Williams, J.C., Haffa, A.L.M., McCulley, J.L., Woodbury, N.W. and Allen, J.P. (2001) Biochemistry 40, 15403–15407.
[15] Mattioli, T.A., Williams, J.C., Allen, J.P. and Robert, B. (1994) Biochemistry 33, 1636–1643.
[16] Sandberg, L. and Edholm, O. (2001) J. Phys. Chem. 105, 273–281.
[17] Artz, K., Williams, J.C., Allen, J.P., Lendzian, F., Rautter, J. and Lubitz, W. (1997) Proc. Natl. Acad. Sci. USA 94, 13582–13587.
[18] Ivancich, A., Artz, K., Williams, J.C., Allen, J.P. and Mattioli, T.A. (1998) Biochemistry 37, 11812–11820.
[19] Jia, Y.W., DiMagno, T.J., Chan, C.K., Wang, Z.Y., Du, M., Hanson, D.K., Schiffer, M., Norris, J.R., Fleming, G.R. and Popov, M.S. (1993) J. Phys. Chem. 97, 13180–13191.
[20] Nagarajan, V., Parson, W.W., Davis, D. and Schenck, C.C. (1993) Biochemistry 32, 12324–12336.
[21] Beekman, L.M.P., Van Stokkum, I.H.M., Monshouwer, R., Rijnders, A.J., McGlynn, P., Visschers, R.W., Jones, M.R. and Van Grondelle, R. (1996) J. Phys. Chem. 100, 7256–7268.
[22] DiMagno, T.J., Laible, P.D., Reddy, N.R., Small, G.J., Norris, J.R., Schiffer, M. and Hanson, D.K. (1998) Spectrochim. Acta, Part A 54, 1247–1267.
[23] Jones, M.R., Heer-Dawson, M., Mattioli, T.A., Hunter, C.N. and Robert, B. (1994) FEBS Lett. 339, 18–24.
[24] McAuley, K.E., Fyfe, P.K., Cogdell, R.J. and Isaacs, N.W. (2000) FEBS Lett. 467, 285–290.
[25] Williams, J.C., Alden, R.G., Murchison, H.A., Peloquin, J.M., Woodbury, N.W. and Allen, J.P. (1992) Biochemistry 31, 11029–11037.
[26] Wachtveitl, J., Farchaus, J.W., Mathis, P. and Oesterhelt, D. (1993) Biochemistry 32, 10894–10904.
[27] Lin, X., Williams, J.C., Allen, J.P. and Mathis, P. (1994) Biochemistry 33, 13517–13523.
[28] Ivancich, A., Mattioli, T.A., Artz, K., Wang, S.J., Allen, J.P. and Williams, J.C. (1997) Biochemistry 36, 3027–3036.
[29] Williams, J.C., Haffa, A.L.M., McCulley, J.L., Woodbury, N.W. and Allen, J.P. (2001) Biochemistry 40, 15403–15407.
[30] Johnson, E.T. and Parson, W.W. (2002) Biochemistry 41, 6483–6494.
[31] Mauk, A.G. and Moore, G.R. (1997) J. Biol. Inorg. Chem. 2, 119–125.
[32] Springs, S.L., Bass, S.E., Bowman, G., Nodelman, I., Schutt, C.E. and McLendon, G.L. (2002) Biochemistry 41, 4321–4328.
[33] Takahashi, E., Wells, T.A. and Wraight, C.A. (2001) Biochemistry 40, 1020–1028.
[34] Kraulis, P.J. (1991) J. Appl. Crystallogr. 24, 946–950.