ENERGETICS OF THE PRIMARY CHARGE SEPARATION IN BACTERIAL PHOTOSYNTHESIS

M. Bixon,[1] Joshua Jortner,[1] and M.E. Michel-Beyerle[2]
[1]School of Chemistry, Tel Aviv University, Tel Aviv 69978, Israel.
[2]Institut für Physikalische und Theoretische Chemie,
Technische Universität München,
Lichtenbergstr. 4, D-85748 Garching, Germany.

Abstract. We consider the free energy relationship for the primary electron transfer (ET) (at T = 295 K) from the electronically excited singlet state of the bacteriochlorophyll dimer in the bacterial photosynthetic native reaction center (RC), some of its single-site mutants and chemically engineered RCs containing accessory 13^2-OH-Ni-bacteriochlorophyll (Ni-B). This analysis resulted in the reasonable value $\lambda_1 = 800\pm250\ cm^{-1}$ for the medium reorganization energy and $\Delta G_1(N) = -480\pm180\ cm^{-1}$ for the energy gap of the native RC. These energetic parameters imply that ET in the native RC corresponds to (nearly) optimal activationless ET. The quantum free energy relation predicts very fast ET time for the Ni-B substituted center ($\Delta G_1 \simeq -1500\ cm^{-1}$) which reflects prosthetic group(s) vibrational excitation induced by ET in the inverted region. The low negative value of $\Delta G_1(N)$ implies that the dominant room temperature ET mechanism for the native RC involves sequential ET.

Key Words: energetics, primary charge separation, Ni-RC, RC mutants.

1. Introduction

All the mechanisms proposed for the primary electron transfer (ET) in the bacterial photosynthetic reaction center (RC) [1-5] attribute a special role to the accessory bacteriochlorophyll (B). The nature of the primary process, i.e., the superexchange [5-15], the sequential [16-27] or the parallel sequential-superexchange [28-30] mechanism, is dominated by the (free) energy gap ΔG_1 of the ion pair state P^+B^-H relative to $^1P^*$. Several sources of information on ΔG_1 in the native RC were advanced: (1) Molecular dynamics simulations [31-33]. These energetic data are intrinsically limited due to incomplete input information, e.g., the location of accessory water molecules and the long-range interactions. (2) Recombination of the ion pair P^+BH^-. Experimental magnetic data [34] resulted in the lower limit $\Delta G_1 > -600\ cm^{-1}$. (3) Kinetic data on ET in the chemically modified pheophytin → bacteriopheophytin RC resulted in $\Delta G_1 = -450\ cm^{-1}$ [27], which is

presumably applicable also for the native RC. In what follows we shall construct a free-energy relationship for the decay rates of $^1P^*$ in the native RC [25,35], in a series of some single-site mutants [25,35-39] and in the chemically modified 13^2-OH-Ni-bacteriochlorophyll (Ni-B) → bacteriochlorophyll (B) RC [40,41]. From this phenomenological analysis we shall extract the energy gap ΔG_1 for the native RC and the medium reorganization energy for the primary ET. The energetics will provide a clue for the mechanism of the primary process.

2. Energetic Parameters for the Native RC and for 'Good' Single-Site Mutants

We have constructed a free-energy relationship for the primary ET in single-site mutants [25,35,36-39] using the following information.

(i) The kinetic data for the decay of $^1P^*$. The kinetic decay of $^1P^*$ is nonexponential, reflecting the effects of static heterogeneity, which are manifested by long time tails [25,30,35,42,43]. On the basis of the representation of the decay of $^1P^*$ in a heterogeneous system in terms of two exponentials [25,35,42,43], as was previously done in the analysis of the experimental data, we showed [30] that the fast decay component provides a reasonable (within 10%-30%) representation of the decay of P^* in the corresponding homogeneous system. Accordingly, we use the experimental fast decay component for the representation of the primary ET rate, which will be denoted by k_{ET}.

(ii) The oxidation potentials $E_R(P^+/P)$ of P in the RCs [25,35,36-39]. It is assumed that for these mutants

$$\Delta G_1 = E_R(P^+/P) + B \quad , \qquad (1)$$

where B is a constant. Eq. (1) implies that equal changes are induced by mutations in the redox potentials of $^1P^*/P^+$ and of P/P^+ and that the mutation perturbs only P and not B_A of H_A.

Single-site local L181 or M208 mutations of *R.capsulatus* [35] and of *R.sphaeroides* do not necessarily obey relation (1), as site mutagenesis may also change the energetics of the prosthetic groups B_A and/or H_A and their ionic states, and also modify the electronic coupling V. A valid free-energy relationship between k_{ET} and $E_R(P^+/P)$ is expected to be obeyed for 'good' single-site mutants, which obey the following constraints regarding the geometry and the interactions:

(A) Minimization of geometrical changes, which may modify V. Such changes will be induced by the replacement of the tyrosine (Y) M210 in *R.sphaeroides* (M208 in *R.capsulatus*) residue by a smaller (i.e., threonine, leucine or isoleucine) or by a bulkier (i.e., tryptophane (W)) residue. On the other hand, phenylalanine (F) and histidine (H) are

closer in size to Y, and H may even hydrogen-bond like Y.
(B) Minimization of the perturbation of the prosthetic groups B_A and H_A by the mutations. All the site mutants of Y M210 (M208) [35,38] are excluded, and so is the GM203 → D (glycine (G) to aspartic acid (D)) mutant, which was specifically designed to affect B_A [36].

Guided by these empirical rules we have taken for the analysis of the free energy relation for 'good' mutants all the L181 mutants of the Chicago-Argonne group [35], the Y-Y mutant from the Munich group [25] and the relevant mutants from the Arizona group [36,37]. For the native RC we have taken the data for *R.capsulatus* [35], and *R.sphaeroides* [25,38]. These data are scattered (due to different samples and interrogation methods) by ± 35%, providing a lower limit for the uncertainty of the experimental data. In Figure 1 we present the experimental k_{ET} data at T = 295 K for the native RC, together with those for the 'good' mutants. These data fit nicely the theoretical free energy dependence

$$k_{ET} = (2\pi V^2/\hbar)\, F(\lambda_1, S_c, \hbar\omega_c, \Delta G_1, T) \quad , \tag{2}$$

where V is the electronic coupling and F is the nuclear Franck Condon factor

$$F = (2\pi k_B T \lambda_1)^{-1/2} \exp(-S_c) \sum_{n=0}^{\infty} \frac{(S_c)^n}{n!} \exp[-(\Delta G_1 + n\hbar\omega_c + \lambda_1/4\lambda_1 k_B T)]. \tag{3}$$

F is characterized by low frequency modes with the medium reorganization energy λ_1 and by the high frequency mode ω_c with a coupling strength S_c. The lifetime for primary ET is given by

$$\tau_{ET} = 1/k_{ET} \quad . \tag{2a}$$

For the 'good' mutants we implicitly assume that V and λ_1 are invariant and ΔG_1 is given by Eq. (1). At this stage we do not commit ourselves to the nature of the charge separation from $^1P^*$, i.e., sequential or superexchange mechanism, although the simple form of (2) implies that one of these mechanisms dominates. The parameters λ_1 (taken to be mutant invariant) and ΔG_1 (mutant dependent according to Eq. (1)) will be determined from the analysis of the experimental data [25,35-37]. Good account of the free energy relationship for τ_{ET} (or k_{ET}) can be accomplished (Fig. 1) with the following parameters: (i) The medium reorganization energy λ_1 = 800 cm^{-1}. (ii) The uniform shift of the (free) energy scale, Eq. (1), is B = -4500 cm^{-1}. (iii) For the high frequency mode we have chosen either the traditional values

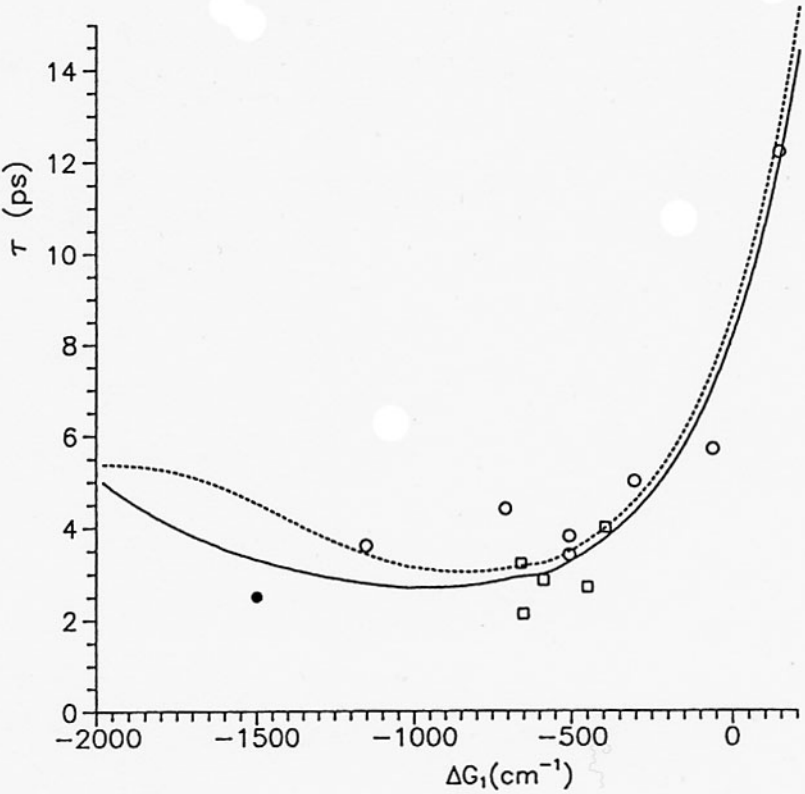

Figure 1. The free-energy relationship for the experimental data of τ_{ET} vs ΔG_1 for the native RC (data marked by a vertical arrow), for 'good' single site mutants and for the chemically modified Ni-B RC at room temperature (T = 295 K). The electronic coupling is V = 20 cm^{-1}. The low frequency 'medium' reorganization energy is λ_1 = 800 cm^{-1}. The high frequency vibration is characterized by S_c = 0.5 and ω_c = 1500 cm^{-1} or ω_c = 1000 cm^{-1}. The experimental data for the single site mutants are taken from: o - references 36,37, [] - reference 35 and • - reference 45. The experimental data point, with the corresponding ΔG_1 values taken from the $E_R(P^+/P)$ redox potentials (references 25,35-37) for the chemically modified Ni-B are taken from reference 45, with $\Delta G_1 \simeq -1500$ cm^{-1} (see text). The theoretical curves are calculated at T = 300 K from Eqs. (2), (2a) and (3) with the parameters given above. Solid curve ω_c = 1000 cm^{-1}, dashed curve ω_c = 1500 cm^{-1}. Note that the free energy relationship is presented by τ_{ET} vs ΔG_1, rather than by the conventional form of $\ell n k_{ET}$ vs ΔG_1.

[28,44] $\hbar\omega_c$ = 1500 cm^{-1} and S_c = 0.5, or a somewhat lower frequency ω_c = 1000 cm^{-1} again with S_c = 0.5. At room temperature (T = 295 K) the calculated free energy relationship is independent of ω_c for ΔG_1 > -1000 cm^{-1} and depends only weakly on ω_c for lower values of ΔG_1 (-1000 cm^{-1} > ΔG_1 > -2000 cm^{-1}) (Fig. 1). (iv) The electronic coupling is taken to be V = 20 cm^{-1}. From this analysis we infer that the energy gap for the native RC, which will be denoted by $\Delta G_1(N)$, is $\Delta G_1(N)$ = -480 cm^{-1}. In view of the spread of the experimental data (Fig. 1) the energetic parameters at room temperature, which provide an adequate fit of these results for the native RC and for the 'good' single-site mutants, are

$$\lambda_1 = 800 \pm 250 \text{ cm}^{-1}$$
$$\Delta G_1(N) = -480 \pm 180 \text{ cm}^{-1} \quad (4)$$

The (free) energy domain spanned by the native RC and by the 'good' single-site mutants corresponds in range from ΔG_1 = -1100 cm^{-1} to ΔG_1 = 100 cm^{-1}. The energetic parameters for the native RC, Eq. (4), are close (within their uncertainty range) to activationless ET, i.e., $-\Delta G_1(N) \simeq \lambda_1$. It is of considerable interest to extend the free energy range for lower ΔG_1 values, which correspond to the strongly exoergic inverted region ($-\Delta G_1 > \lambda_1$). This analysis will clearly bring up the role of quantum effects on the Franck-Condon factor F, which originates from the coupling with the intramolecular vibrational modes of the prosthetic groups [44]. This can be accomplished by the analysis of the k_{ET} vs ΔG_1 relation for the Ni-B RC [40].

3. Energetic Parameters for the Ni-B RC

The incorporation of the Ni substituted prosthetic groups into the photosynthetic RC of *R.sphaeroides* R26, replacing the two accessory bacteriochlorophylls B_A and B_B, results in a drastic change in the energy of the ion pair state $P^+(Ni\text{-}B)^-$ relative to that of P^+B^- in the native RC. The redox potential of the Ni substituted chlorophyll $(NiCh\ell)^-/(NiCh\ell)$ in solution is lower by 0.29 eV than the corresponding value for the redox potential of $Ch\ell^-/Ch\ell$ [40]. From the thermally activated participation of $P^+(Ni\text{-}B)^-$ in the recombination dynamics of the radical pair $P^+H_A^-$ an energy gap between the two states of less than 500 cm^{-1} can be estimated [45] and we shall tentatively take ΔG_1 = -1500 cm^{-1} for the (free) energy gap for (Ni-B) chemically substituted RC. Obviously, the primary ET in this chemically engineered RC corresponds to the inverted region. In spite of this drastic reduction of ΔG_1, which corresponds to the lowest value of the gap achieved either by mutagenesis and/or chemical substitution,

the primary ET lifetime is very close to that of the native RC. The decay of $^1P^*$ in the (Ni-B) substituted RC at T = 295 K is characterized by a biexponential decay with the short component τ_{ET} = 2.5 ps (amplitude 69%) and a longer component τ_L = 7 ps (amplitude 31%) [45]. The short decay component for the primary ET in the (Ni-B) substituted RC is very close (within 30%, which corresponds to experimental uncertainty) to τ_{ET} for the native RC (Fig. 1). The very weak energy gap dependence of τ_{ET} in the range $\Delta G_1 \simeq -400$ cm^{-1} to $\Delta G_1 \simeq -1500$ cm^{-1} (Fig. 1), can be well accounted for by the ET theory, i.e., Eqs. (2) and (3) with the invariant value of λ_1, Eq. (4). In the inverted region, i.e., $-\Delta G_1 \geq \lambda_1$, the contribution of the quantum modes is major, resulting in a marked deviation from the classical result (which corresponds to $S_c = 0$), which is attributed to the intramolecular vibrational excitation of the prosthetic groups induced by ET [44]. As is evident from Fig. 1, the energy gap dependence of τ_{ET} for the Ni-B substituted RC is reasonably well accounted for by the ET theory, with ω_c = 1000 cm^{-1}

4. Concluding Remarks

The energetics of the primary ET in the native photosynthetic RC, some of its single-site 'good' mutants and the Ni-B chemically engineered RC, can be accounted for in terms of the quantum free energy relationship, Eqs. (2) and (3). The major results of the analysis, Eq. (4), resulted in the (mutant and chemically substituted invariant) value of the medium reorganization energy λ_1 = 800±250 cm^{-1} and in the (free) energy gap $\Delta G_1(N)$ = -480±180 cm^{-1} for the native RC. From these results we conclude that:

1) The reorganization energy λ_1 for the primary ET incorporates all the "low frequency" vibrational modes of the protein and of the prosthetic groups (for which the frequencies are $\hbar\omega < k_B T$ at T = 295 K) which involve the protein modes and the intramolecular modes of the dimer. The value of λ_1 obtained herein is considerably higher than the surprisingly low value ($\lambda_1 \leq 250$ cm^{-1} at T = 295 K), which was previously inferred from the analysis of single site mutants [25, 35-38]. Our value of λ_1 is larger than the spectroscopic electron-phonon coupling for the electronic excitation $P \rightarrow {}^1P^*$, which gives the spectroscopic reorganization energy λ_S = 250 cm^{-1} [46, 47]. Indeed, λ_S is expected to provide the lower limit for λ_1. Finally, we note that λ_1 is lower than the reorganization energy $\lambda_T >$ 1300 cm^{-1} inferred from magnetic field effects [48] on the recombination of the ion pair $P^+BH^- \rightarrow {}^3P^*$
2) The values of λ_1 and $\Delta G_1(N)$, Eq. (4), imply that ET in the native RC is close (within the uncertainty range of the energetic

parameters) to activationless ET, i.e., $-\Delta G_1 \simeq \lambda_1$. This physical situation corresponds to (nearly) optimal ET (i.e., nearly shortest τ_{ET}) in the native RC.

3) The (free) energy gap relation, which incorporates vibrational quantum effects, predicts a very fast ET for the Ni-B chemically substituted RC. τ_{ET} for this system, which is characterized by the lowest currently available value of ΔG_1 ($\simeq -1500$ cm^{-1}), is close to the ET lifetime for the native RC. This prediction, pertaining to the inverted region, is borne out by the experimental results.
4) The low value of $\Delta G_1(N)$ for the native RC is in good agreement with the lower limit $\Delta G_1 > -600$ cm^{-1} previously inferred from the analysis of magnetic data for the P^+BH^- ion pair [34] and the value $\Delta G_1 = -450$ cm^{-1} derived from time-resolved data for the pheophytin $\rightarrow$ H chemically modified RC [27].
5) The low value of $\Delta G_1(N) = -480 \pm 180$ cm^{-1} obtained herein for the native RC is considerably lower in its absolute value than the energy gap $\Delta G = -2000$ cm^{-1} between $^1P^*$ and P^+BH^- [49,50]. This result implies that the first ET step in the native RC at room temperature is not a direct superexchange mediated ET to H.
6) The dominance of the sequential mechanism for primary ET prevails for the room temperature inverted region, i.e., the Ni-B chemically modified RC and the single-site mutants presented in Fig. 1. For larger values of ΔG_1 (≥ -300 cm^{-1}), which correspond to double and to triple mutants [39] at room temperature and at low temperatures [39] (which were not analyzed herein), as well as for some single site mutants at low temperatures, the contribution of the superexchange mechanism is substantial [30]. Even for the native RC at low temperature, when heterogeneity effects are incorporated, a contribution ($\sim$ 10%) of the superexchange is expected to prevail [30] and to contribute to the long time tail of the decay of $^1P^*$ [30].

There are some pitfalls in our analysis. From the experimental point of view, we have utilized in Fig. 1 experimental data for different samples from different laboratories, increasing experimental uncertainty. In our analysis we have invoked the invariance of λ_1 for different mutants and for the chemically engineered Ni-B RC. Finally, we have provided only a heuristic account of heterogeneity effects. This important issue is addressed elsewhere [30,51].

References

1. J. Jortner and M.E. Michel-Beyerle, in: Antennas and Reaction Centers of Photosynthetic Bacteria, ed. M.E. Michel-Beyerle (Springer, Berlin, 1985) p. 345-354.

2. The Photosynthetic Bacterial Reaction Center. Structure and Dynamics, eds. J. Breton and A. Vermeglio (Plenum NATO ASI Series, New York, 1988).
3. Reaction Centers of Photosynthetic Bacteria, ed. M.E. Michel-Beyerle (Springer Verlag, Berlin, 1990).
4. The Photosynthetic Bacterial Reaction Center II, NATO-ASI series A, J. Breton and A. Verméglio (Life Sciences, Plenum Press, New York, 1992) vol. 237.
5. The Photosynthetic Reaction Center, J. Deisenhofer and J.R. Norris (Academic Press, San Diego, 1993).
6. N.W. Woodbury, M. Becker, D. Middenforf and W.W. Parson, Biochemistry 24 (1985) 7516.
7. S.F. Fischer, I. Nussbaum and P.O.J. Scherer, in: Antennas and Reaction Centers of Photosynthetic Bacteria, ed. M.E. Michel-Beyerle (Springer, Berlin, 1985) p. 256.
8. A. Ogrodnik, N. Remy-Richter, M.E. Michel-Beyerle and R. Feick, Chem. Phys. Lett. 135 (1987) 576.
9. J.R. Norris, D.E. Budil, D.M. Tiede, J. Tang, S.V. Kolaczkowski, C.H. Chang and M. Schiffer, in: Progress in Photosynthetic Research, ed. J. Biggins (Martinus Nijhoff, Dordrecht, 1987) vol. I, p. 1.4.363.
10. M.E. Michel-Beyerle, M. Plato, J. Deisenhofer, H. Michel, M. Bixon and J. Jortner, Biochim. Biophys. Acta 932 (1988) 52.
11. M. Bixon, J. Jortner, M. Plato and M.E. Michel-Beyerle, in: The photosynthetic bacterial reaction center. Structure and Dynamics, eds. J. Breton and A. Vermeglio (Plenum NATO ASI Series, New York, 1988) p. 399.
12. M. Plato, K. Möbius, M.E. Michel-Beyerle, M. Bixon and J. Jortner, J. Am. Chem. Soc. 110 (1988) 7279.
13. M. Bixon, M.E. Michel-Beyerle and J. Jortner, Isr. J. Chem. 28 (1988) 155.
14. M. Bixon, J. Jortner, M.E. Michel-Beyerle and A. Ogrodnik, Biochim. Biophys. Acta 977 (1989) 273.
15. R.A. Friesner and Y. Won, Biochim. Biophys. Acta 977 (1989) 99.
16. R.A. Marcus, Isr. J. Chem. 28 (1988) 205.
17. R. Haberkorn, M.E. Michel-Beyerle and R.A. Marcus, Proc. Natl. Acad. Sci. USA 70 (1979) 4185.
18. R.A. Marcus, Chem. Phys. Lett. 133 (1987) 471.
19. S.V. Chekalin, Ya.A. Matveetz, A.Ya. Shkuropatov, V.A. Shuvalov and A.P. Yartzev, FEBS Lett. 216 (1987) 245.
20. R.A. Marcus, Chem. Phys. Lett. 146 (1977) 13.
21. S. Creighton, J.-K. Hwang, A. Warshel, W.W. Parson and J. Norris, Biochem. 27 (1988) 774.
22. W. Holzapfel, U. Finkele, W. Kaiser, D. Oesterhelt, H. Scheer, H.U. Stilz and W. Zinth, Chem. Phys. Lett. 160 (1989) 1.

23. W. Holzapfel, U. Finkele, W. Kaiser, D. Oesterhelt, H. Scheer, H.U. Stilz and W. Zinth, Proc. Nat. Acad. Sci. USA 87 (1990) 5168.
24. C. Lauterwasser, U. Finkele, H. Scheer and W. Zinth, Chem. Phys. Lett. 183 (1991) 471.
25. P. Hamm, K.A. Gray, D. Oesterhelt, R. Feick, H. Scheer and W. Zinth, Biochim. Biophys. Acta 1142 (1993) 99.
26. T. Arlt, S. Schmidt, W. Kaiser, C. Lauterwasser, M. Meyer, H. Scheer and W. Zinth, Proc. Natl. Acad. Sci. USA 90 (1993) 11757.
27. P.M. Schmidt, T. Arlt, P. Hamm, H. Huber, T. Nägele, J. Wachtveitel, M. Meyer, H. Scheer and W. Zinth, Chem. Phys. Lett. 223 (1994) 116
28. M. Bixon, J. Jortner and M.E. Michel-Beyerle, Biochim. Biophys. Acta 1056 (1991) 301.
29. M. Bixon, J. Jortner and M.E. Michel-Beyerle, reference 4 (1992) 291.
30. M. Bixon, J. Jortner and M.E. Michel-Beyerle, Chem. Phys. 179 (1995) 389.
31. W.W. Parson, Z.T. Chu and A. Warshel, Biochem. Biophys. Acta 1017 (1990) 251.
32. M. Mercht, J.N. Gehlen, D. Chandler and M. Newton, J. Am. Chem. Soc. (1993) 4178.
33. A. Warshel, Z.T. Chu and W.W. Parson, Photochem. and Photobiology A82 (1994) 123.
34. M. Volk, A. Ogrodnik and M.E. Michel-Beyerle, in: Anoxygenic Photosynthetic Bacteria, eds. R.E. Blankenship, M.T. Madigan and C.E. Bauer, pp. 595 (Kluwer Acad., Dordrecht, 1995).
35. Y. Jia, T.J. DiMagno, C.-K. Chan, Z. Wang, M. Du, D.K. Hanson, M. Schiffer, J.R. Norris, G.R. Fleming and M.S. Popov, J. Phys. Chem. 97 (1993) 13180.
36. J.C. Williams, R.G. Alden, H.A. Murchison, J.M. Peloquin, N.W. Woodbury and J.P. Allen, Biochemistry 31 (1992) 11029.
37. H.A. Murchison, R.G. Alden, J.P. Allen, J.M. Peloquin, A.K.W. Taguchi, N.W. Woodbury and J.C. Williams, Biochemistry 32 (1993) 3498.
38. V. Nagarajan, W.W. Parson, D. Davis and C.C. Schenck, Biochemistry 32 (1993) 12324.
39. N.W. Woodbury, S. Lin, X. Lin, J.M. Peloquin, A.K.W. Taguchi, J.C. Williams and J.P. Allen, Chem. Phys. 197 (1995) 405.
40. G. Hartwich, Ph.D. Thesis, TU München (1994).
41. G. Hartwich, M. Friese, H. Scheer, A. Ogrodnik, and M.E. Michel-Beyerle, Chem. Phys. (in press) 1995.
42. M. Du, S.J. Rosenthal, X. Xie, T.J. DiMagno, M. Schmidt, D.K. Hanson, M. Schiffer, J.R. Norris and G.R. Fleming, Proc.

Natl. Acad. Sci. USA 89 (1992) 8517.
43. M.G. Müller, K. Griebenow and A.R. Holzwarth, Chem. Phys. Lett. 199 (1992) 465.
44. M. Bixon and J. Jortner, J. Phys. Chem. 95 (1991) 1942.
45. T. Häberle, H. Lossan, M. Friese, G. Hatwich, A. Ogrodnik, H. Scheer and M.E. Michel-Beyerle (this volume).
46. R. Raja, S. Reddy, P.A. Lyle and G.J. Small, Photosynth. Res. 31 (1992) 167.
47. P.A. Lyle, S. Kolaczkowski and G.J. Small, J. Phys. Chem. 97 (1993) 6924.
48. M. Bixon, J. Jortner and M.E. Michel-Beyerle, Z. Phys. Chem. 180 (1993) 193.
49. A. Ogrodnik, M. Volk. R. Letterer, R. Feick and M.E. Michel-Beyerle, Biochim. Biophys. Acta 936 (1988) 361.
50. R.A. Goldstein, L. Takiff and S.G. Boxer, Biochim. Biophys. Acta 934 (1988) 253.
51. M. Bixon, J. Jortner and M.E. Michel-Beyerle (this volume).