

M.-E. Michel-Beyerle (Editor)

# Reaction Centers of Photosynthetic Bacteria

Feldafing-II-Meeting

With 165 Figures

Springer-Verlag Berlin Heidelberg New York  
London Paris Tokyo Hong Kong Barcelona

# Contents

---

## PART I Native Reaction Centers: Structure and Spectroscopy

---

F. REISS-HUSSON, B. ARNOUX, A. DUCRUIX, M. ROTH, M. PICAUD and C. ASTIER : Reaction Center from Wild Type Y <i>Rhodobacter sphaeroides</i> : Primary Structure of the L and M Subunits; Carotenoid and Detergent Structures in the Three-Dimensional Crystals . . . . .	3
Q. ZHOU, T. A. MATTIOLI and B. ROBERT Probing Reaction Center Asymmetry Using Low Temperature Absorption Spectroscopy of <i>Rhodobacter sphaeroides</i> Reaction Centers Containing Bacteriopheophytin Anions . . . . .	11
A. VERMEGLIO, D. GARCIA and J. BRETON Cytochrome Arrangement in Reaction Centers of Different Species of Photosynthetic Bacteria . . . . .	19
W. MÄNTELE, M. LEONHARD, M. BAUSCHER, E. NABEDRYK, J. BRETON and D.A. MOSS Infrared Difference Spectroscopy of Electrochemically Generated Redox States in Bacterial Reaction Centers . . . . .	31
E.J. LOUS, M. HUBER, R.A. ISAACSON and G. FEHER EPR and ENDOR Studies of the Oxidized Primary Donor in Single Crystals of Reaction Centers of <i>Rhodobacter sphaeroides</i> R-26 . . . . .	45
F. LENDZIAN, B. ENDEWARD, M. PLATO, D. BUMANN, W. LUBITZ and K. MÖBIUS ENDOR and TRIPLE Resonance Investigation of the Primary Donor Cation Radical P <sub>865</sub> <sup>+</sup> in Single Crystals of <i>Rhodobacter sphaeroides</i> R-26 Reaction Centers . . . . .	57
A. ANGERHOFER, J. GREIS, V. AUST, J.U. VON SCHÜTZ and H.C. WOLF Triplet State ADMR of Bacterial Reaction Centers at Low Transition Frequencies . . . . .	69
S. BUCHANAN and H. MICHEL Investigation of Quinone Reduction in <i>Rhodopseudomonas viridis</i> by FTIR Difference Spectroscopy and X-Ray Diffraction Analysis . . . . .	75
D.L. THIBODEAU, J. BRETON, C. BERTHOMIEU, K.A. BAGLEY, W. MÄNTELE and E. NABEDRYK Steady-State and Time-Resolved FTIR Spectroscopy of Quinones in Bacterial Reaction Centers . . . . .	87

-----  
PART II            **Native Reaction Centers: Electron Transfer Dynamics**  
-----

G.J. SMALL, R. JANKOWIAK, M. SEIBERT, C.F. YOCUM and D. TANG Spectral Hole Burning Studies of Photosystem II Reaction Centers: Excited State Structure, Charge Separation and Energy Transfer Dynamics . . . . .	101
C. KIRMAIER and D. HOLTEN Evidence for an Inhomogeneous Distribution of Reaction Centers on the Timescale of the Primary Electron Transfer Events . . . . .	113
U. FINKELE, K. DRESSLER, C. LAUTERWASSER and W. ZINTH Analysis of Transient Absorption Data from Reaction Centers of Purple Bacteria . . . . .	127
K. DRESSLER, U. FINKELE, C. LAUTERWASSER, P. HAMM, W. HOLZAPFEL, S. BUCHANAN, W. KAISER, H. MICHEL, D. OESTERHELT, H. SCHEER, H.U. STILZ and W. ZINTH Similarities of the Primary Charge Separation Process in the Photosynthesis of <i>Rhodobacter sphaeroides</i> and <i>Rhodospseudomonas viridis</i> . . . . .	135
G.H. ATKINSON, H. HAYASHI, M. TASUMI and S. KOLACZKOWSKI Picosecond Resonance Raman Spectroscopy of <i>Rhodobacter sphaeroides</i> Reaction Centers . . . . .	141
S.G. BOXER, D.J. LOCKHART, S. FRANZEN and S.H. HAMMES Electric Field Modulation of the Fluorescence Lineshape for Photosynthetic Reaction Centers: A New Probe of the Primary Electron Transfer Mechanism . . . . .	147
A. OGRODNIK, U. EBERL, R. HECKMANN, M. KAPPL, R. FEICK and M.E. MICHEL-BEYERLE One Step Electron Transfer to P <sup>+</sup> H <sup>-</sup> in Reaction Centers of <i>Rhodobacter sphaeroides</i> Derived from Dichroic Excitation Spectra of Electric Field Modulated Fluorescence Yield . . . . .	157
M.G. MÜLLER, K. GRIEBENOW and A.R. HOLZWARTH Energy Transfer and Charge Separation Kinetics in the Reaction Center of <i>Chloroflexus aurantiacus</i> Studied by Picosecond Time-Resolved Fluorescence Spectroscopy . . . . .	169
R. FEICK, J.L. MARTIN, J. BRETON, M. VOLK, G. SCHEIDEL, T. LANGENBACHER, C. URBANO, A. OGRODNIK and M.E. MICHEL-BEYERLE Biexponential Charge Separation and Monoexponential Decay of P <sup>+</sup> H <sup>-</sup> in Reaction Centers of <i>Chloroflexus aurantiacus</i> . . . . .	181
P. SEBBAN, P. PAROT, L. BACIOU, P. MATHIS and A. VERMEGLIO Analog Effects of Low Temperature and Lipid Rigidity on the Distribution of Two Conformational States of the Reaction Centers . . . . .	189

## PART III

**Modified Reaction Centers:  
Effects of Mutagenic and Chemical Modifications**

I. SINNING, J. KOEPKE and H. MICHEL Recent Advances in the Structure Analysis of <i>Rhodospseudomonas</i> <i>viridis</i> Reaction Center Mutants .....	199
M.M. YANG, W.J. COLEMAN and D.C. YOUVAN Genetic Coding Algorithms for Engineering Membrane Proteins .....	209
M. HUBER, E.J. LOUS, R.A. ISAACSON, G. FEHER, D. GAUL and C.C. SCHENCK EPR and ENDOR Studies of the Oxidized Donor in Reaction Centers of <i>Rhodobacter sphaeroides</i> Strain R-26 and two Heterodimer Mutants in which Histidine M202 or L173 was Replaced by Leucine .....	219
C.C. SCHENCK, D. GAUL, M. STEFFEN, S.G. BOXER, L. McDOWELL, C. KIRMAIER and D. HOLTEN Site-Directed Mutations Affecting Primary Photochemistry in Reaction Centers: Effects of Dissymmetry in the Special Pair .....	229
W.W. PARSON, V. NAGARAJAN, D. GAUL, C.C. SCHENCK, Z.-T. CHU and A. WARSHEL Electrostatic Effects on the Speed and Directionality of Electron Transfer in Bacterial Reaction Centers: The Special Role of Tyrosine M-208 .....	239
K.A. GRAY, J.W. FARCHAUS, J. WACHTVEITL, J. BRETON, U. FINKELE, C. LAUTERWASSER, W. ZINTH and D. OESTERHELT The Role of Tyrosine M210 in the Initial Charge Separation in the Reaction Center of <i>Rhodobacter sphaeroides</i> .....	251
H.U. STILZ, U. FINKELE, W. HOLZAPFEL, C. LAUTERWASSER, W. ZINTH and D. OESTERHELT Site-Directed Mutagenesis of Threonine M222 and Tryptophan M252 in the Photosynthetic Reaction Center of <i>Rhodobacter sphaeroides</i> .....	265
W.J. COLEMAN, E.J. BYLINA, W. AUMEIER, J. SIEGL, U. EBERL, R. HECKMANN, A. OGRODNIK, M.E. MICHEL-BEYERLE and D.C. YOUVAN Influence of Mutagenic Replacement of Tryptophan M250 on Electron Transfer Rates Involving Primary Quinone in Reaction Centers of <i>Rhodobacter capsulatus</i> .....	273
S.J. ROBLES, J. BRETON and D.C. YOUVAN Transmembrane Helix Exchanges Between Quasi-Symmetric Subunits of the Photosynthetic Reaction Center .....	283
J. BRETON, J.-L. MARTIN, J.-C. LAMBRY, S.J. ROBLES and D.C. YOUVAN Ground State and Femtosecond Transient Absorption Spectroscopy of a Mutant of <i>Rhodobacter capsulatus</i> which Lacks the Initial Electron Acceptor Bacteriopheophytin .....	293

N.W. WOODBURY, A.K. TAGUCHI, J.W. STOCKER and S.G. BOXER	
Preliminary Characterization of pAT-3, a Symmetry Enhanced Reaction Center Mutant of <i>Rhodobacter capsulatus</i> . . . . .	303
A. STRUCK, D. BEESE, E. CMIEL, M. FISCHER, A. MÜLLER, W. SCHÄFER and H. SCHEER	
Modified Bacterial Reaction Centers: 3. Chemically Modified Chromophores at Sites B <sub>A</sub> , B <sub>B</sub> and H <sub>A</sub> , H <sub>B</sub> . . . . .	313
K. WARNCKE and P.L. DUTTON	
Effect of Cofactor Structure on Control of Electron Transfer Rates at the Q <sub>A</sub> Site of the Reaction Center Protein . . . . .	327

---

PART IV Reaction Centers: Modelling of Structure/Function-Relationship

---

A. FREIBERG and T. PULLERITS	
Energy Transfer and Trapping in Spectrally Disordered Photosynthetic Membranes . . . . .	339
M.R. WASIELEWSKI, G.L. GAINES, III, M.P. O'NEIL, W.A. SVEC, M.P. NIEMCZYK and D. M. TIEDE	
Multi-Step Electron Transfer in Rigid Photosynthetic Models at Low Temperature: Requirements for Charge Separation and Spin-Polarized Radical Ion Pair Formation . . . . .	349
P.O.J. SCHERER, W. THALLINGER and S.F. FISCHER	
Electronic Couplings for Light Induced Charge Transfer in Covalently Bonded Donor-Acceptor Systems . . . . .	359
J. FAJER, K.M. BARKIGIA, K.M. SMITH, E. ZHONG, E. GUDOWSKA-NOWAK and M.D. NEWTON	
Micro-Environmental Effects on Photosynthetic Chromophores . . . . .	367
A. SCHERZ, J.R.E. FISHER and P. BRAUN	
Simulation of the Absorption and Circular Dichroism Spectra for the Primary Electron Donor in Reaction Centers of Purple Bacteria and Photosystem II . . . . .	377
M. BIXON, J. JORTNER and M.E. MICHEL-BEYERLE	
On the Primary Charge Separation in Bacterial Photosynthesis . . . . .	389
P.O.J. SCHERER	
Multiple Excited States of Photosynthetic Reaction Centers . . . . .	401
J. VRIEZE and A.J. HOFF	
Exciton Band Mixing in <i>Rhodospseudomonas viridis</i> . . . . .	409

A.L. MORRIS, J.R. NORRIS and M.C. THURNAUER  
 An Extended Model for Electron Spin Polarization in Photosynthetic  
 Bacteria ..... 423

E.W. KNAPP and L. NILSSON  
 Can Electron Transfer be Influenced by Protein Dynamics:  
 The Transfer from Cytochrome C to the Special Pair in  
 Photosynthetic Reaction Centers ..... 437

---

**Concluding Remarks**

---

M.E. MICHEL-BEYERLE and H. SCHEER  
 Beyond Native Reaction Centers ..... 453

**Subject Index** ..... 464

# Similarities of the Primary Charge Separation Process in the Photosynthesis of *Rhodobacter sphaeroides* and *Rhodospseudomonas viridis*

K. Dressler<sup>1</sup>, U. Finkeler<sup>1</sup>, C. Lauterwasser<sup>1</sup>, P. Hamm<sup>1</sup>, W. Holzapfel<sup>1</sup>, S. Buchanan<sup>2</sup>, W. Kaiser<sup>1</sup>, H. Michel<sup>2</sup>, D. Oesterheld<sup>3</sup>, H. Scheer<sup>4</sup>, H. U. Stiltz<sup>3</sup>, and W. Zinth<sup>1</sup>

<sup>1</sup> Physik Department E11, Technische Universität München, W 8000 München 2, FRG

<sup>2</sup> Max-Planck-Institut für Biophysik, W 6000 Frankfurt a. M., FRG

<sup>3</sup> Max-Planck-Institut für Biochemie, W 8033 Martinsried, FRG

<sup>4</sup> Botanisches Institut der Ludwig-Maximilians-Universität, W 8000 München 19, FRG

Photosynthetic conversion of light into chemical energy starts via a series of electron transfer reactions in pigment-protein complexes called reaction centers (RC's). The most direct access to the primary reaction dynamics offers time resolved optical spectroscopy. During the past few years, this technique has been continuously improved permitting advanced experiments with high temporal and amplitude resolution. In this paper, we show, that RC's from Rhodobacter(Rb.) sphaeroides and Rhodospseudomonas (Rps.) viridis exhibit common features in the absorption transients. This points to a substantial similarity of the elementary molecular processes. This fact is not self-evident, since different polypeptides and different types of bacteriochlorophylls (BChl) and bacteriopheophytins (BPh) are present in various reaction centers, e.g. BChl a and BPh a are essential pigments in the reaction centers of Rb. sphaeroides while BChl b and BPh b are active in the RC's of Rps. viridis. For both reaction centers x-ray structures are now available /1-3/. It was shown that the prosthetic groups and neighbouring amino acids are in a very similar arrangement: most importantly are two BChl molecules in close contact which act as the primary electron donor P. The other pigments are arranged in two branches, A and B. Starting from the primary donor, the special pair P, one finds a monomeric bacteriochlorophyll (B), a bacteriopheophytin (H), and a quinone (Q) on each branch. It was shown that the electron transfer occurs via the A-branch and that after about 3 - 4 ps a radical pair  $P^+H_A^-$  is formed. Approximately 200 ps later the electron reaches the quinone  $Q_A$  building the intermediate  $P^+Q_A^-$ . The role of the monomeric bacteriochlorophyll  $B_A$  is still in debate /4,5/. Recent experiments on Rb. sphaeroides have proven the existence of a previously undetected

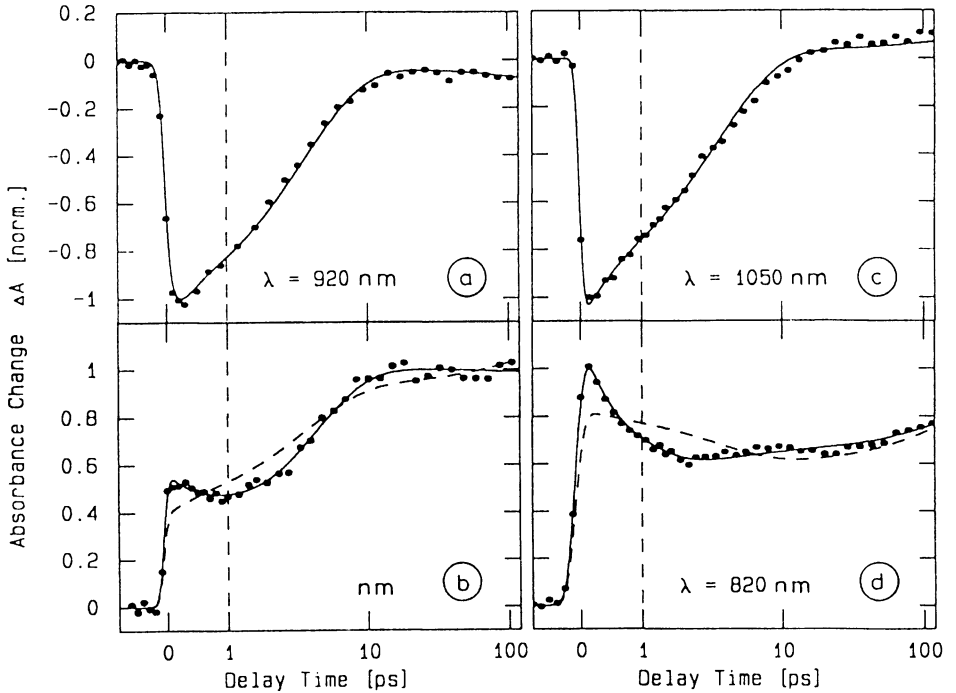


Fig.1 Transient absorption data for reaction centers from *Rb.sphaeroides* (a,b) and *Rps. viridis* (c,d). The filled circles represent the experimental data, the solid lines correspond to model calculations with time constants given in the text. The broken lines are calculated without the fast (0.9 ps or 0.65 ps, respectively) kinetic. The excitation wavelengths are 860 nm and 955 nm for *Rb. sphaeroides* and *Rps. viridis*, respectively.

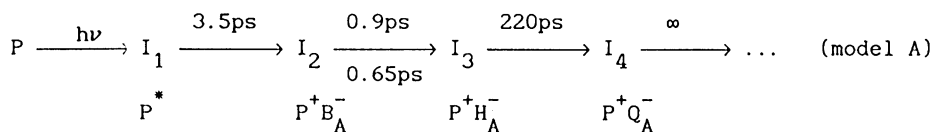
0.9 ps kinetic. A straightforward interpretation relates the corresponding transient to the radical pair  $P^+B_A^-$ , a real intermediate formed prior to  $P^+H_A^-$  /6,7/.

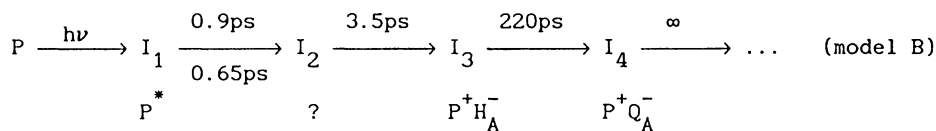
The experiments presented here were performed using the excite and probe technique with weak subpicosecond pulses (pulse duration below 150 fs) generated by two different laser-amplifier systems with repetition rates of 10 Hz. The samples were excited in the lowest energy band of P (at 860 nm for *Rb.sphaeroides* and at 955 nm for *Rps. viridis*). Probing was performed by a 5 nm to 20 nm wide fraction of a femtosecond white light continuum. Exciting and probing pulses were parallel polarized. The reaction centers were prepared as described in Ref. /6/ and /8/. They were kept at room temperature under stirring.



Time-resolved absorption data for both types of bacteria are shown in Fig.1 for different probing wavelengths. The decay of the excited electronic state of the special pair is studied at  $\lambda_{pr} = 920$  nm for Rb. sphaeroides (Fig.1a) and  $\lambda_{pr} = 1050$  nm for Rps. viridis (Fig.1c). Both probing wavelengths are located on the long-wavelength side of the P absorption band (see Fig.2a and 2b), where the population of the first excited electronic state is readily detected via its stimulated emission. As shown in Fig.1a and 1c the rapidly appearing gain decays with a time constant around 3.5 ps. Quite different is the situation at wavelengths close to the absorption band of the monomeric bacteriochlorophylls. Here an additional fast kinetic component becomes evident (Fig.1b, 1d). In Rb. sphaeroides at 785 nm (Fig.1b) a very fast first absorption increase at time zero is followed by a brief relative absorption decrease before the absorption rises again with 3.5 ps. For Rps. viridis one finds the additional fast kinetic component quite clearly at 820 nm near the peak of the BChl absorption band (Fig.1d). Extensive studies at more than twenty different wavelengths gave the following numbers for the time constants for both RC's: the fastest process occurs with 0.9 ps +/- 0.4 ps in Rb. sphaeroides and with 0.65 ps +/- 0.3 ps in Rps. viridis. The other time constants are 3.5 ps +/- 0.4 ps, 220 ps +/- 50 ps and infinity in both RC's. The transient absorption measurements also supply amplitudes of the various kinetic components which allow to calculate difference spectra of the cross-sections of the intermediate states for specific sequential reaction models /7,9/.

The experiments clearly show, that the absorption curves can be described well by a multiexponential function with a minimum of four time constants. As a consequence, the reaction model comprises at least four intermediate states. However, the reaction scheme cannot be deduced uniquely from the transient absorption data. A certain reaction model can only be accepted if the deduced spectra of all intermediate states are not in contradiction with any other information. In the following, we discuss two linear models, which are distinguished by their different order of the early intermediates:





The difference cross-section spectra of intermediates  $I_1$ ,  $I_3$  and  $I_4$  do not depend on the specific model A or B. The spectrum of intermediate  $I_1$  is depicted in Fig. 2c (*Rb. sphaeroides*) and Fig. 2d (*Rps. viridis*). Intermediate  $I_1$  exhibits a pronounced absorption decrease on the long-wavelength

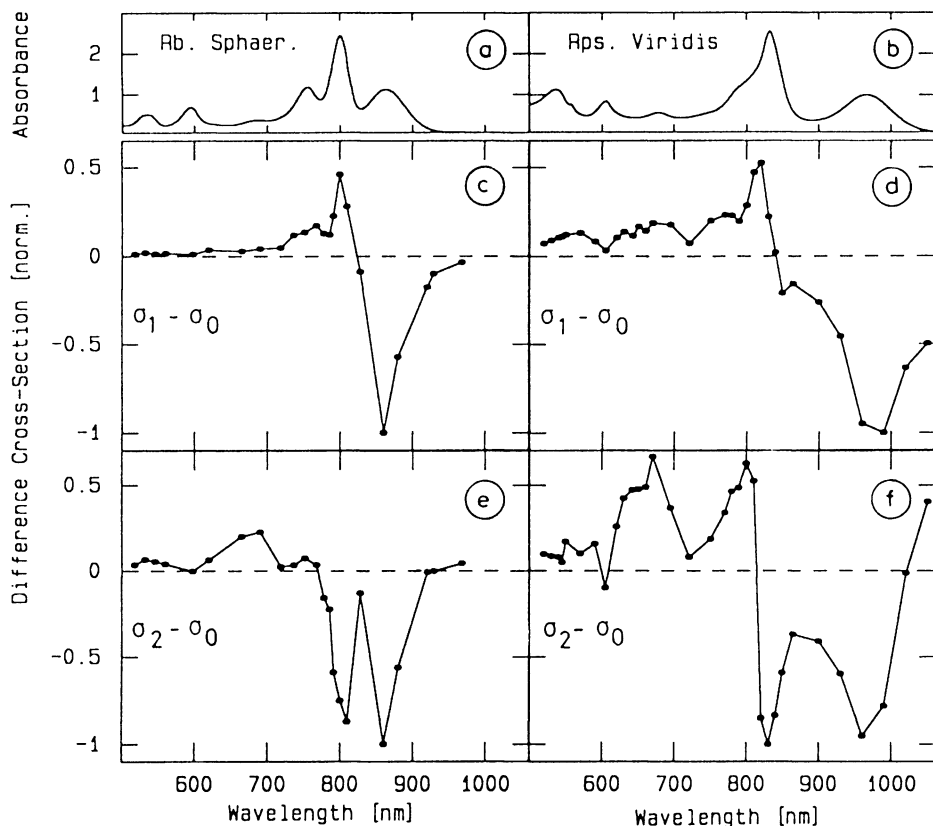


Fig.2 Spectral data for *Rb. sphaeroides* (a,c,e) and *Rps. viridis* (b,d,f). (a,b) give the absorption spectra, (c,d,e,f) show difference spectra calculated according to model A for the intermediates  $I_1$  ( $\sigma_1 - \sigma_0$ ) and  $I_2$  ( $\sigma_2 - \sigma_0$ ).

side of the P absorption band, which is due to optical gain from the electronically excited special pair P\*. Excited state absorption is strong around 800 nm. The spectra of the intermediate  $I_2$  differ significantly for the two reaction models. In model B the intermediate  $I_2$  displays the same salient spectral features (not shown here) as  $I_1$ . However, its gain is reduced by 30%, a relative absorption decrease occurs in the  $Q_y$  band of the BChl and an increased absorption is found around 660 nm (in the anion band of the tetrapyrrols). These observations indicate, that intermediate  $I_2$  (in model B) contains an electronically excited special pair. The spectral difference between  $I_1$  and  $I_2$  may be explained by an excited state relaxation process or by a mixing of P\* with a charge-transfer state  $P^+B_A^-$ . According to model B the bacteriopheophytin  $H_A$  would be the primary electron acceptor.

Quite different is the situation in model A where the 3.5 ps decay precedes the 0.9 ps (0.65 ps) process. Fig. 2e, 2f show the difference spectra of intermediate  $I_2$  for model A. The salient features are: (i) disappearance of the absorption of the special pair P, (ii) absorption changes characteristic for  $P^+$ , (iii) strong absorption decrease in the  $Q_y$  band of the monomeric bacteriochlorophylls (at 800 nm for Rb. sphaeroides, at 820 nm for Rps. viridis), (iv) pronounced absorption increase around 660 nm in the BChl and BPh anion bands. (v) Furthermore transient dichroism experiments for Rb. sphaeroides indicate that the transition moment of the 660 nm band of  $I_2$  is parallel to the direction expected for the BChl anion  $B^-/6$ . It is remarkable that all five points support the assignment of  $I_2$  being the radical pair  $P^+B_A^-$ . Thus the monomeric bacteriochlorophyll  $B_A$  should be the primary electron acceptor.

In conclusion: We have shown that the primary electron transfer in the bacterial reaction centers from Rb. sphaeroides and Rps. viridis proceeds according to a common reaction scheme, where a subpicosecond reaction is involved. We discussed two linear reaction models. So far the experiments cannot decide conclusively between the two. Model B leads to an additional excited electronic state ( $I_2$ ) of which the functional relevance is unknown. On the other hand the structural arrangement of the chromophors in the RC's and all the spectral features (i) to (v) of intermediate  $I_2$  in model A favour the radical pair  $P^+B_A^-$  as a real transient in the electron transfer process. According to this model the electron transfer proceeds as follows: From the excited electronic state of the special pair P\* an electron moves to the monomeric bacteriochlorophyll  $B_A$  within 3.5 ps for-

ming the radical pair state  $P^+B_A^-$ , which decays more rapidly with 0.9 ps (0.65 ps) to the radical pair state  $P^+H_A^-$ .

### References

- 1 J. Deisenhofer, H. Michel: *EMBO J.* 8, 2149 (1989)
- 2 G. Feher, J. P. Allen, M. Y. Okamura, D. C. Rees: *Nature* 339, 111 (1989)
- 3 C.-H. Chang, D. Tiede, J. Tang, U. Smith, J. Norris, M. Schiffer: *FEBS Lett.* 205, 82 (1986)
- 4 J.L. Martin, J. Breton, A.J. Hoff, A. Migus, A. Antonetti: *Proc. Natl. Acad. Sci. USA* 83, 957 (1986)
- 5 J. Breton, J.L. Martin, A. Migus, A. Antonetti, A. Orszag: *Proc. Natl. Acad. Sci. USA* 83, 5121 (1986)
- 6 W. Holzapfel, U. Finklele, W. Kaiser, D. Oesterhelt, H. Scheer, H.U. Stolz, W. Zinth: *Chem. Phys. Lett.* 160, 1 (1989)
- 7 W. Holzapfel, U. Finklele, W. Kaiser, D. Oesterhelt, H. Scheer, H.U. Stolz, W. Zinth: *Proc. Natl. Acad. Sci. USA* 87, 5168 (1990)
- 8 H. Michel: *J. Mol. Biol.* 158, 567 (1982)
- 9 U. Finklele, K. Dressler, C. Lauterwasser, W. Zinth: this volume