

M.-E. Michel-Beyerle (Editor)

# Reaction Centers of Photosynthetic Bacteria

Feldafing-II-Meeting

With 165 Figures

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# Contents

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## 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 *Rhodobacter sphaeroides*:  
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 *Rhodobacter sphaeroides*  
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 *Rhodobacter sphaeroides* 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  $P_{865}^+$  in Single Crystals of *Rhodobacter sphaeroides*  
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 *Rhodospseudomonas viridis* 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>Rhodopseudomonas 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. OGRONNIK, 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**  
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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

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**Concluding Remarks**

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

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

absorption measurements on this system in order to characterize the intermediate and kinetics of electron transfer further.

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## REFERENCES

- J.P. Allen, G. Feher, T. O. Yeates, H. Komiya, D.C. Rees: *Proc. Natl. Acad. Sci. USA* 84, 6162-6166 (1987)
- R.E. Blankenship, W.W. Parson: *Biochim. Biophys. Acta* 545, 429-444 (1979)
- E.J. Bylina, C. Kirmaier, L. McDowell, D. Holten, D.C. Youvan: *Nature* 336, 182-1 (1988)
- E.J. Bylina, S. Ismail, D.C. Youvan: *Plasmid* 16, 175-181 (1986)
- E.J. Bylina, D.C. Youvan: *Proc. Natl. Acad. Sci. USA* 85, 7226-7230 (1988)
- C.H. Chang, D. Tiede, J. Tang, V. Smith, J. Norris, M. Shiffer: *FEBS Lett.* 205, 82-86 (1986)
- R.K. Clayton: *Photochem. Photobiol.* 1, 201-210 (1962)
- J. Diefenhofer, O. Epp, K. Miki, R. Huber, H. Michel: *Nature* 318, 618-624 (1985)
- J.D. Gust, T.A. Moore, D.K. Luttrull, G.R. Seely, E. Bittersmann, R.V. Bensasson M. Rougee, E.J. Land, F.C. DeSchryver, M. Van der Auweraer: *Photochem. Photobiol* 51, 419-426 (1990)
- C. Kirmaier, D. Holten, W.W. Parson: *Biochim. Biophys. Acta* 810, 49-61 (1985)
- C. Kirmaier, D. Holten, E.J. Bylina, D.C. Youvan: *Proc. Natl. Acad. Sci.* 85, 756: 7566 (1988)
- D.J. Lockhart, S.G. Boxer: *Biochemistry* 26, 664-668 (1987)
- L.J. Mancino, D.P. Dean, R.E. Blankenship: *Biochim. Biophys. Acta* 764, 46-54 (1984)
- W.W. Parson: in *Photosynthesis* (J. Amesz, ed.) Elsevier, Amsterdam (1986)
- R.C. Prince, D.C. Youvan: *Biochim. Biophys. Acta* 890, 286-291 (1987)
- N.W. Woodbury, W.W. Parson: *Biochim. Biophys. Acta* 850, 197-210 (1986)
- N.W. Woodbury, E. Bittersmann: in *Proceedings of the VIIIth International Congress on Photosynthesis*, Stockholm, Sweden (1989) (in press)
- D.C. Youvan, S. Ismail, E.J. Bylina: *Gene* 38, 19-30 (1985)



# Modified Bacterial Reaction Centers:

## 3. Chemical Modified Chromophores at Sites $B_A$ , $B_B$ and $H_A$ , $H_B$

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### Introduction

The recently demonstrated exchange of the "monomeric" bacteriochlorophylls (BChl)  $B_A$  and  $B_B$ , has opened a new route to investigate structure-function relationships in photosynthetic reaction centers (RC) of *Rhodobacter sphaeroides* R26 [1-4]. The function of these pigments is still controversial [5-22]. However, the finding of a new kinetic intermediate at ambient temperature between the initial excited state  $P^*$  of the primary donor, and the charge separated state  $P^{+}H_A^{-}$  in which the photoejected electron resides on the bacteriopheophytin (BPhe) of the A-branch, has provided strong evidence that one of them ( $B_A$ ) is an intermediate acceptor in the primary charge separation process [15,20]. A similar role has been proposed for  $B_B$  in triplet energy transfer from P to the carotenoid in wild-type RC [18]. To evaluate the details of electron transfer and pigment interaction it seemed desirable to introduce pigments with altered spectral and/or redox properties. As first pigments showing considerably blue-shifted absorption spectra, we have incorporated bacteriochlorophylls containing 3-vinyl- or 3-hydroxyethyl-substituents instead of the 3-acetyl group [4]. Here, we wish to summarize results on these and some other pigment modifications, including in particular the exchange of bacteriopheophytins as well. An overview is given on the pigment modifications tested and some steady state spectra are shown.

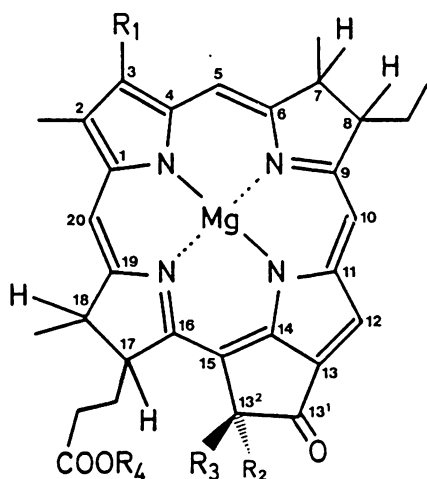
## Materials and Methods

Reaction centers: *Rb. sphaeroides* R26 and 2.4.1. RC were prepared by repeated solubilization of chromatophores and subsequent chromatography on DEAE-cellulose as described previously [2]. Final purification was achieved by density-gradient centrifugation (0.2 to 0.8 M sucrose in 10 mM tris-HCl buffer, pH 7.6, containing 0.08 % LDAO, 20 hours, 240000·g). RC were enriched in the 0.6 M region. They were withdrawn, dialyzed against tris-HCl buffer (10 mM, pH 7.6, containing 0.08% LDAO) and stored at -20 C. *Rhodospirillum rubrum* and *Rhodospseudomonas viridis* reaction centers were prepared by standard procedures, and purified as the ones from *Rb. sphaeroides*.

Pigments: Bchl a<sub>P</sub> and Bchl a<sub>BE</sub> were extracted from *Rb. sphaeroides* and *Rs rubrum*, respectively. Esters with other alcohols were obtained by transesterification. Pigments with opened isocyclic ring and with a 13<sup>2</sup>-oxo-group were obtained by the phase test reaction [23-25]. Demetalation to the respective pheophytins was done with dilute HCl. [3-acetyl]-chlorophyll a was obtained by oxidation of Bchl a with high-potential quinones [26]. 13<sup>2</sup>-OH-Bchl (I) and pigments containing a 3-vinyl- (V,VII) or 3-hydroxyethylsubstituent (VI,VIII) were prepared as published before [4]. Pyrolysis to remove the 13<sup>2</sup>-COOCH<sub>3</sub> group was done according to [27]. All products were characterized by absorption, fluorescence, <sup>1</sup>H-NMR and mass spectroscopy. A full account of the chemistry will be given separately.

Exchange experiments were performed as previously [1-4] using a 20 fold excess of exogenous pigments over the BChl contained in the RC. All experiments with *Rb. sphaeroides* R26-RC were done under the same exchange conditions, viz. incubation of the reaction centers at 42.5° C for 90 minutes in a 20 mM tris/ 0.08% LDAO/ 10% MeOH buffer, containing the modified pigments in a 20 fold excess. Afterwards, excess pigments were removed from the incubation mixture by repeated chromatography on DEAE-cellulose. The temperature was optimized for other species, and in the case of *Rs. rubrum*, LDAO was replaced by Triton-X100 (0.1%).

Extraction of pigments from RC for HPLC analysis was done as described in [3,4]. The HPLC analysis was done on silica or reversed phase as reported [3,4,28].

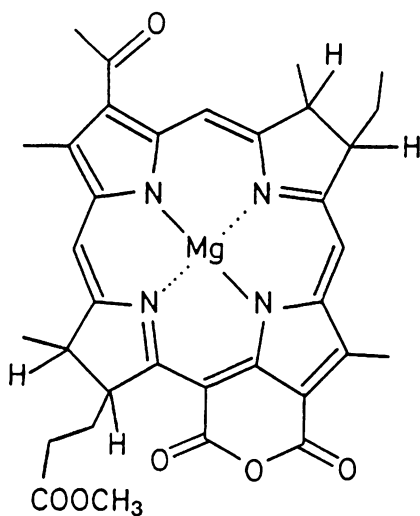


		R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>
Ia	Bchl a <sub>p</sub>	COCH <sub>3</sub>	COOCH <sub>3</sub>	H	C <sub>20</sub> H <sub>39</sub>
Ib	Bchl a' <sub>p</sub>	COCH <sub>3</sub>	H	COOCH <sub>3</sub>	C <sub>20</sub> H <sub>39</sub>
II	Bchl a <sub>pp</sub>	COCH <sub>3</sub>	COOCH <sub>3</sub>	H	C <sub>20</sub> H <sub>39</sub>
III	13 <sup>2</sup> -OH-Bchl a <sub>pp</sub> <sup>a)</sup>	COCH <sub>3</sub>	COOCH <sub>3</sub>	OH	C <sub>20</sub> H <sub>39</sub>
IV	13 <sup>2</sup> -OH-Bchl a <sub>pp</sub>	COCH <sub>3</sub>	COOCH <sub>3</sub>	OH	C <sub>20</sub> H <sub>39</sub>
V	[3-Vinyl]-Bchl a <sub>p</sub>	C <sub>2</sub> H <sub>5</sub>	COOCH <sub>3</sub>	H	C <sub>20</sub> H <sub>39</sub>
VI	[3-Hydroxyethyl]-Bchl a <sub>pp</sub> <sup>b)</sup>	CHOH-CH <sub>2</sub>	COOCH <sub>3</sub>	H	C <sub>20</sub> H <sub>39</sub>
VII	[3-vinyl]-13 <sup>2</sup> -hydroxy-Bchl a <sub>pp</sub> <sup>a)</sup>	C <sub>2</sub> H <sub>5</sub>	COOCH <sub>3</sub>	OH	C <sub>20</sub> H <sub>39</sub>
VIII	[3-Hydroxyethyl]-13 <sup>2</sup> -hydroxy-Bchl a <sub>pp</sub> <sup>a)</sup>	CHOH-CH <sub>2</sub>	COOCH <sub>3</sub>	OH	C <sub>20</sub> H <sub>39</sub>
IX	Pyro-bchl a <sub>p</sub>	COCH <sub>3</sub>	H	H	C <sub>20</sub> H <sub>39</sub>
X	[17'-Methyl]-13 <sup>2</sup> -hydroxy bchl a <sub>pp</sub> <sup>a)</sup>	COCH <sub>3</sub>	COOCH <sub>3</sub>	OH	CH <sub>3</sub>
XI	[17'-Ethyl]-13 <sup>2</sup> -hydroxy-Bchl a <sub>pp</sub> <sup>a)</sup>	COCH <sub>3</sub>	COOCH <sub>3</sub>	OH	C <sub>2</sub> H <sub>5</sub>
XII	[17'-Fluorethyl]-13 <sup>2</sup> -hydroxy-Bchl a <sub>pp</sub> <sup>a)</sup>	COCH <sub>3</sub>	COOCH <sub>3</sub>	OH	CH <sub>2</sub> CH <sub>2</sub> F
XIII	[17'-Trifluorbutyl]-13 <sup>2</sup> -hydroxy-Bchl a <sub>pp</sub> <sup>a)</sup>	COCH <sub>3</sub>	COOCH <sub>3</sub>	OH	(CH <sub>2</sub> ) <sub>3</sub> CF <sub>3</sub>

a) 13<sup>2</sup>-Epimer mixture

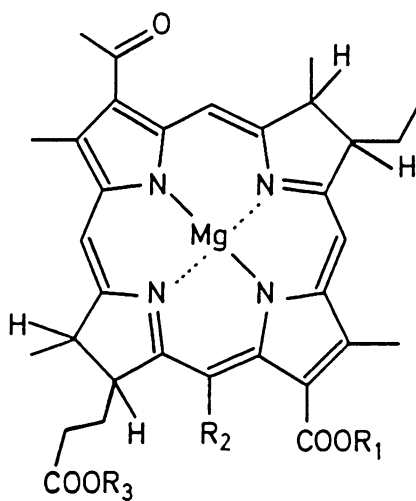
b) Stereochemistry at C undefined

**Table 1:** Bacteriochlorophylls with intact isocyclic ring



XIV 13a-Oxa-[13<sup>2</sup>-Oxo]-[17<sup>1</sup>-methyl]-BChl a    COCH<sub>2</sub>    -O    CH<sub>3</sub>

Table 2: Bacteriochlorophyll with enlarged isocyclic ring



	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
XV Bacteriochlorin e <sub>6</sub> -trimethylester	CH <sub>3</sub>	CH <sub>2</sub> COOCH <sub>3</sub>	CH <sub>3</sub>
XVI Bacteriochlorin e <sub>6</sub> -triethylester	C <sub>2</sub> H <sub>5</sub>	CH <sub>2</sub> COOC <sub>2</sub> H <sub>5</sub>	C <sub>2</sub> H <sub>5</sub>

Table 3: Bacteriochlorophylls with opened isocyclic ring

## Results

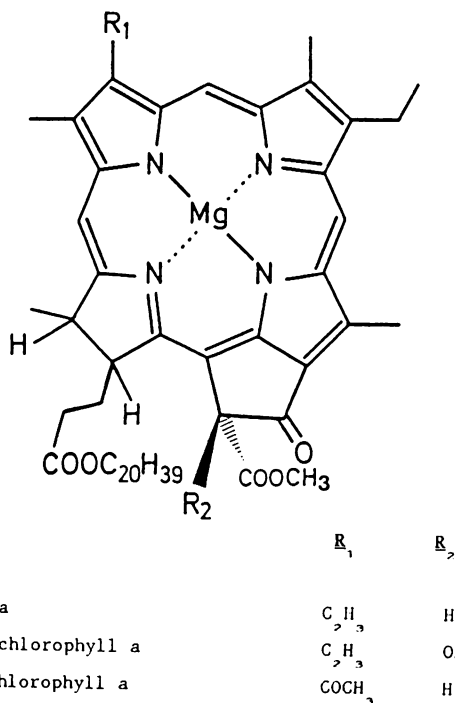
### Modified pigments

The pigments investigated, involve modifications at rings A,B,D and the isocyclic ring, and several of the chromophores have been prepared as magnesium complexes, e.g. (bacterio)chlorophylls, and as free bases, e.g. (bacterio)pheophorbides (Tables 1-5). The results of the exchange experiments are summarized in Table 6. All exchanges were performed under basically the same conditions. The analyses of the experiments were always done by HPLC coupled with absorption spectroscopy [3,4]. Only a few examples of the pigment modifications and their exchanges shall be discussed in this brief survey. Hydroxylation at C-13<sup>2</sup> gives only minor differences in the absorption spectrum, as compared to the respective non hydroxylated pigments in organic solvent. The absorption spectrum of 13<sup>2</sup>-OH-BChl a (III) is shown in Fig. 1 (dash-dotted line). It is nearly the same as for unmodified BChl a (I), except for a small but characteristic blue-shift of the Q<sub>x</sub>-band. Another modification concentrated on the 3-acetyl substituent of BChl a (see Table 1, V-VIII). Absorption spectra of [3-vinyl]-13<sup>2</sup>-OH-BChl a (VII) and [3-vinyl]-BPh a (XXIV) in organic solvents are shown in Fig. 2 and Fig. 3 (dash-dotted lines). There is a short-wavelength shift of 26 and 24 nm, of the Q<sub>v</sub>-band versus the 3-acetyl compound BChl a (I) and BPh a (XX), respectively. These pigments are of special interest, because they represent a structural link between plant chlorophyll a and BChl a. The only remaining difference is in the hydrogenation state of the macrocycle.

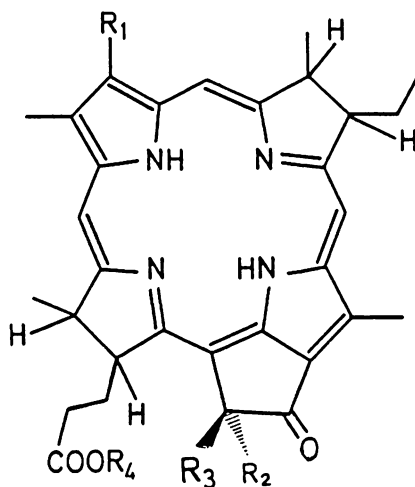
### Exchange of B<sub>A,N</sub> in *Rb. sphaeroides* R26:

Pigments, like (Ia, II, III, IV, V, VI, VII, VIII, X, XI), in which the central Mg atom is present, exchange selectively with the monomeric BChl (B<sub>A,N</sub>) of the RC. In no case an exchange with BPhe at sites H<sub>A,N</sub> was observed as determined by HPLC. There was also no indication that the special pair is exchangeable in any of the experiments. Pigments with more extensive modifications at the isocyclic ring, like (IX) and (XIV) do not exchange under the conditions of the experiment. By contrast hydroxylation at C-13<sup>2</sup> is not only allowed, but even tends to increase the exchange rate, and probably also the stability of RC. The spectral changes upon introducing modified pigments, parallel the changes of the absorptions in monomeric (e.g. ether) solution between the respective pigment and Bchl a.

Pigments like (II) in which only the 17<sup>3</sup> propionic acid ester is modified show no changes in the absorption spectra. Minor changes in the Q<sub>x</sub> region are observed in the absorption spectra after exchange with pigments of RC like (III) and (IV), which are hydroxylated at C-13<sup>2</sup>. An absorption spectrum of RC after exchange with (III) is displayed in Fig. 1 (solid line). The only distinctive difference is a shift of the Q<sub>x</sub> band from about 600 nm (native RC, data not shown) to 593 nm (modified ones) [3], corresponding to a similar shift in monomeric solution (see above). Corresponding results were obtained for (X, XI). Pigment modifications, which affect the absorption spectrum in organic solvents more strongly, show on the other hand similarly pronounced changes at all regions of monomeric BChl absorption in the spectrum of RC, provided an exchange is possible. As an example, the absorption spectrum of reaction centers after an exchange with (VII) is presented in Fig. 2 (solid line). This pigment carries a 3-vinyl-substituent like Chl a, but has the bacteriochlorophyll conjugation system. An exchange is also possible with BChl a bearing a 3-hydroxyethyl-substituent. Obviously, modifications at C-3 are tolerated by the B<sub>A,B</sub> binding pockets [4]. The absorption spectra of the modified RC show hardly any change (as compared to the native ones) in the 870 nm band of the pri-



**Table 4:** Chlorophylls



		$R_1$	$R_2$	$R_3$	$R_4$
XX	Bphe $a_p$	$\text{COCH}_3$	$\text{COOCH}_3$	H	$\text{C}_{20}\text{H}_{39}$
XXI	[13 <sup>2</sup> -hydroxy]-Bphe $a_p^{a)}$	$\text{COCH}_3$	$\text{COOCH}_3$	OH	$\text{C}_{20}\text{H}_{39}$
XXII	[3-hydroxyethyl]-Bphe $a_p^{b)}$	$\text{CHOHCH}_3$	$\text{COOCH}_3$	H	$\text{C}_{20}\text{H}_{39}$
XXIII	[3-hydroxyethyl]-13 <sup>2</sup> -hydroxy-Bphe $a_p^{a, b)}$	$\text{CHOHCH}_3$	$\text{COOCH}_3$	OH	$\text{C}_{20}\text{H}_{39}$
XXIV	[3-vinyl]-Bphe $a_p$	$\text{C}_2\text{H}_3$	$\text{COOCH}_3$	H	$\text{C}_{20}\text{H}_{39}$
XXV	[3-vinyl]-13 <sup>2</sup> -hydroxy-Bphe $a_p^{a)}$	$\text{C}_2\text{H}_3$	$\text{COOCH}_3$	OH	$\text{C}_{20}\text{H}_{39}$

a) 13<sup>2</sup>-Epimer mixture

b) Stereochemistry at C<sup>1</sup> undefined

**Table 5:** Bacteriopheophytins

Table 6: Exchangeability of pigments in reaction centers of *Rhodobacter spheroides*

	P <sub>870</sub>	B <sub>L/M</sub>	H <sub>L</sub>	H <sub>M</sub>
Ia	-	+	-	-
Ib	-	+ <sup>b)</sup>	-	-
II	-	+	-	-
III	-	+	-	-
IV	-	+	-	-
V	-	+ <sup>a)</sup>	-	-
VI	-	+ <sup>a)</sup>	-	-
VII	-	+	-	-
VIII	-	+	-	-
IX	-	-	-	-
X	-	+ <sup>a)</sup>	-	-
XI	-	+ <sup>a)</sup>	-	-
XII	-	?	-	-
XIII	-	?	-	-
XIV	-	-	-	-
XV	-	-	-	-
XVI	-	-	-	-
XVIIa	-	-	-	-
XVIIb	-	-	-	-
XVIII	-	-	-	-
XIX	-	-	-	-
XX	-	-	?	?
XXI	-	-	?	+
XXII	-	-	?	+
XXIII	-	-	?	+
XXIV	-	-	+	+
XXV	-	-	?	+

a) Partial exchange

b) Partially formed under exchange conditions

? Results unclear



mary donor and the 765 nm band of  $H_{A,B}$  but significant blueshifts of the 800 nm and part of the 600 nm band assigned commonly to the  $Q_V$  and  $Q_X$  bands, respectively, of the monomeric  $B_A$  and  $B_B$ . Qualitatively the same result is achieved with pigments (V, VI, VIII) [4].

Pigments like XVII, XVIII and XIX bearing a chlorin macrocycle characteristic for plant chlorophylls, do not exchange under the reaction conditions. The same is true for pigments like (XV, XVI) in which the isocyclic ring V is absent.

#### Exchange of $H_{A,B}$ in *Rb. sphaeroides* R26:

Pigments like (XX, XXI, XXII, XXIII, XXIV or XXV) which lack the central Mg atom, exchange selectively with the BPhe in the  $H_{A,B}$  pockets. The absorption spectrum of reaction center after an exchange with (XXIV) is presented in Fig. 3. The absorption spectra of the modified RC show hardly any change (as compared to the native ones) in the 870 nm band of the primary donor and in the 800 nm band of the monomeric bacteriochlorophylls, but significant blue-shifts of the 760 nm and 540 nm band assigned commonly to the  $Q_V$  and  $Q_X$  bands, respectively, of the monomeric  $H_{A,B}$ . The experiments also indicate, that one of the two bacteriopheophytins, (probably  $H_B$ ), is more readily exchangeable (data not shown). An exchange rate above 50% of the bacteriopheophytins is more difficult, but possible as shown in Fig. 3. Data collection on the functionality of RC modified in such a manner is in progress.

#### Other bacterial reaction centers:

Experiments with reaction centers from *Rb. sphaeroides* 2.4.1, the wild type strain, allow an exchange of Bchl not higher than 25% of total bacteriochlorophyll. Structurally, the major difference between R26 and 2.4.1 reaction centers is the presence of the carotenoid sphaeroiden(on)e, on the M branch of the latter one, close to  $B_B$ . We conclude therefore, that in 2.4.1 RC, there is only one pocket accesible, the  $B_A$  pocket, and that the carotenoid blocks exchange of  $B_B$ . Results with the carotinoid-less mutant *Rs. rubrum* G9 show an exchange like in *Rb. sphaeroides* R26, but the very low protein stability in LDAO renders a comparison more difficult.

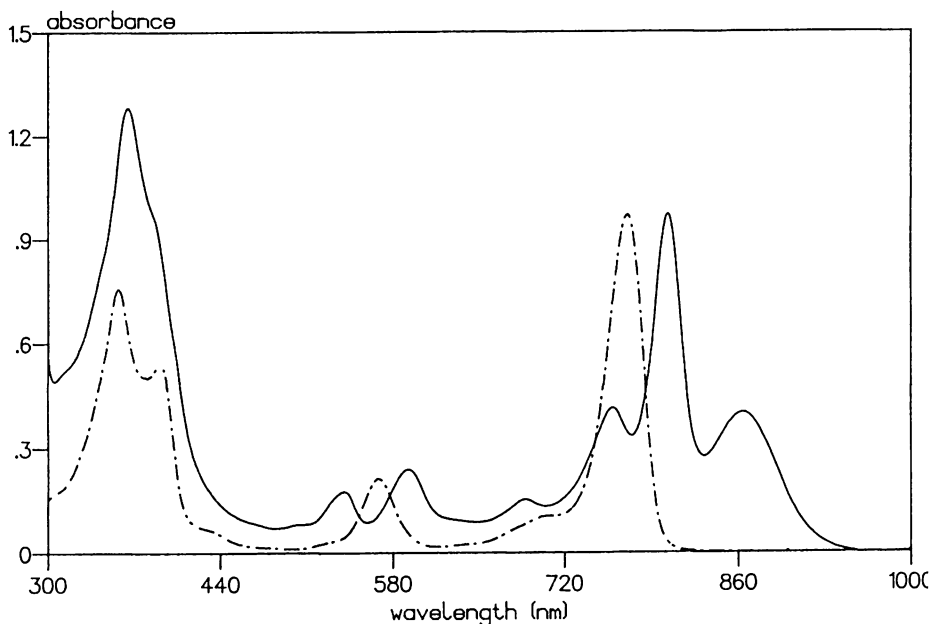


Fig. 1 Absorption spectra of 13<sup>2</sup>-OH-BChl a (III) in ether (-----) and of RC from *Rb. sphaeroides* R26 after exchange with the same pigment (—).

## Discussion

It has been shown that bacteriochlorophylls with modified propionic acid esters are "accepted" selectively by the binding pockets for the monomeric pigments B<sub>A</sub> and B<sub>B</sub>. The same was shown for bacteriochlorophylls modified at the position 3 and/or 13<sup>2</sup>. It has also been shown that bacteriopheophytins modified at the position 3 and/or 13<sup>2</sup> are "accepted" selectively by the binding pockets H<sub>A,B</sub> for the monomeric pheophytins, with indication for a preferential exchange of the inactive (?) H<sub>B</sub>. In these pigments, only the presence or absence of Mg seems to determine whether the pigment exchanges with the Bchl (=Mg-complexes) located originally at B<sub>A,B</sub>, or with the BPhe (= free base) located originally at H<sub>A,B</sub>. Evolution [29,30] or site directed mutagenesis [31,32] has led to several RC in which BPhe replaces BChl, and vice versa. These changes are always related to a replacement of a polar, metal-coordinating residue close to the central Mg of a BChl, e.g. histidine in native RC, with a non-polar one, like isoleucine, or vice versa. In all these cases, there is a high degree of discrimination between the BChl and BPhe by the binding sites. This finding has obvious implications on the assembly of the RC *in vivo*.

The lack of exchange of pigments with a chlorine macrocycle parallels observations of Loach and coworkers [33,34] on B870 complexes of *Rs. rubrum*. It may be important in understanding pigment/protein structure relationships. It is presently unclear, if the bacteriochlorine macrocycle is essential

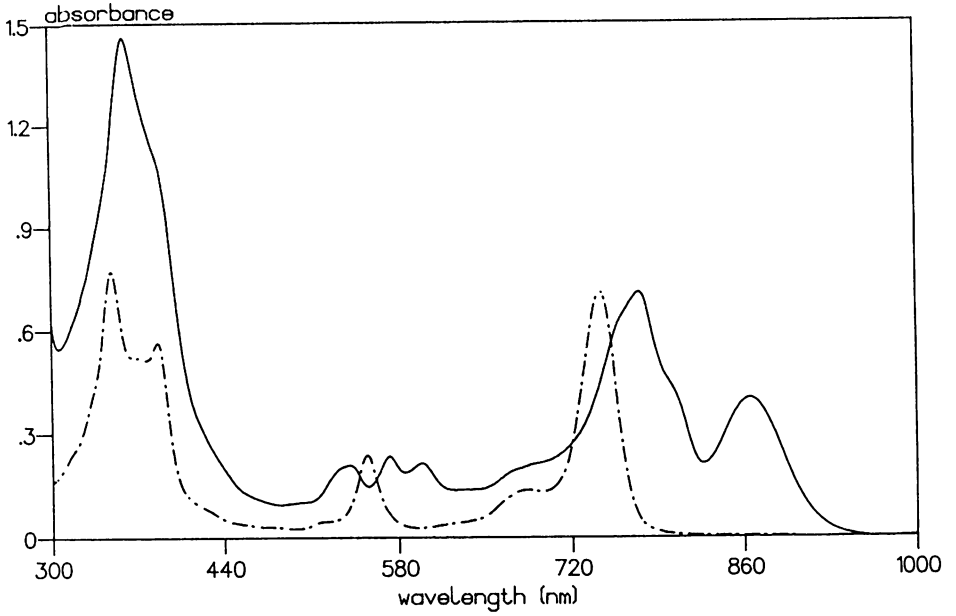


Fig. 2 Absorption spectra of [3-vinyl]-13<sup>2</sup>-OH-BChl a (VII) in ether (----) and of RC from *Rb. sphaeroides* R26 after exchange with the same pigment (—).

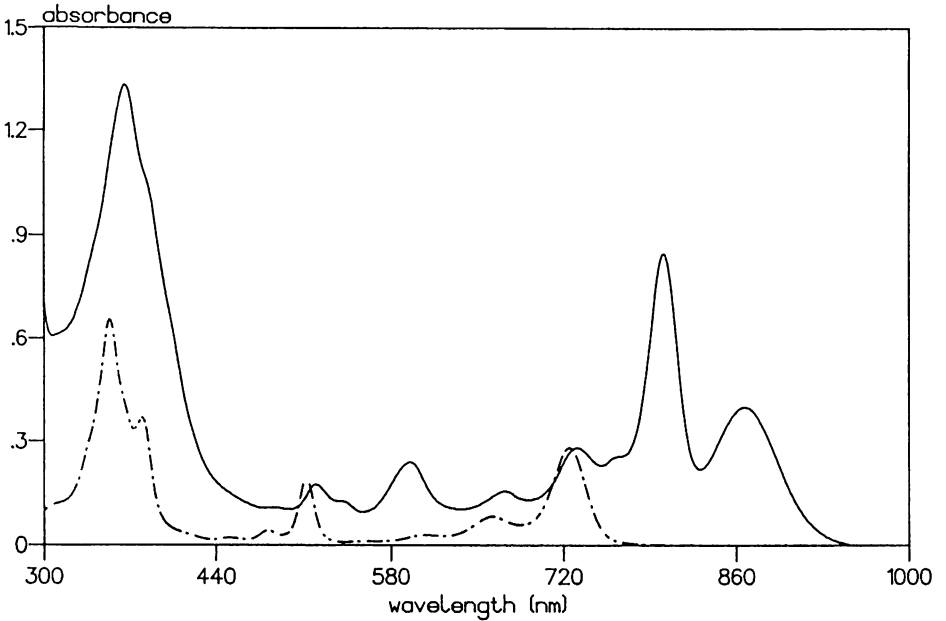


Fig. 3 Absorption spectra of [3-vinyl]-BPhe a (XXIV) in ether (----) and of RC from *Rb. sphaeroides* R26 after exchange with the same pigment (—).

for the stability of the whole reaction center protein, or if chlorins do not fit into the  $B_{\text{A},\text{B}}$  pockets. It should be mentioned in this context, that we have a number of observations, that the absence of BChl or BPhe destabilize RC.

An interesting spectroscopic result is the low electronic coupling of the different pigment domains. The lack of singlet state interactions between P and  $B_{\text{A},\text{B}}$  is confirmed by CD-data [4]. At the same time, this allows a fairly unambiguous assignment of the upper exciton of the primary donor to the 810 nm band remaining after shift of the  $B_{\text{A},\text{B}}$  monomer band in RC containing e.g. pigment VIII at the latter sites. By a similar comparison, there is also ENDOR evidence of a low coupling between the pigments at  $B_{\text{T},\text{M}}$  and the doublet state  $P^{++}$  (Lubitz *et al.*, unpublished), but that there is a significant interaction with the triplet state  $P^T$  (Angerhofer *et al.*, unpublished).

Functionally important is the fact, that in all the complexes which accept modified bacteriochlorophylls, the reversible photobleaching is retained. Kinetik and other spectroscopic work is in progress to further characterize RC with modified pigments, in order to understand the structure/function relationship of the tetrapyrroles in bacterial RC.

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### References

- [ 1 ] Scheer, H., D. Beese, R. Steiner, and A. Angerhofer (1988) In Structure of Bacterial Reaction Centers: X-ray Crystallography and Optical Spectroscopy with Polarized Light (Edited by J. Breton and A. Vermeghlio), pp. 101-112, Plenum, New York
- [ 2 ] Beese, D. (1989) Dissertation, Universität München
- [ 3 ] Struck, A. and H. Scheer (1990) FEBS Lett. 261, 385-388
- [ 4 ] Struck, A., E. Cmiel, I. Katheder, and H. Scheer (1990) FEBS Lett. 268, 180-184
- [ 5 ] Michel, H. and J. Deisenhofer (1988) Pure Appl. Chem. 60, 953-958
- [ 6 ] Chang, C. H., D. Tiede, J. Tang, U. Smith, J. R. Norris, and M. Schiffer (1986) FEBS Lett. 205, 82-86.

- 7] Allen, J. P., G. Feher, T. O. Yeates, H. Komiya, and D. C. Rees (1987) Proc. Natl. Acad. Sci. USA 84, 6162-6166
- 8] Becker, M., D. Middendorf, N. W. Woodbury, W. W. Parson, and R. E. Blankenship (1986) . In Ultrafast Phenomena V. (Edited by G. R. Fleming and A. E. Siegmann), Springer S. Chem. Phys., pp. 374-378, Springer, Heidelberg
- 9] Michel-Beyerle, M.E. and A. Ogrodnik (1990) in: Current Research in Photosynthesis, Vol 1, Ed. M. Baltscheffsky, p.19-26, Kluwer, Amsterdam
- 10] Bixon, M., J. Jortner, M. E. Michel-Beyerle, and A. Ogrodnik (1989) Biochim. Biophys. Acta 977, 273-286
- 11] Marcus, R. (1990) in: Current Research in Photosynthesis, Vol 1, Ed. M. Baltscheffsky, p.1-10, Kluwer, Amsterdam
- 12] Breton, J., J.-L. Martin, A. Migus, A. Antonetti, and A. Orszag (1986) Proc. Natl. Acad. Sci. USA 83, 5121-5225.
- 13] Friesner, R. A. and Y. Won (1990) in: Perspectives in Photosynthesis. (Edited by J. Jortner and B. Pullman), pp. 337-346, Academic press, New York
- 14] Hu, Y. and S. Mukamel (1990) In Perspectives in Photosynthesis. (Edited by J. Jortner and B. Pullman), pp. 171-184, Academic press, New York
- 15] Kirmaier, C. and D. Holten (1990) Proc. Natl. Acad. Sci. USA 87, in press.
- 16] Shuvalov, V. A., A. O. Ganago, A. V. Klevanik, and A. Y. Shkuropatov (1988) In The Photosynthetic Bacterial Reaction Center - Structure and Dynamics. (Edited by J. Breton and A. Verméglio), NATO ASI. Ser. A: Life Sciences, pp. 205-220, Plenum, New York
- 17] Wasielewski, M. R. and D. M. Tiede (1986) FEBS Lett. 204, 368-372
- 18] Frank, H. A. and C. A. Violette (1989) Biochim. Biophys. Acta 976, 222-232.
- 19] Holzapfel, W., U. Finklele, W. Kaiser, D. Oesterhelt, H. Scheer, H. U. Stilz, and W. Zinth (1989) Chem. Phys. Lett. 160, 1-7.
- 20] Zinth, W., W. Holzapfel, U. Finklele, W. Kaiser, D. Oesterhelt, H. Scheer, and H. U. Stilz (1990) In: Current Research in Photosynthesis, Vol 1, Ed. M. Baltscheffsky, p.27-30, Kluwer, Amsterdam
- 21] Beese, D., R. Steiner, H. Scheer, A. Angerhofer, B. Robert, and M. Lutz (1987) Photochem. Photobiol. 46, 293-304.
- 22] Ditson, S. L., R. C. Davis, and R. M. Pearlstein (1984) Biochim. Biophys. Acta 766, 623-629.
- 23] Fischer, H. and H. Orth (1940) Die Chemie des Pyrrols, Vol. II, 2nd half., Akademische Verlagsgesellschaft, Leipzig, reprinted: Johnson Reprint Corp., New York, 1968
- 24] Lötjönen, S. and P. H. Hynninen (1980) Int. J. Meth. Synth. Org. Chem. 7, 541-544.
- 25] Porra, R. J., W. A. Thompson, and P. E. Kriedemann (1989) Biochim. Biophys. Acta 975, 384-394

- [26] Smith, J. R. L. and M. Calvin (1966) *J. Amer. Chem. Soc.* 88, 4500-4506.
- [27] Pennington, F. C., H. H. Strain, W. A. Svec, and J. J. Katz (1964) *J. Amer. Chem. Soc.* 86, 1418-1426.
- [28] Watanabe, T., A. Hongu, K. Honda, M. Nakazato, M. Konno, and S. Saitoh (1984) *Anal. Chem.* 56, 251-256.
- [29] Ovchinnikov, Y. A., N. G. Abdulaev, B. E. Shmuckler, A. A. Zargarov, M. A. Kutuzov, I. N. Telezhinskaya, N. B. Levina, and A. S. Zolotarev (1988) *FEBS. Lett.* 232, 364-368
- [30] Shiozawa, J. A., F. Lottspeich, D. Oesterhelt, and R. Feick (1989) *Eur. J. Biochem.* 180, 75-84
- [31] Bylina, E. J. and D. C. Youvan (1988) *Proc. Natl. Acad. Sci. USA* 85, 7226-7230, see also this volume
- [32] Schenck, C. and D. Gaul (1990) private communication, in part see: Boxer, S.G., D. G. Lockart, S. Hammes, L. Mazzola, C. Kirmaier, D. Holten, D. Gaul, and C. Schenck, in: *Current Research in Photosynthesis*, Vol 1, Ed. M. Baltscheffsky, p.113-116, Kluwer, Amsterdam; see also this volume
- [33] Loach, P. A., T. Michalski, P.S. Parkes-Loach (1990) in: *Current Research in Photosynthesis*, Vol II, Ed. M. Baltscheffsky, p.69-72, Kluwer, Amsterdam
- [34] Parkes-Loach, P. S., T. J. Michalski, W. J. Bass, U. J. Smith, and P. A. Loach (1990) *Biochemistry* 29, 2951-2960.