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The Photosynthetic Bacterial Reaction Center II

Structure, Spectroscopy,
and Dynamics

Edited by

Jacques Breton

CEN Saclay
Gif-sur-Yvette, France

and

André Verméglio

CEN Cadarache
Saint Paul lez Durance, France

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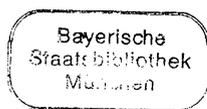
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CONTENTS

The 3-D Structure of the Reaction Center from <i>Rhodospseudomonas viridis</i> J. Deisenhofer, H. Michel.....	1
Correlation between the Polarized Light Absorption and the X-Ray Structure of Single Crystals of the Reaction Center from <i>Rhodobacter sphaeroides</i> R-26 H.A. Frank, M. L. Aldema	13
Symmetrical Inter-subunit Suppressors of the Bacterial Reaction Center cd-Helix Exchange Mutants S.J. Robles, T. Ranck, D.C. Youvan	21
Mutations that Affect the Donor Midpoint Potential in Reaction Centers from <i>Rhodobacter sphaeroides</i> J.C. Williams, N.W. Woodbury, A.K.W. Taguchi, J.M. Peloquin, H.A. Murchison, R.G. Alden, J.P. Allen.....	25
Suggestions for Directed Engineering of Reaction Centers : Metal, Substituent and Charge Modifications J. Fajer, L.K. Hanson, M.C. Zemer, M.A. Thompson	33
Potential Energy Function for Photosynthetic Reaction Center Chromophores: Energy Minimisations of a Crystalline Bacteriopheophytin a Analog N. Foloppe, J. Breton, J.C. Smith.....	43
Bacterial Reaction Centers with Plant-type Pheophytins H. Scheer, M. Meyer, I. Katheder	49
Trapping of a Stable Form of Reduced Bacteriopheophytin and Bacteriochlorophyll in <i>Ectothiorhodospira</i> sp. Photoreaction Center T. Mar, G. Gingras	59
Triplet-minus-singlet Absorbance Difference Spectroscopy of <i>Heliobacterium</i> <i>chlorum</i> Monitored with Absorbance-detected Magnetic Resonance J. Vrieze, E.J. van de Meent, A.J. Hoff	67
Mid- and Near-IR Electronic Transitions of P ⁺ : New Probes of Resonance Interactions and Structural Asymmetry in Reaction Centers W.W. Parson, E. Nabedryk, J. Breton.....	79
¹⁵ N ENDOR Experiments on the Primary Donor Cation Radical D ⁺ in Bacterial Reaction Center Single Crystals of <i>Rb. sphaeroides</i> R-26 F. Lendzian, B. Bönigk, M. Plato, K. Möbius, W. Lubitz	89
EPR and ENDOR Studies of the Primary Donor Cation Radical in Native and Genetically Modified Bacterial Reaction Centers J. Rautter, C. Geßner, F. Lendzian, W. Lubitz, J.C. Williams,. H.A. Murchison, S. Wang, N.W. Woodbury, J.P. Allen	99

Molecular Orbital Study of Electronic Asymmetry in Primary Donors of Bacterial Reaction Centers M. Plato, F. Lendzian, W. Lubitz, K. Möbius.....	109
Near-infrared-excitation Resonance Raman Studies of Bacterial Reaction Centers V. Palaniappan, D.F. Bocian.....	119
Asymmetric Structural Aspects of the Primary Donor in Several Photosynthetic Bacteria: the Near-IR Fourier Transform Raman Approach T.A. Mattioli, B. Robert, M. Lutz.....	127
<i>Rhodocyclus gelatinosus</i> Reaction Center: Characterization of the Quinones and Structure of the Primary Donor I. Agalidis, B. Robert, T. Mattioli, F. Reiss-Husson.....	133
FTIR Characterization of Leu M160→His, Leu L131→His and His L168→Phe Mutations Near the Primary Electron Donor in <i>Rb. sphaeroides</i> Reaction Centers E. Nabedryk, J. Breton, J.P. Allen, H.A. Murchison, A.K.W. Taguchi, J.C. Williams, N.W. Woodbury.....	141
FTIR Spectroscopy of the P+QA ⁻ /PQA State in Met L248→Thr, Ser L244→Gly, Phe M197→Tyr, Tyr M210→Phe, Tyr M210→Leu, Phe L181→Tyr and Phe L181-Tyr M210→Tyr L181-Phe M210 Mutants of <i>Rb. sphaeroides</i> E. Nabedryk, J. Breton, J. Wachtveitl, K.A. Gray, D. Oesterhelt.....	147
Light-induced Charge Separation in Photosynthetic Bacterial Reaction Centers Monitored by FTIR Difference Spectroscopy: The QA Vibrations J. Breton, J.-R. Burie, C. Berthomieu, D.L. Thibodeau, S. Andrianambinintsoa, D. Dejonghe, G. Berger, E. Nabedryk.....	155
Time-resolved Infrared and Static FTIR Studies of QA→QB Electron Transfer in <i>Rhodopseudomonas viridis</i> Reaction Centers R. Hienerwadel, E. Nabedryk, J. Breton, W. Kreutz, W. Mäntele.....	163
Is Dispersive Kinetics from Structural Heterogeneity Responsible for the Nonexponential Decay of P870* in Bacterial Reaction Centers? S.V. Kolaczkovski, P.A. Lyle, G.J. Small.....	173
Effect of Charge Transfer States on the Zero Phonon Line of the Special Pair in the Bacterial Reaction Center E.J.P. Lathrop, R.A. Friesner.....	183
Theoretical Studies on the Electronical Structure of the Special Pair Dimer and the Charge Separation Process for the Reaction Center <i>Rhodopseudomonas viridis</i> P.O.J. Scherer, S.F. Fisher.....	193
Recent Experimental Results for the Initial Step of Bacterial Photosynthesis T.J. DiMagno, S.J. Rosenthal, X. Xie, M. Du, C.-K. Chan, D.Hanson, M. Schiffer, J.R. Norris, G.R. Fleming.....	209
Model Calculations on the Fluorescence Kinetics of Isolated Bacterial Reaction Centers from <i>Rhodobacter sphaeroides</i> A.R. Holzwarth, M.G. Müller, K. Griebenow.....	219
Femtosecond Spectroscopy of the Primary Electron Transfer in Photosynthetic Reaction Centers W. Zinth, P. Hamm, K. Dressler, U. Finkele, C. Lauterwasser.....	227
Femtosecond Optical Characterization of the Excited State of <i>Rhodobacter capsulatus</i> DLL M.H. Vos, F. Rappaport, J.-C. Lambry, J. Breton, J.-L. Martin.....	237

Electron Transfer in <i>Rhodospseudomonas viridis</i> Reaction Centers with Prereduced Bacteriopheophytin BL V.A. Shuvalov, A.Ya. Shkuropatov, A.V. Klevanik	245
Fast Internal Conversion in Bacteriochlorophyll Dimers U. Eberl, M. Gilbert, W. Keupp, T. Langenbacher, J. Siegl, I. Sinning, A. Ogrodnik, S.J. Robles, J. Breton, D.C. Youvan, M.E. Michel-Beyerle.....	253
Primary Charge Separation in Reaction Centers: Time-resolved Spectral Features of Electric Field Induced Reduction of Quantum Yield A. Ogrodnik, T. Langenbacher, U. Eberl, M. Volk, M.E. Michel-Beyerle	261
Electric Field Effects on the Quantum Yields and Kinetics of Fluorescence and Transient Intermediates in Bacterial Reaction Centers S.G. Boxer, S. Franzen, K. Lao, D.J. Lockhart, R. Stanley, M. Steffen, J.W. Stocker	271
Radical Pair Dynamics in the Bacterial Photosynthetic Reaction Center M. Bixon, J. Jortner, M.E. Michel-Beyerle.....	283
The Primary Charge Separation in Bacterial Photosynthesis. What Is New ? M. Bixon, J. Jortner, M.E. Michel-Beyerle.....	291
Multi-Mode Coupling of Protein Motion to Electron Transfer in the Photosynthetic Reaction Center: Spin-Boson Theory Based on a Classical Molecular Dynamics Simulation D. Xu, K. Schulten	301
Pulsed Electric Field Induced Reverse Electron Transfer from Ground State BChl ₂ to the Cytochrome c Hemes in <i>Rps. viridis</i> G. Alegria, C.C. Moser, P.L. Dutton.....	313
Structural Changes Following the Formation of D ⁺ QA ⁻ in Bacterial Reaction Centers: Measurement of Light-induced Electrogenic Events in RCs Incorporated in a Phospholipid Monolayer P. Brzezinski, M.Y. Okamura, G. Feher	321
Charges Recombination Kinetics in Bacterial Photosynthetic Reaction Centers: Conformational States in Equilibrium Pre-exist in the Dark B. Schoepp, P. Parot, J. Lavorel, A. Verméglio.....	331
Protein Relaxation Following Quinone Reduction in <i>Rhodobacter capsulatus</i> : Detection of Likely Protonation-linked Optical Absorbance Changes of the Chromophores D.M. Tiede, D.K. Hanson	341
Study of Reaction Center Function by Analysis of the Effects of Site-specific and Compensatory Mutations M. Schiffer, C.-K. Chan, C.-H. Chang, T.J. DiMagno, G.R. Fleming, S. Nance, J.R. Norris, S. Snyder, M. Thurnauer, D.M. Tiede, D.K. Hanson.....	351
Proton Transfer Pathways in the Reaction Center of <i>Rhodobacter sphaeroides</i> : a Computational Study P. Beroza, D.R. Fredkin, M.Y. Okamura, G. Feher.....	363
Electrostatic Interactions and Flash-induced Proton Uptake in Reaction Centers from <i>Rb. sphaeroides</i> V.P. Shinkarev, E. Takahashi, C.A. Wraight.....	375

Initial Characterization of the Proton Transfer Pathway to QB in <i>Rhodospseudomonas viridis</i> : Electron Transfer Kinetics in Herbicide-resistant Mutants W. Leibl, I. Sinning, G. Ewald, H. Michel, J. Breton	389
Study of Reaction Centers from <i>Rb. capsulatus</i> Mutants Modified in the QB Binding Site L. Baciou, E.J. Bylina, P. Sebban	395
Calculations of Proton Uptake in <i>Rhodobacter sphaeroides</i> Reaction Centers M.R. Gunner, B. Honig	403
Chlorophyll Triplet States in the CP47-D1-D2-cytochrome b-559 Complex of Photosystem II P.J.M. van Kan, M. L. Groot, S.L.S. Kwa, J.P. Dekker, R. van Grondelle	411
Light Reflections II G. Feher	421
Index	427

BACTERIAL REACTION CENTERS WITH PLANT-TYPE PHEOPHYTINS

Hugo Scheer, Michaela Meyer, and Ingrid Katheder

Botanisches Institut der Universität München,
Menzinger Str. 67, D-8000 München 19

SUMMARY

The exchangeability of the bacteriopheophytins at sites H_A and H_B with modified (bacterio)pheophytins ($=(\mathbf{B})\mathbf{Phe}^*$) was tested in reaction centers (RC) of *Rhodobacter spheroides* R26. An exchange at both sites is possible with Pyro-**BPhe a** lacking the 13^2 -COOCH₃ group, and with three plant-type pheophytins: **Phe a** (which contains a 3-vinyl-group), 13^2 -hydroxy-**Phe a** (which contains in addition a hydroxy group), and [3-acetyl]-**Phe a** (which differs from **BPhe a** only by the unsaturated ring II). In all cases, the exchange appears to be easier at the H_B -site. An exchange only at this site, was obtained with **BPhe a**_{gg} in which the esterifying phytol is replaced by geranyl-geraniol, and with 13^2 -hydroxy-**Phe a**. Environment-induced red-shifts (EIRS) are observed with all pigments, and they are in the range of the ones known for the native **BPhe a**. Strong optical activity is induced in most pigments. Shifts in the absorptions of the monomeric **BChls** at sites $B_{A,B}$ indicate an interaction with the **BPhe**s sites $H_{A,B}$, or an indirect structural effect.

INTRODUCTION

In reaction centers (RC) of *Rhodobacter spheroides* (Rb.), the tetrapyrrole pigments at the sites $B_{A,B}$ ("monomeric" bacteriochlorophylls,

*) **Abbreviations:** **Chl** = chlorophyll, **Phe** = pheophytin, **BChl** = bacteriochlorophyll, **BPhe** = bacteriopheophytin; the subscripts refer to the esterifying alcohols ("p" or none for phytol, "gg" for geranylgeraniol), RC = reaction centers, Rb. = *Rhodobacter*, cd = circular dichroism, P = primary donor site, B = site of monomeric BChl, H = site of BPhe in RC. The subscripts "A" and "B" refer to the active ("L") and inactive branch ("M"), respectively, of the electron transport chain.

MATERIALS AND METHODS

RC of *Rb. spheroides* R26 were prepared from chromatophores by repeated solubilization with increasing concentrations of LDAO and NaCl (modified after Feher and Okamura, 1978) and purified on DEAE-cellulose (Struck, 1990).

BPhe a_p, **BPhe a_{gg}** and **Phe a** were extracted from *Rb. spheroides* 2.4.1, *Rhodospirillum rubrum* G9 and *Spirulina gleitleri* (SOSA Texcoco), resp., by standard procedures and purified on DEAE-cellulose (Sato and Murata, 1978). Demethoxycarbonylation of **BPhe a_p** to **Pyro-BPhe a_p** was done according to Pennington *et al.* (1963). [3-acetyl]-**Phe a** was made from **Phe a** (Smith and Calvin, 1966). 13²-hydroxy-**Chl a** was obtained as a by-product during the isolation of **Chl a** and pheophytinized according to Rosenbach-Belkin (1988). Structures of the pigments were verified by VIS-NIR absorption, ¹H-NMR and mass spectroscopy.

The conditions for the exchange experiments of **BPhe a** against the modified pigments as described by Struck (1990), were optimized. The incubation temperature of the RC was increased to 43.5°C. The modified pigments were added in a 10-fold excess, the solvent for the pigments was 100% acetone, its final concentration 10%. After incubation, the excess of free pigments was removed by repeated chromatography on DEAE-cellulose.

Extraction of the pigments from RC was done with CHCl₃/CH₃OH = 5:1 (v/v). The extract was dried under a stream of argon, dissolved in toluene, and then subjected without delay to HPLC-analysis according to Watanabe *et al.* (1984).

RESULTS

Three plant-type pheophytins were tested: i) [3-acetyl]-**Phe a**, which differs from **BPhe a** only by the unsaturation of ring II. ii) **Phe a**, which has in addition the 3-acetyl- replaced by a vinyl-group. iii) 13²-hydroxy-**Phe a**, which contains furthermore an OH-group instead of the enolizable 13²-proton.

The absorption spectra of **Phe a** and 13²-hydroxy-**Phe a** are essentially identical (Fig. 1). In comparison to **BPhe a**, there are two characteristic blue-shifted Q_X-bands for the two plant-type pheophytins, and the Q_Y-bands are blue-shifted by 70-80 nm, too. Unlike **Phe a**, [3-acetyl]-**Phe a** shows a split Soret-band as do the bacteriopheophytins. Providing the same assignments, they are red-shifted by 24 nm (B_X) and 28 nm (B_Y). The main peak of the Soret-band of **Phe a** is red-shifted by ≈52 nm compared to B_X of **BPhe a**.

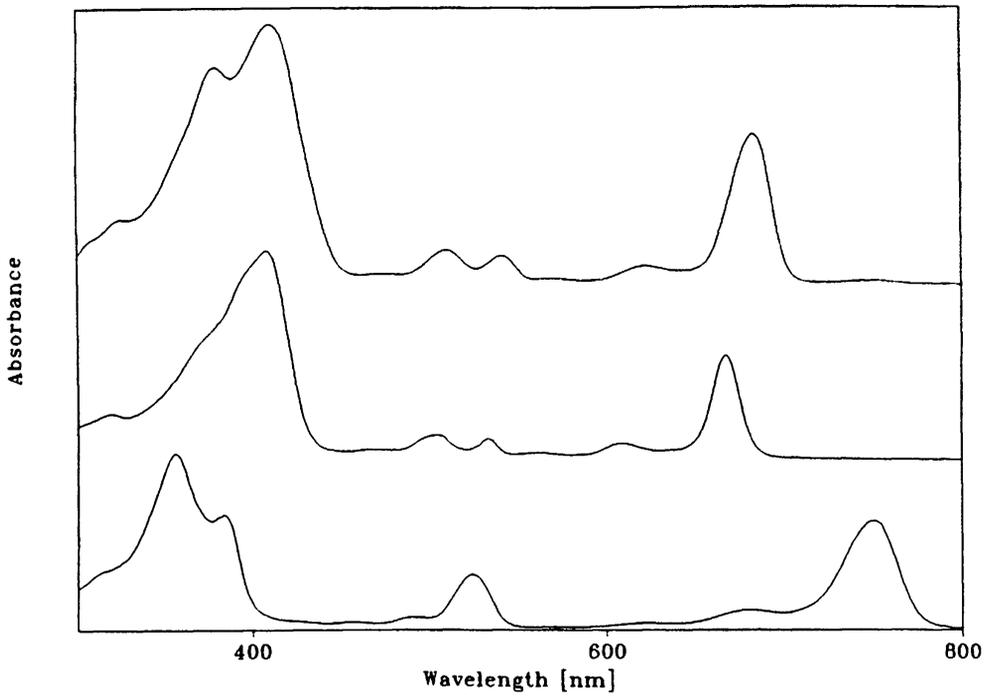


Fig. 1. Absorption spectra of pigments in ether solution: Bacteriopheophytin a (BPhe a, bottom), pheophytin a (Phe a, center) and [3-acetyl]-pheophytin a ([3-acetyl]-Phe a, top).

Table 1. Exchange rates of some plant-type pheophytins and bacterial-type pheophytins

Pigment	exchange-rates (%)	
BPhe a _{agg}	38	
Pyro-BPhe a	35 (1)	68 (2)
Phe a	80 (1)	95 (2)
[3-Acetyl]-Phe a	68	
13 ² -OH-Phe a	35	

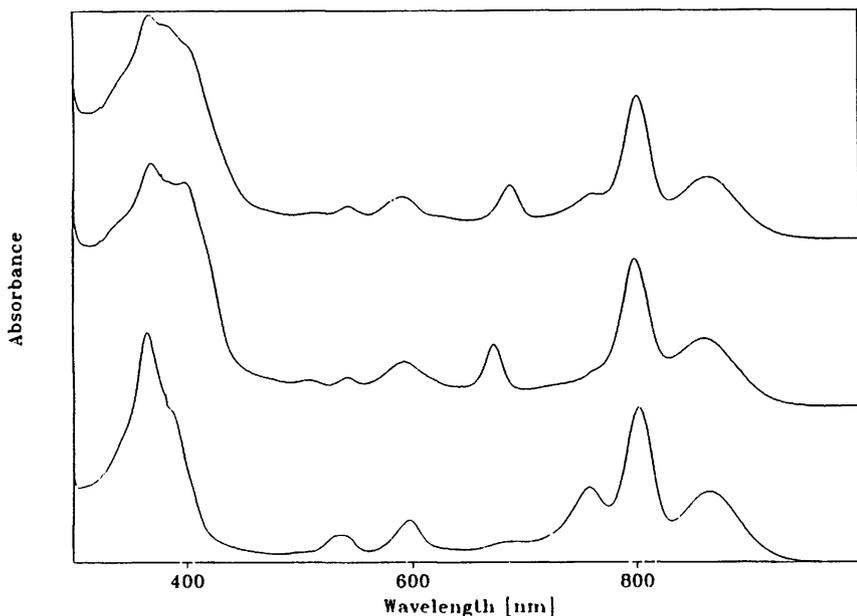


Fig. 2. Absorption spectra of reaction centers with modified pheophytins: in tris-HCl buffer (20 mM, pH 8) containing LDAO (0.1%). Native RC from *Rhodospirillum rubrum* R26 (bottom), RC after double exchange of BPhe a against Phe a (center), and RC after single exchange of BPhe a against [3-acetyl]-Phe a (top). Spectra were normalized to the same absorption at the dimer band (≈ 865 nm).

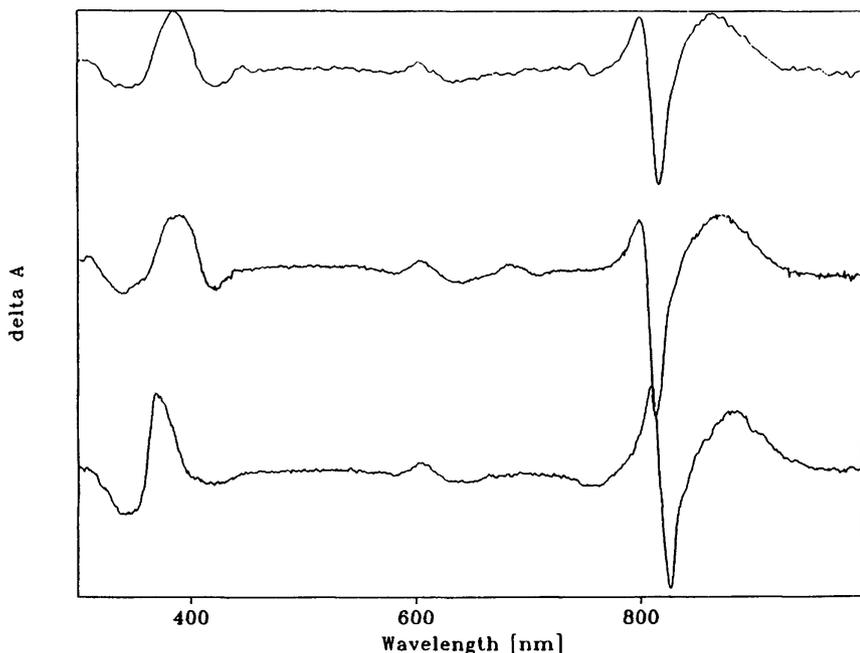


Fig. 3. Circular dichroism spectra of reaction centers with modified pheophytins, in tris-HCl buffer (20 mM, pH 8) containing LDAO (0.1%). Native RC from *Rhodospirillum rubrum* R26 (bottom), RC after exchange of BPhe a against Phe a (center), and RC after single exchange of BPhe a against [3-acetyl]-Phe a (top). Spectra were normalized to the same absorption at the dimer band (≈ 865 nm).

The absorption spectra of the three bacteriopheophytins tested (BPhe a_p, BPhe a_{gg} and Pyro-BPhe a_p) are nearly identical and not shown.

The exchange-rates of these (B)Phe_s are summarized in Table 1. Phe a and [3-acetyl]-Phe a exchange readily to >50%, e.g. they exchange both in H_A- and H_B-sites of the RC. The HPLC-chromatogram of RC after repeated (double) exchange of BPhe a against Phe a (Fig. 4), shows only traces of the former (> 90% exchange). Because of the dehydrogenation of ring II, Phe a has a shorter retention time than BPhe a.

The absorption spectra of the RC modified with plant-type pheophytins (exchange rates >90%) show distinct changes as compared to the native ones. These changes follow the differences in the solution spectra of the respective pigments (Fig. 2). The Q_X(0-1)-band shows a blue-shift of about 22 nm (Phe a) or 14 nm ([3-acetyl]-Phe a) and the Q_X(0-0)-band a red-shift of about 7 nm (Phe a) or 17 nm ([3-acetyl]-Phe a), as compared to the center of the BPhe a-band Q_X(0-0) in native RC. The Q_Y-band of BPhe a ($\lambda_{\max} \approx 758$ nm in native RC) is replaced by a strongly blue-shifted one. Comparing RC containing Phe a and [3-acetyl]-Phe a, the relative band positions of the solution spectra are preserved. The Q_Y-band of the latter is in particular red-shifted compared to the former.

In the cd-spectra, the bands assigned to BPhe a are diminished. RC containing Phe a show instead a distinct, s-shaped feature at the position of

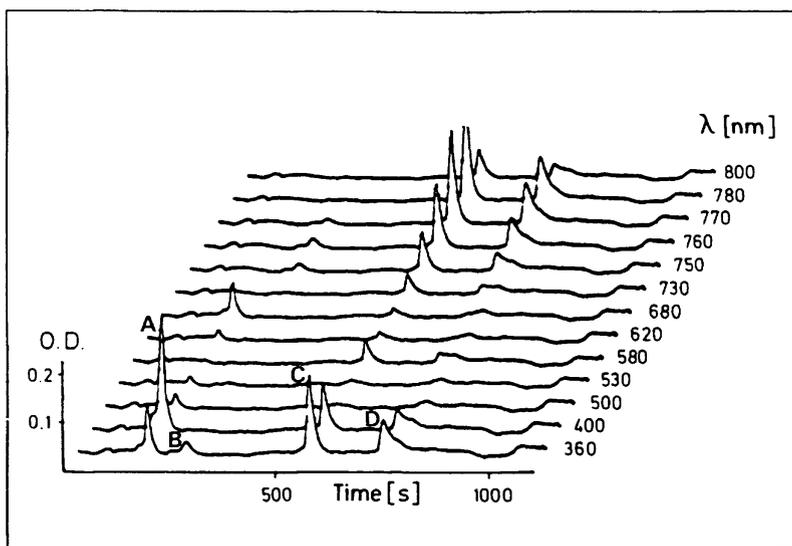


Fig. 4. HPLC chromatogram of RC after repeated exchange of BPhe a against Phe a: (chromatography system of Watanabe *et al.* (1984), detection with HP diode array). The detection wavelengths are given on the right-hand side of the individual traces. Peak assignments: A: Phe a, B: BPhe a, C: BChl a, D: 13-hydroxy-BChl a

Table 2. Absorption maxima [nm] of (bacterio)pheophytins in ether solution, of the same pigments in the H-sites of RC, and the resulting environment-induced red-shifts (EIRS) (nm and cm^{-1}).

Pigment	Ether [nm]		Protein [nm]		red-shift [nm] (cm^{-1})	
	Q _x	Q _y	Q _x	Q _y	Q _x	Q _y
BPhe a _p	524	750	537	758	13 (462)	8 (141)
BPhe a _{gg}	524	749	539	758	15 (531)	9 (159)
Pyro-BPhe a	527	749	538	757	11 (388)	8 (141)
Phe a	504/533	667	509/542	674	5/9 (195/312)	7 (156)
[3-Acetyl]-Phe a	510/541	680	516/544	689	6/3 (228/102)	9 (192)
13 ² -OH-Phe a	502/531	667	506/539	673	4/8 (157/280)	6 (134)

the Q_y-band of the newly introduced pigment, but the latter is not obvious in the spectrum of RC containing [3-acetyl]-Phe a. It is noteworthy, that there is also an effect of the cd assigned to the monomeric BChl, e.g. a decrease of the exciton band of the monomeric BChls in the Q_x-region and at about 380 nm (Fig. 3).

DISCUSSION

All (B)Phes investigated, exchanged selectively into the H-binding site(s) of BPhe a. The presence or absence of the central Mg-atom again (Struck *et al.*, 1990) then seems to determine whether the pigment is accepted in H_{A,B} or B_{A,B}, resp. This complements site directed mutagenesis of amino acids: BPhe replaces BChl if a suitable ligand (his, glu, ser, thr) is introduced, and *vice versa* (Schenck *et al.*, 1990; Woodbury *et al.*, 1990; Coleman and Youvan, 1990).

Compared to the structural variations allowed for exchange of BChls into the B_{A,B} binding sites, the results indicate that the H_{A,B} sites allow for considerably more extensive structural changes. It is particularly noteworthy, that there is a ready exchange possible with the plant-type Phes in the H_{A,B} binding sites, because the plant-type Chls (= Mg-complexes) were not accepted in previous experiments in the BChl-binding sites (Struck, 1990). The efficiency of the exchange with Phe a is greater than with 13²-hydroxy-Phe a and [3-acetyl]-Phe a (Table 1). Remarkable is also the preference of Phe a (= 3-vinyl) over all other pigments containing the 3-acetyl-group, which is characteristic for the native BPhe a. Although exchangeability is strictly an operational criterion, these results indicate a greater structural plasticity at the H- than at the B-sites.

Most of the pigments investigated, showed exchanges amounting to >50%, which by repeated incubation led to replacements $\leq 95\%$. This clearly shows that both binding sites are accessible to these pigments. The asymmetry introduced by glu-100, has then generally not a strongly selecting influence. BPhe a_{gg} is an exception. This pigment has a more unsaturated esterifying alcohol (four double bonds instead of one), which changes the flexibility, polarity and spatial structure of this part of the molecule. It should be noted, that the different arrangement of the esterifying alcohols of BPhe-H_A and BPhe-H_B is one of the distinctive symmetry breaking elements in RC, which indicates a specific function of the alcohol in binding. No such specificity was observed in BChl exchanges at the B_{A,B} sites (Struck, 1990), and neither is there a comparable asymmetry.

At the B_{A,B}-sites, differential exchange kinetics were observed, with B_B exchanging more rapidly than B_A. A similar difference was seen in earlier BPhe exchange experiments. The present results with plant-type pheophytins indicate no obvious difference with these pigments, but due to band-overlap in the Q_X-region this result needs further studies, e.g. at low temperature.

Comparing the pigments in ether solution and in the RC environment, an environment-induced red-shift (EIRS) of the Q_X and Q_Y-bands in the protein is found for all pigments. This shift shows only relatively small variations (Table 2). It is not clear if this is a result of protein-chromophore or protein-protein-interactions (Scherz *et al.*, 1990), or both. At least in Phe a, there is also a concomitant increase in optical activity. Both effects are compatible with a non-planar distortion of the macrocyclic system.

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INDEX

- Absorption spectra
 low temperature, 247, 359-360
 ground, excited and radical pair states, 193
 primary electron donor, 63
 polarized light, 13
- ABNR (Adopted Basis Newton-Raphson), 45
- ADMR (Absorption Detected Magnetic Resonance)
 67
- Amino acids sequence, 1
- Alanine
 M246, 357
 M247, 357
- Arginine
 L217, 8, 368, 391, 405
 H177, 368
- Asparagine
 L173, 405
 L213, 8, 364, 375, 405
 L210, 368, 405
- Aspartic acid
 L213, 164
 M43, 8
- Antenna complexes, 33, 67, 173
- ATP, 313
- Bacteriochlorin, 34
- Bacteriopheophytin
 crystalline, 43
- Biexponentiality
 charge recombination, 210, 292, 331
 charge separation, 173, 174, 220, 229, 292
- Carotenoid, 2
- Charge recombination
 between
 $P^+B_A^-$, 27
 $P^+H_A^-$, 27
 $P^+Q_A^-$, 27, 156, 331, 341, 375
 $P^+Q_B^-$, 166, 341, 375, 399
 electric effect on, 278
 mutation effect on, 27
- Charge transfer states, 254, 291
- CHARMM, 44, 205, 404
- Chloroflexus aurantiacus*, 193
- Chromatium minutissimum*, 246
- Chromophore exchange, 33
- Circular dichroism, 53
- Completely Neglected Differential Orbital, 112
- Configuration Interaction (CI), 81, 193
- Crystals, 1, 13, 43, 89, 99, 114, 183
- Cytochrome, 133, 165
 b559, 166, 411
 c, 151, 245, 313
 c553, 314
 c559, 313
 electron transfer, 313
- Decay Associated Spectra (DAS), 220
- Ectothiorhodospira* sp, 59
- Electric field
 effect on quantum yield, 253, 261, 271
 fluorescence lineshape, 257, 277
 fluorescence yield, 255, 274
 pulse, 313
 reverse electron transfer, 313
- Electrochromism, 38, 193
- Electron transfer rates, 7, 245
 pH dependence, 378
 protein dynamics, 378
 quinone reduction, 321, 341, 355
 recombination,
 $P^+Q_A^-$, 27, 321, 331, 341, 399
 $P^+Q_B^-$, 166, 341, 375, 399
- Electron Nuclear Double Resonance (ENDOR),
 26, 89; *see also* Magnetic resonance
- Electron-phonon coupling, 242
- Electron Spin Echo Envelope Modulation (ESEEM)
 89
- EXAFS, 33
- Exciton band
 high energy, 37, 63, 194, 241, 249
- Femtosecond flash photolysis, 27, 60, 209, 227,
 237, 253
- Fluorescence
 lifetime, 209, 219, 255, 271
- Franck-Condon factor, 118, 191, 257, 261, 283,
 288, 293
- Fourier Transform Infrared Spectroscopy (FTIR),
 26, 87, 105, 141, 155, 163
- Fourier Transform Raman (FT Raman) *see* Raman

- Glutamic acid
 H173, 368
 H177, 8
 L104, 8, 125
 L212, 8, 163, 326, 347, 353, 368, 375,
 391, 405
 L213, 353
 L225, 353
 M232, 5
 M234, 368, 405
- Glycine
 M188, 2
Halobacterium halobium, 390
Heliobacterium chlorum, 67, 35
Heliobacterium gestii, fasciculum, 35
Heliobacillus mobilis, 35
 Helix exchange, 21
 Heme, 2, 44
 Heterogeneity, 173, 209, 221, 234
 Heterodimer HL(M202), 81, 103
 Highest Occupied Molecular Orbital (HOMO), 37,
 79, 104, 116, 200
 HFC'S electron nuclear hyperfine coupling, 99
- Histidine
 C124, 4
 L131, 101, 141
 L153, 21
 L168, 26, 105, 112, 131, 141, 147
 L173, 7, 123
 L190, 5, 364
 L230, 5, 364
 M160, 26, 101, 101, 141
 M200, 7, 142
 M202, 120
 M217, 5
 M264, 5
 M280, 193
- Hole burning, 173, 233, 292
 Huang-Rhys S value, 174, 184
 Hückel MO, 104, 110
- Intermediate Neglect of Differential Overlap
 (INDO), 35, 90, 112, 193, 342
 Inter-subunit suppressor, 21
- Isoleucine
 L229, 396
- Iterative Extended Hückel (IEH) *see* Hückel
- Langmuir-Blodgett films, 29, 262
 Lennard-Jones potential, 44
 Linear dichroism, 13, 142, 152, 193, 241
 Lowest Unoccupied Molecular Orbital (LUMO),
 37, 79, 118, 200
- Low temperature
 absorption spectra of native reaction center,
 247, 359
 absorption spectra of Photosystem II, 414
 ADMR, MIA, 68
 electron transfer in model systems, 308
 femtosecond kinetics, 27, 60, 209, 227, 237, 253
 inverse temperature dependence on P⁺ decay, 28
- Low temperature (*con'd*)
 P⁺QA⁻ recombination, 27, 88, 156, 331, 341, 375
 phototrapping, 61
 Raman, 121
- Lysine
 H130, 368
- Magnetic resonance
 ENDOR and TRIPLE, 87, 89, 99, 109
 EPR, 20, 36, 60, 67, 89, 99, 114, 141
 NMR, 356 51
- Metal binding site, 33
 Methyl-bacteriopheophobide, 43
- Methionine
 L248, 147
- MOPAC, 45
- Mutants
 around P, 25, 99, 141, 147, 211, 276
 around QA or QB, 33, 321, 353, 375, 395
 around Bchl and Bph, 33, 50, 216
 DLL, 237, 253, 271, 292
 helix, 21
- Newton Raphson Minimisation Algorithm, 45
 Non-monoexponentiality, 233, 272, 331
- Oscillation features, 230, 237, 292
- P⁺ electronic transition, 19, 89
- Pigments exchange, 33, 231
- Phenylalanine
 L181, 147, 210
 L183, 292
 L241, 7
 M197, 105, 141, 147
 M210, 147
- pH effect on electron transfer rate, 378
- Phospholipid monolayer, 321
- Photosystem I (PSI), 36, 67
- Photosystem II (PSII), 25, 74, 185, 389, 411
- Phototrapping, 59
- Plant type pheophytins, 49
- Proton transfer, 363, 375, 389, 403
- Quantum yield
 temperature dependence, 416
- Quinone
 binding, 352
 characterization, 134
 electron transfer between, 163, 341
 time resolved FTIR, 147
- Radical pair, 278, 283
- Raman spectroscopy, 34, 119, 127, 133, 183, 240
- Reorganization energy, 29, 212, 284, 288, 294
- Rhodobacter capsulatus*, 21, 87, 101, 125, 184, 193,
 209, 237, 253, 292, 341, 352, 395
- Rhodobacter sphaeroides*, 13, 25, 49, 60, 74,
 80-81, 89, 99, 109, 119, 127, 141, 147, 155,
 173, 184, 193, 219, 228, 237, 261, 271, 321,
 331, 341, 351, 363, 375, 389, 395, 403

Rhodocyclus gelatinosus, 131, 133
Rhodopseudomonas viridis, 1, 35, 73, 80, 89, 109, 127, 156, 163, 174, 193, 228, 241, 245, 253, 267, 292, 301, 313, 331, 341, 351, 365, 375, 389, 395
Rhodospirillum rubrum, 51, 95, 101, 132, 331, 371
 RHF-INDO/SP, 90, 105, 110
 RINDO, 194
 Rydberg state, 194, 203

Salt effect, 381
 Serine
 L223, 9, 364, 391
 L224, 123, 143, 147
 Spin Boson Theory, 301
Spirulina gleitleri, 51
 Spontaneous emission, 209, 246, 292
 Stark effect, 38, 87, 184, 202, 216, 254, 261, 279, 342
 Stimulated emission, 209, 229, 239, 246, 253, 292
 Superexchange mechanism, 174, 214, 227, 237, 261, 272, 288, 291

Temperature dependence
 electron transfer rates, 326, 336
 Terbutryne, 395

Threonine
 L226, 366, 396
 Triplet state
 electric effect on quantum yield, 278
 Tryptophane
 L100, 8
 M250, 8
 M266, 8
 Tyrosine
 L162, 8
 M195, 7, 147
 M208, 7, 210
 M210, 155, 292

Unidirectionality, 222, 234, 259

Valine
 L220, 391
 Van de Waals forces, 6, 44
 Vibronic coupling, 185

Zeners parametrization, 194
 Zero-Phonon line, 178, 183, 233, 292
 Zero-Phonon hole, 176
 Zero-Field-Splitting parameters, 68

