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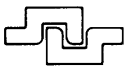

Series A: Life Sciences

The Photosynthetic Bacterial Reaction Center II

Structure, Spectroscopy, and Dynamics

Edited by

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CONTENTS

BACTERIAL REACTION CENTERS WITH PLANT-TYPE PHEOPHYTINS

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SUMMARY

The exchangeability of the bacteriopheophytins at sites H_A and H_B with modified (bacterio)pheophytins (=**(B)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-$COOCH_3$ 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 **BChl**s 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.

The Photosynthetic Bacterial Reaction Center II
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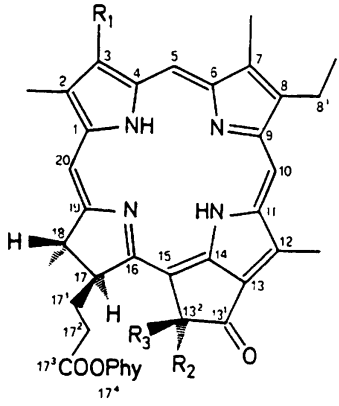
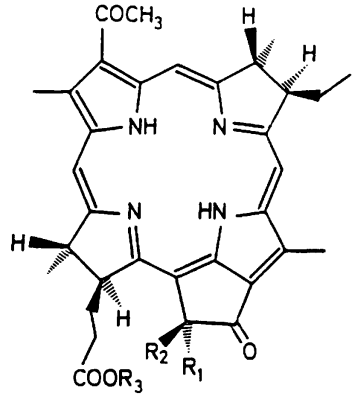
BChl-$B_{A,B}$) and $H_{A,B}$ (bacteriopheophytins, **BPhe**-$H_{A,B}$) are exchangeable against chemically modified pigments. **BChl**-$B_{A,B}$ could be exchanged with a variety of modified **BChl**s, but neither with plant-type chlorophylls (**Chl**), nor with bacteriopheophytins (**BPhe**) or plant-type pheophytins (**Phe**) (Struck *et al.*, 1990). The exchangeability of the bacteriopheophytins at sites $H_{A,B}$ has been studied less. Struck (1990) has shown, that they are exchangeable against some modified **BPhe**s, but not against any Mg-containing **(B)Chl**s. The selectivity of the sites according to the presence or absence of the central Mg-atom, rsp., corroborates results from site-directed mutagenesis (Coleman and Youvan, 1990; Woodbury *et al.*, 1990; Schenck *et al.*, 1990). However, little is presently known on the influence of the reduction level (chlorin *vs.* bacteriochlorin) or the peripheral substituents of **(B)Phe**s on the exchangeability. We wish to report exchange experiments with **BPhe**s modified at C-13^2 and C-17^4, and in particular with plant-type **Phe**s containing an unsaturated ring II.



Plant-type Pheophytins
*) Epimer mixture

Pigment	R_1	R_2	R_3
Phe a	$CHCH_2$	$COOCH_3$	H
Phe a'	$CHCH_2$	H	$COOCH_3$
[3-Acetyl]-Phe a	$COCH_3$	$COOCH_3$	H
13^2-OH-Phe a*	$CHCH_2$	$COOCH_3$	OH



Bacterial-type Pheophytins

Pigment	R_1	R_2	R_3
BPhe a_p	$COOCH_3$	H	$C_{20}H_{39}$
BPhe a_p'	H	$COOCH_3$	$C_{20}H_{39}$
BPhe a_{gg}	$COOCH_3$	H	$C_{20}H_{33}$
Pyro-BPhe a	H	H	$C_{20}H_{39}$

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), rsp., by standard procedures and purified on DEAE-cellulose (Satoh 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). 13^2-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, ^{1}H-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 $CHCl_3/CH_3OH$ = 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) 13^2-hydroxy-**Phe a**, which contains furthermore an OH-group instead of the enolizable 13^2-proton.

The absorption spectra of **Phe a** and 13^2-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**.

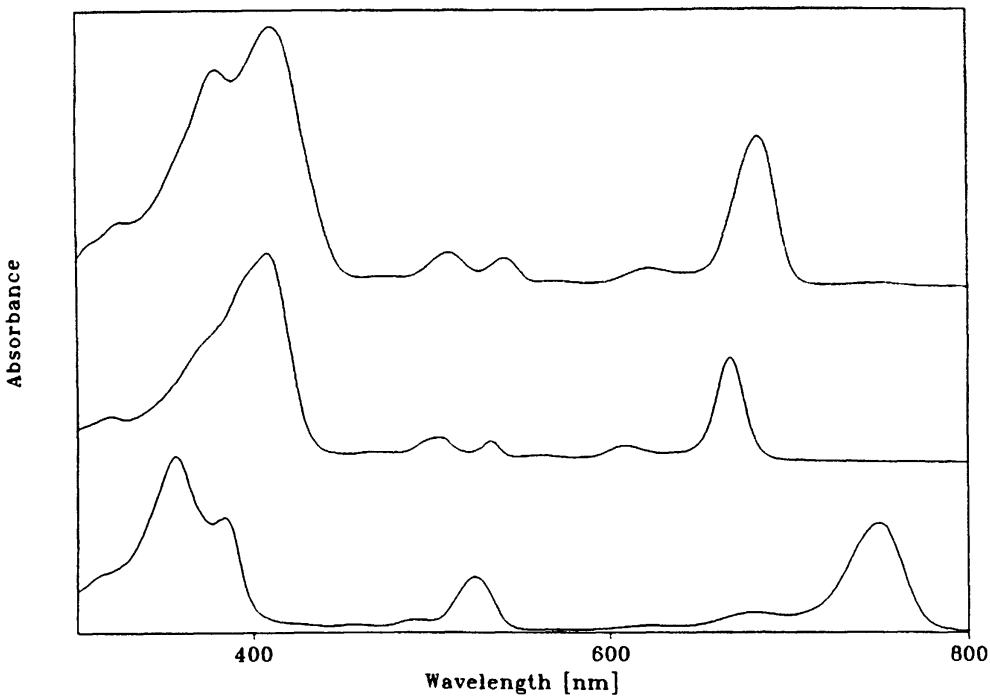


Fig. 1. Absorption spectra of pigments in ether solution: Bacteriopheophytin a (**BPhe a**, bottom), pheophytin a (**Phe a**, center) and [3-acetyl]-pheophytin a ([3-acetyl]-**Phe a**, top).

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

Pigment	exchange-rates [%]
BPhe a_{gg}	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

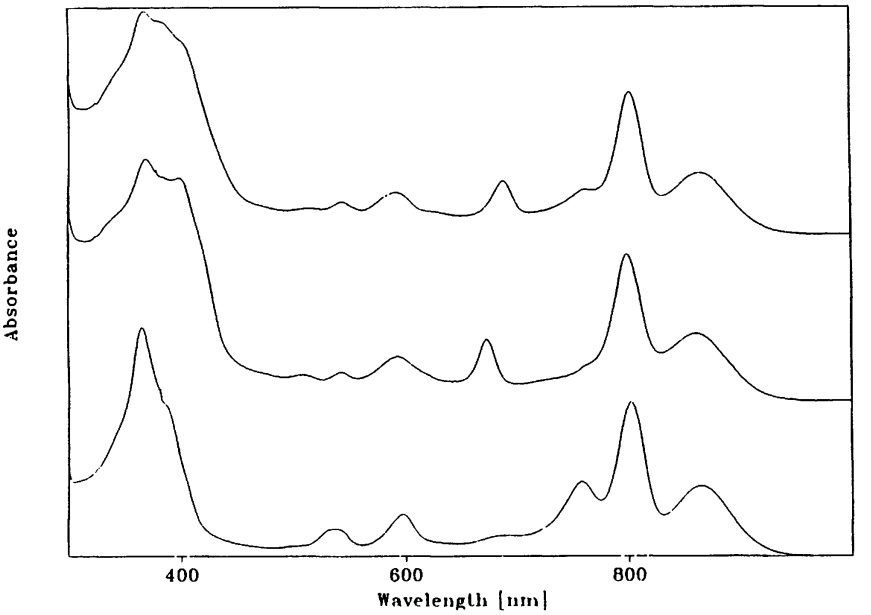


Fig. 2. Absorption spectra of reaction centers with modified pheophytins: in tris-HCl buffer (20 mM, pH 8) containing LDAO (0.1%). Native RC from *Rhodobacter spheroides* R26 (bottom), RC after double exchange of **BPhe a** against **Phe a** (center), and RC after single exchange of **BPhe a** against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

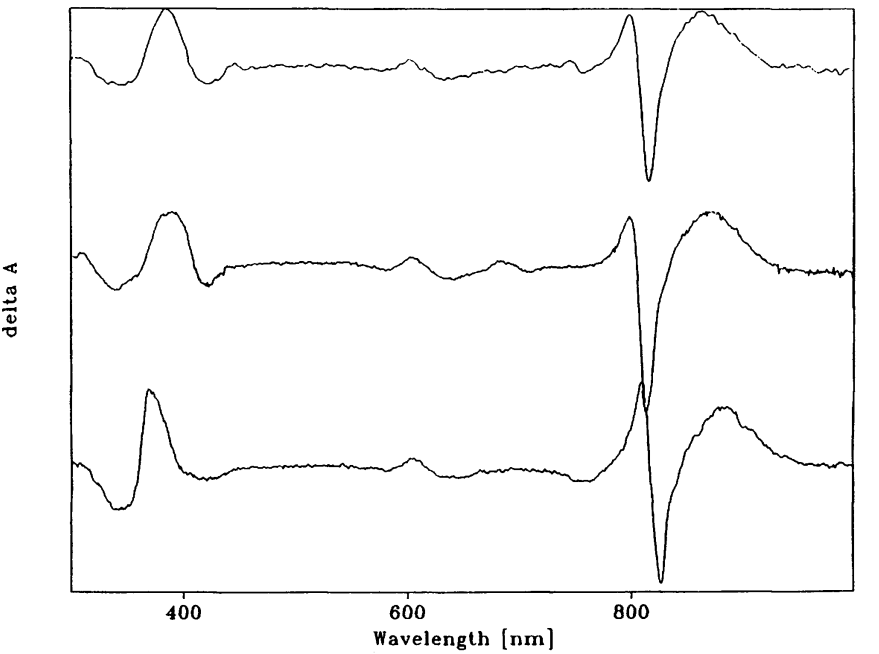


Fig. 3. Circular dichroism spectra of reaction centers with modified pheophytins, in tris-HCl buffer (20 mM, pH 8) containing LDAO (0.1%). Native RC from *Rhodobacter spheroides* R26 (bottom), RC after exchange of **BPhe a** against **Phe a** (center), and RC after single exchange of **BPhe a** against [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)Phes** 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

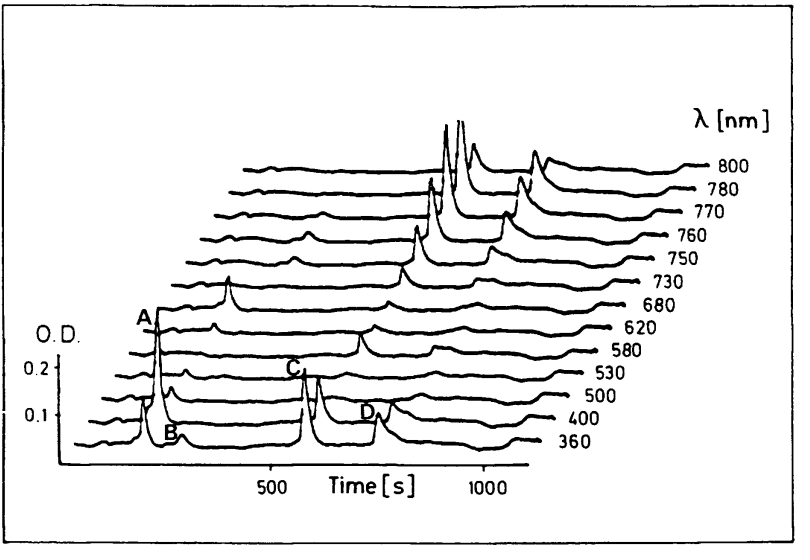


Fig. 4. HPLC chromatogram of RC after repeated exchange of BPhe a against Phe a: (chromatography system of Watanabe *et al.* (1984), detection with HP diode array). The detection wavelengths are given on the right-hand side of the individual traces. Peak assignments: A: **Phe a**, B: **BPhe a**, C: **BChl a**, D: 13^2-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 **BChl**s in the Q_X-region and at about 380 nm (Fig. 3).

DISCUSSION

All **(B)Phe**s 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 $B_{A,B}$, rsp. 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 **BChl**s 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 **Phe**s in the $H_{A,B}$ binding sites, because the plant-type **Chl**s (= 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 13^2-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 ≤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** $\mathbf{a}_{gg}$ 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 braking 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 that 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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REFERENCES

Coleman W.J. and Youvan D.C., 1990, *Ann. Rev. Biophys.* 895:63

Feher G. and Okamura M.T., 1978, *Nature* 339:111

Pennington F.C., Strain H.H., Svec W.A., and Katz J.J., 1963, *J. Am. Chem. Soc.* 86:1418

Rosenbach-Belkin V., 1988, Ph. D. thesis; The Weizmann Institute of Science, Rehovot, Israel

Schenck C.C., Gaul D., Steffen M., Boxer S.G., McDowell L., Kirmaier C., and Holten D., 1990, in: "Reaction Centers of Photosynthetic Bacteria", ed. Michel-Beyerle M.-E., Springer, Berlin, pp. 229

Scherz A., Fischer J.R.E., and Braun P., 1990, in: "Reaction Centers of Photosynthetic Bacteria", ed. Michel-Beyerle M.-E., Springer, Berlin, pp. 377

Smith J.R.L. and Calvin M., 1966, *J. Am. Chem. Soc.* 88:4500

Struck A., 1990, Ph. D. thesis, Ludwig-Maximilians-Universität, München, Germany

Struck A., Beese D., Cmiel E., Fischer M., Müller A., Schäfer W., and Scheer H., 1990, in: "Reaction Centers of Photosynthetic Bacteria", ed. Michel-Beyerle M.-E., Springer, Berlin, pp. 313

Struck A., Cmiel E., and Katheder I., 1990, *FEBS Lett.* 268:180

Watanabe T., Hongu A., Konda K., Nakazato M., Konno M., and Saitoh S., 1984, *Anal. Chem.* 56:251

Woodbury N.W., Taguchi A.K., Stocker J.W., and Boxer S.G., 1990, in: "Reaction Centers of Photosynthetic Bacteria, ed. Michel-Beyerle M.-E., Springer, Berlin, pp. 303

INDEX