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Probing native-like orientation of pigments in modified reaction centers from *Rhodobacter sphaeroides* R26 by linear dichroism

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Abstract Site-specific pigment modifications are useful to investigate structure-function relationships in photosynthesis. In reaction centers bearing modified (bacterio)pheophytins, changed electron transfer kinetics have been related to the changed redox potentials of the pigments introduced (Huber, H. et al. (1995) Chemical Physics, Special Issue, vol 197 (Hochstrasser, R.M. and Hofacker, G.L. eds.) pp. 297–305; [1]). In order to analyze potentially interfering structural changes induced in these reaction centers by the exchange procedure, in particular mispositioning or misorientation of the pigments, low-temperature linear dichroism spectra have been measured for reaction centers from *Rhodobacter sphaeroides* containing modified bacteriopheophytins and bacteriochlorophylls at the sites $H_{A,B}$ and $B_{A,B}$, respectively. They show that all modified pigments are oriented similar to the native ones, and that they do not affect significantly the linear dichroism of the monomeric bacteriochlorophylls and bacteriopheophytins or of the primary donor.

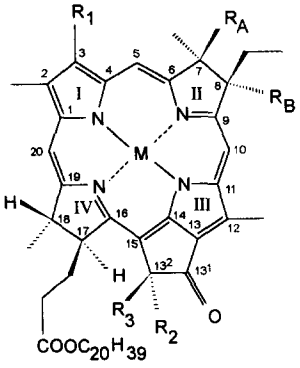
Key words: Pigment modification; Low-temperature spectroscopy; Reaction center; Asymmetry; Gel squeezing; Linear dichroism

1. Introduction

The native bacteriopheophytin *a* (BPhe) in reaction centers (RC) of *Rhodobacter sphaeroides* R26 can be exchanged with chemically modified bacterial- and plant-type pheophytins (Phe). Depending on the structure of the individual pigment exchanged, replacements of the native BPhe of $<50\%$ or $\geq 90\%$ have been observed [2]. These numbers correspond to an exchange of only one or both BPhes, respectively, and have been interpreted to reflect either selective exchange at one site only, probably H_B, or exchange at both sites, H_A and H_B [2,3]. Bacteriochlorophyll *a* (BChl) at the 'monomeric' sites B_A and B_B can likewise be replaced by modified bacteriochlorophylls [4]. In this case, site selectivity is less pronounced, but a selective exchange at site B_A is possible in RC containing a carotenoid, e.g. from wild-type *R. sphaeroides* 2.4.1, probably by some shielding of the nearby B_B site [5,6]. Spectroscopic investigations with such modified RC are useful to address questions concerning structure-function relationships in reaction centers [4]. An important conclusion from these studies was that the monomeric BChl *a* at site B_A is a real intermediate in the ultrafast electron transfer from P to H_A [7].

In all these studies, it has tacitly been assumed that the modified pigments are introduced into the binding site with the same orientation as the pigment original present and that the replacement or the treatment during exchange does not change the structure of the complex. These assumptions can be validated only by a combination of spectroscopic and structure-analytical tools. Low-temperature linear dichroism spectroscopy has been shown in the past to be a sensitive tool in this respect [8,9]. We here report linear dichroism spectra of RC in which BPhe *a* has been replaced with the related pigments [3-vinyl]BPhe *a*, [3-acetyl]Phe *a*, and Phe *a* [2], or the BChls at sites $B_{A,B}$ with modified ones, in order to modify their redox potentials and spectra [10].

The results show all modified pigments to be arranged in the RC in the same way as the native pigments and to have no



Pigment	R_A	R_B	M	R_1	R_2	R_3	Remarks
BPhe	H	H	H_2	$COCH_3$	$COOCH_3$	H	
BPhe'	H	H	H_2	$COCH_3$	H	$COOCH_3$	
[3-vinyl]-BPhe	H	H	H_2	$CHCH_2$	H	$COOCH_3$	
13^2-OH-BChl	H	H	Mg	$COCH_3$	$COOCH_3$	OH	
[3^1,13^2-OH]-BChl	H	H	Mg	OH	$COOCH_3$	OH	
[3-vinyl]-13^2-OH-BChl	H	H	Mg	$CHCH_2$	$COOCH_3$	OH	
[3-acetyl]-Phe	-	-	H_2	$COCH_3$	$COOCH_3$	H	a
Phe	-	-	H_2	$CHCH_2$	$COOCH_3$	H	a

a: 7,8 double-bond

Fig. 1. Structures of the modified pigments.

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Abbreviations: $B_{A,B}$, binding sites for monomeric BChls at the active and inactive branches, respectively; $B_{x,y}$, near-UV absorption bands polarized in the molecular *x*- and *y*-directions, respectively; BChl, bacteriochlorophyll; BPhe, bacteriopheophytin; Chl, Chlorophyll; $H_{A,B}$, binding sites for BPhes at the active and inactive branches, respectively; LD, linear dichroism; LDAO, *N,N*-dimethyldodecylamine *N*-oxide; Phe, pheophytin; P, primary donor; $Q_{X,Y}$, visible and near-infrared absorption bands polarized in the molecular *x*- and *y*-directions, respectively; RC, reaction center; TL buffer, 20 mM Tris, pH 8, containing 0.1% LDAO.

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detectable effect on the configuration of the tetrapyrrole pigments at the neighbouring binding sites.

2. Materials and methods

Modified pigments of *R. sphaeroides* and exchanged RC were prepared and analyzed as described previously [2,11]. The exchange procedures with the pigments used were repeated at least twice to ascertain high pigment replacements.

For the LD measurements, the RC were polymerized in an acrylamide/glycerol gel and then squeezed to a factor of 1.2–1.4 [8]. Absorption and LD spectra at low temperature (10 K) of these orientated RC were measured as described previously [9].

3. Results and discussion

3.1. Modified pigments

The (bacterio)pheophytins studied have modifications at rings I and II (Fig. 1). [3-Vinyl]BPhe *a* is a bacteriochlorin but differs from BPhe *a* by its C_3 substituent. [3-Acetyl]Phe *a* carries the native substituent at C-3 but is a chlorin (saturated ring II) rather than a bacteriochlorin (unsaturated ring II, Fig. 1). Phe *a* carries both changes: It is a chlorin and bears a [3-vinyl] group. For detailed information on the tested pigments and on the characterization of RC containing them at sites

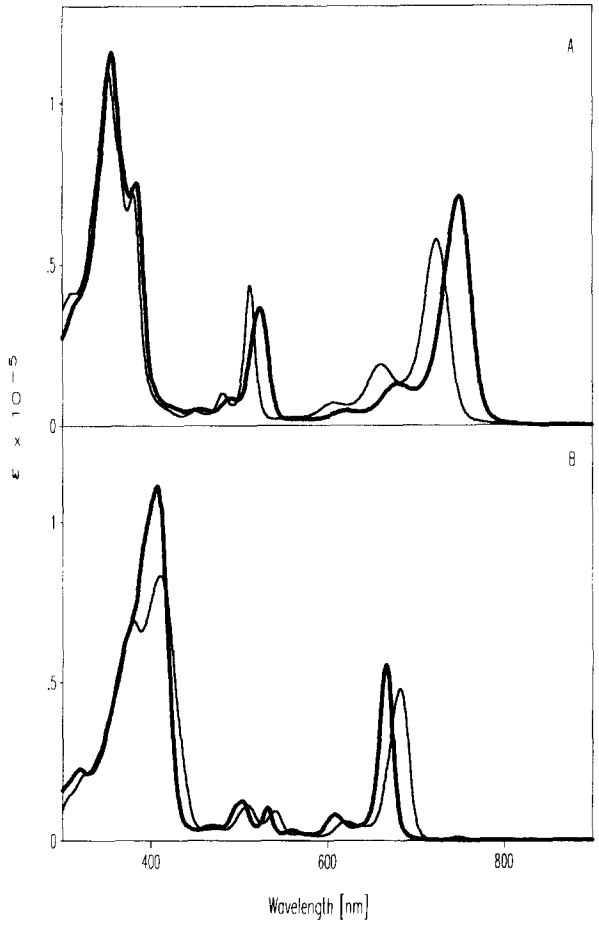

Fig. 2. (A) Absorption spectra of BPhe *a* (heavy line) and [3-vinyl]BPhe *a* (thin line) in ether solution. Molar extinction coefficients (ε) given in M^{-1} cm^{-1}. (B) Absorption spectra of Phe *a* (heavy line) and [3-acetyl]Phe *a* (thin line) in ether solution. Molar extinction coefficients (ε) given in M^{-1} cm^{-1}.

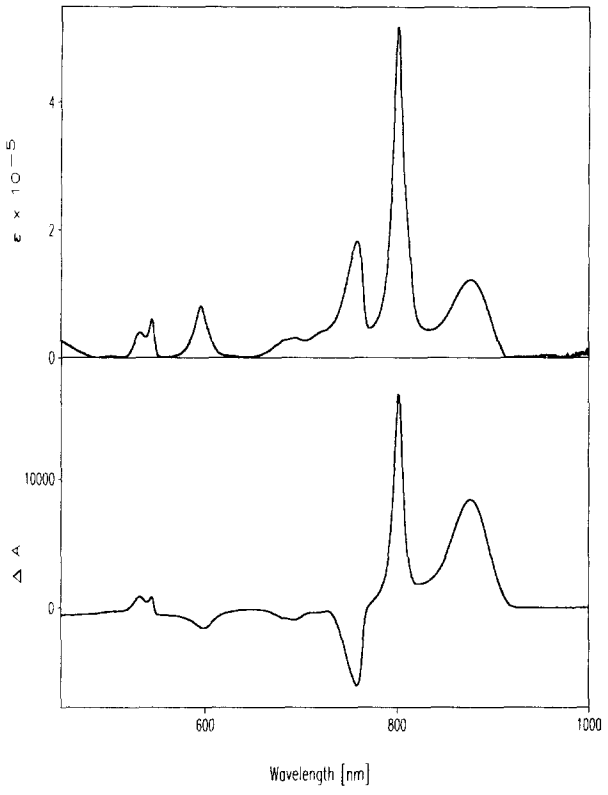

Fig. 3. Absorption (top) and LD spectrum (bottom) of native RC in TL buffer at 10 K. Molar extinction coefficients (ε) given in M^{-1} cm^{-1}.

$H_{A,B}$, readers are referred to [2,12]. These structural modifications lead to distinct spectral changes (Fig. 2) and to modifications in the redox potentials [10], by comparison with native BPhe *a*. Modifications at C-3 result in only moderate spectral shifts. There is a blue shift from 524 to 512 nm (Q_X) and from 750 to 724 nm (Q_Y) upon replacement of the [3-acetyl]- by a vinyl group in BPhe *a*. Oxidation of the bacteriochlorin to a chlorin at ring II gives rise to more pronounced spectral changes. There is a split Q_X band at 510 and 541 nm [13], a strongly blue-shifted Q_Y band (680 nm), and a red-shifted Soret-band system, if BPhe *a* is compared to the corresponding chlorin, viz. [3-acetyl]Phe *a*. The Q_X and Q_Y bands of all pigments are red shifted when they become bound to the $H_{A,B}$ sites, but the relative displacements of the respective bands among the four pigments are retained.

In the BChls investigated, changes have been introduced again at C-3 and also at C-13^2 located at the isocyclic ring. No chlorins can be introduced into the $B_{A,B}$ binding sites [4]. The spectral changes upon modifying C-3 are similar to those in the BPhe *a*; those related to the 13^2-OH substituents are negligible for the Q_Y and < 5 nm for the Q_X band [11].

The redox potentials of the modified pigments in solution show effects of both the C-3 substituent and the oxidation state at ring II, while the effect of the 13^2-OH group is negligible [10]. It is assumed that the relative ordering and redox potential differences determined in vitro are maintained when pigments are introduced into the RC.

3.2. Low-temperature absorption and LD spectra of the modified RC

The main difference between the room temperature spec-

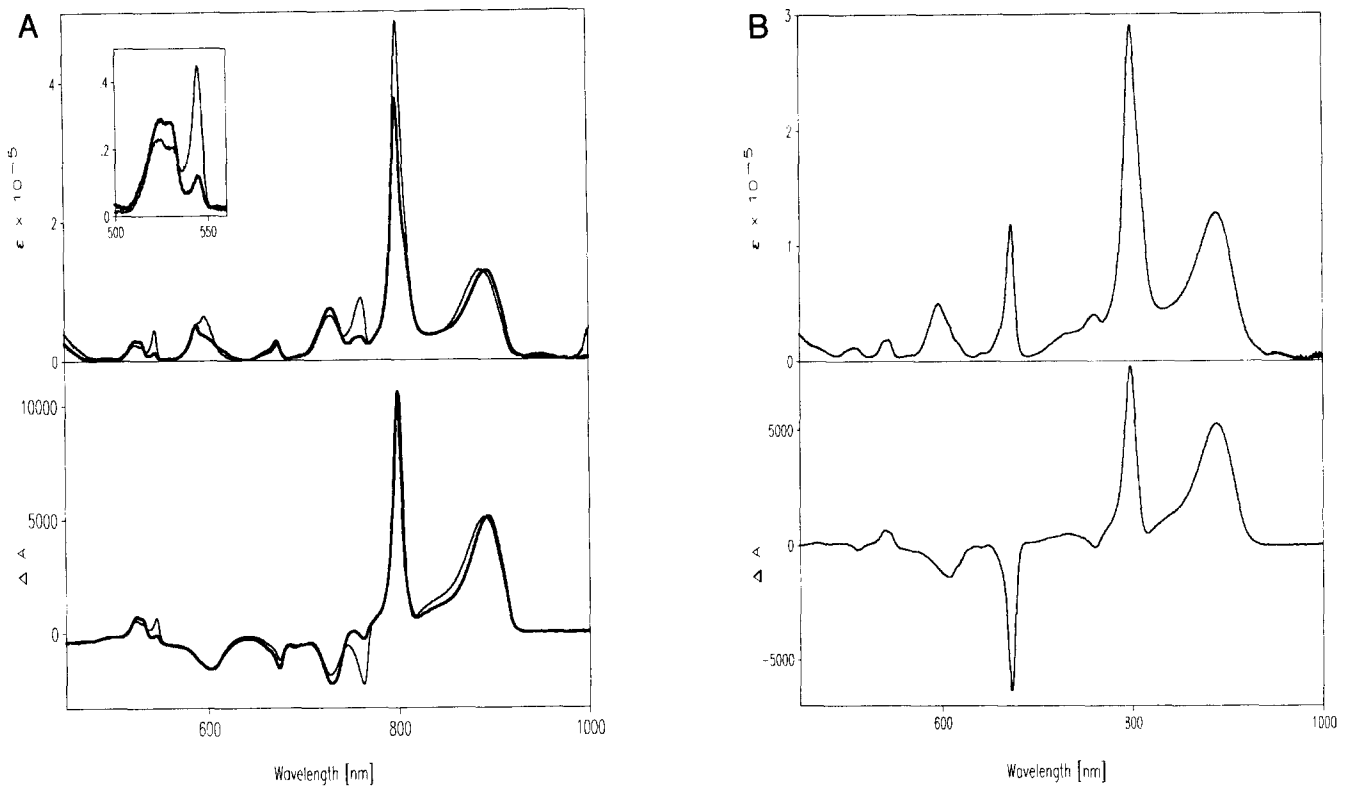

Fig. 4. (A) Absorption (top) and LD spectra (bottom) at 10 K of RC exchanged with [3-vinyl]BPhe *a* in TL buffer. The spectra show the RC exchanged once (thin line, 50% replacement) and twice (heavy line, 93% replacement). Molar extinction coefficients (ε) given in M^{-1} cm^{-1}. The inset shows only the Q_X region of the pigments at sites $H_{A,B}$. (B) Absorption (top) and LD spectra (bottom) at 10 K of RC in which BPhe *a* is exchanged to 95% with Phe *a* in TL buffer at 10 K. Molar extinction coefficients (ε) given in M^{-1} cm^{-1}.

trum and the absorption spectrum at 10 K of native RC is the splitting of the Q_X band of the BPhes at sites $H_{A,B}$ (Fig. 3). The short-wavelength band at 533 nm belongs to BPhe *a* located in the inactive branch at site H_B, whereas the red-shifted band at 545 nm belongs to H_A [14]. These splitting and shifts are also present in the LD spectrum with both bands giving a positive signal. The Q_X bands of the four BChl pigments contribute an unresolved LD signal with net negative sign. In the Q_Y region, the signs of the LD of the BChls ($B_{A,B}$, P) are positive, while that of the single Q_Y band of the BPhes is negative.

The split Q_X band of the (B)Phes can be used to follow the pigment exchanges at the two BPhe sites. Multiple exchanges have been made with all pigments to ensure maximum replacements. In all cases the exchange with modified pigments occurs stepwise, i.e. it is more rapid at the inactive site H_B and then the active BPhe *a* will be replaced by the modified pigment. As an example, two subsequent steps for the reaction with [3-vinyl]BPhe *a* are shown in Fig. 4A. After the first exchange, where the pigment replacement is about 50%, the short-wavelength band at 533 nm is decreased by 40% and replaced by a band at ≈525 nm, but the band at 544 nm is still present. This observation leads to the conclusion that, at this exchange level, only the BPhe at the inactive branch was replaced by the modified pigment and native BPhe *a* remains at the active site. Judging from the sign of the signal, the orientation of the *x*- and *y*-axes of the modified pigment is identical to that of the native BPhe *a*. Both Q_X bands have LD of the same positive sign, supporting a similar orientation of BPhe *a* and [3-vinyl]BPhe *a* at H_B. After the second exchange, the H_A-BPhe is also replaced by [3-vinyl]BPhe *a* (exchange rate >90%), which causes an increase of the band at 525 nm and a decrease at 544 nm. The positive LD of this band is again maintained. Likewise the Q_Y band at 758 nm (BPhe) is now almost completely replaced by a band blue-shifted to 729 nm ([3-vinyl]BPhe *a*) (Table 1). The Q_Y band shifting to 729 nm always exhibits a negative LD. This is evidence that the native and the modified pigments of both binding sites have similar orientations with respect to the membrane plane.

Only the spectra of maximally exchanged RC are shown for the two chlorins. In the case of Phe *a*, there are two Q_X bands around 507 and 541 nm (Fig. 4B), consistent with the absorption spectrum of the pigment in ether solution (Fig. 2B). The signs of the LD bands are the same as for BPhe *a* (Table 1), showing that the chlorins, too, have the same orientation of the *x*- and *y*-axes in the RC as do the native BPhes at the sites H_A and H_B. Similar changes were seen in RC modified with [3-acetyl]Phe *a* (Table 1, spectra not shown). The Q_Y bands of the monomeric BChls and of the special pair in the absorption and LD spectra and the signs of their LD bands in no case showed a visible effect of the modified BPhes, introduced into the RC, on the BChls.

Basically similar results were obtained upon replacement of the native BChl *a* at sites $B_{A,B}$ (Table 1). Here, both pigments are exchangeable in all cases studied. Again, the LD of the newly introduced pigments are the same as those of the native ones, and no change is seen of the LD of the pigments at the other binding sites (P, $H_{A,B}$).

It should be noted that LD can only distinguish pigment

Table 1
Absorption maxima and LD extrema of native and modified reaction centers

Pigment used for exchange	Pigment (site)	Absorption λ_{max} (nm)	LD λ_{max} (nm) (sign)
Native RC	BChl (P)	877	878 (+)
	BChl ($B_{A,B}$)	802	801 (+)
	BPhe ($H_{A,B}$)	759	759 (−)
[3-Vinyl]BPhe *a*	BChl (P)	890	891 (+)
	BChl ($B_{A,B}$)	801	800 (+)
	BPhe[a] ($H_{A,B}$)	762	763 (−)
	[3-vinyl]BPhe ($H_{A,B}$)	729	729 (−)
[3-Acetyl]Phe *a*	BChl (P)	891	891 (+)
	BChl ($B_{A,B}$)	801	800 (+)
	BPhe[a] ($H_{A,B}$)	760	762 (−)
	[3-acetyl]Phe ($H_{A,B}$)	690	691 (−)
Phe *a*	BChl (P)	894	895 (+)
	BChl ($B_{A,B}$)	798	798 (+)
	BPhe[a] ($H_{A,B}$)	762	762 (−)
	Phe ($H_{A,B}$)	673	674 (−)
13^2-OH-BChl *a*	BChl (P)	886	888 (+)
	(13^2-OH)-BChl ($B_{A,B}$)	803	803 (+)
	BPhe ($H_{A,B}$)	760	760 (−)
[3^1,13^2-OH]BChl *a*	BChl (P)	888	888 (+)
	BChl[a] ($B_{A,B}$)	801	801 (+)
	[3^1,13^2-OH]BChl ($B_{A,B}$)	760	760 (−)
	BPhe a ($H_{A,B}$)	760	760 (−)
[3-Vinyl]13^2-OH-BChl *a*	BChl (P)	885	883 (+)
	BChl[a] ($B_{A,B}$)	800	800 (+)
	[3-vinyl]13^2-OH-BChl ($B_{A,B}$)	771	773 (+)
	BPhe ($H_{A,B}$)	760	760 (−)

[a]Residual native pigment, occupation < 5%.

orientations in which the *x*- or *y*-axis have been rotated from their original positions by specific ranges of angles [15]. In particular, it would not be possible to notice rotations around one or both of the axes by values close to 180°. Such a situation may be envisioned with the more symmetric porphyrins, where furthermore the *x*- and *y*-polarized transitions are generally only difficult to distinguish. It is however, very improbable with the (bacterio)chlorins, which show a highly asymmetric substitution pattern, and allow furthermore a ready distinction of the two axes of orientations by the well separated Q_X and Q_Y bands. This distinction is principally also possible in the B bands, but more difficult due to extensive overlap of the six pigments present in RC.

In conclusion, all modified pigments investigated, show similar orientations to those of the native ones in bacterial reaction centers. The investigations of finer details in the orientation needs, if necessary, more sensitive methods like X-ray analyses, which are in progress.

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