LINEAR DICHROISM OF LIGHT-INDUCED ABSORBANCE CHANGES OF REACTION CENTERS OF *RHODOSPIRILLUM RUBRUM*

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1. Introduction

Reaction centers, isolated from chromatophores of *Rhodopseudomonas sphaeroides,* contain three protein subunits, four molecules of bacteriochlorophyll (BChl), two molecules of bacteriopheophytin (Bph), one or two molecules of ubiquinone and one atom of iron [1–3]. Absorption and circular dichroism (CD) spectra of reaction centers, isolated from various photosynthetic bacteria, are similar [1,4]. In reaction centers from *Rps. sphaeroides* [2,5], *Rhodospirillum rubrum* [6] and *Chromatium vinosum* [7] the molecules of BChl *a* have absorption bands at 870 nm or 890 nm (band 1), at 800 nm (band 2) and 600 nm (band 3), while the molecules of Bph *a* have absorption bands at 535–545 nm and 760 nm. In reaction centers from *Rhodopseudomonas viridis* the molecules of BChl *b* absorb light at 960 nm (band 1), 830 nm (band 2) and 600 nm (band 3), while the molecules of Bph *b* absorb light at 545 nm and 790 nm [8,9].

Upon illumination, the oxidation of the reaction centers is accompanied by bleaching of BChl bands 1 and 3. BChl absorbing in band 1 is denoted as a primary electron donor, P. If the 'primary' electron acceptor, the complex of ubiquinone and Fe [10], is in the reduced form, a light-flash induces in the reaction center the formation of a pigment anion–cation biradical, P^+Bph^-, which has a lifetime of 6–10 ns [11–14]. Oxidized ubiquinone accepts an electron from the biradical during ~200 ps with formation of P^+ [13]. Ferrocytochrome is oxidized by the biradical with formation of Bph^- and ferricytochrome [14–16].

BChl band 2 splits into two bands with opposite signs in CD-spectra of reaction centers isolated from various bacteria [4,17]. In reaction centers of *Rps. viridis* the formation of P^+ is accompanied by the disappearance of negative CD-band at 847 nm [4,9], while the formation of Bph^- results in the disappearance of positive CD-band at 827 nm [9]. As a direct interaction between P and Bph is not revealed from difference absorption and CD spectra [4,9,17], the exciton interaction of all the pigment molecules in the reaction center has been assumed to occur via a BChl dimer, absorbing in band 2, which is intercalated between P and Bph [9].

To estimate the arrangement of pigment molecules in reaction centers, the relative orientation of vectors of Q_x and Q_y transition electric-dipole moments of pigment molecules should be known. With this aim the linear dichroism of light-induced absorbance changes under photooxidation of BChl-870 has been studied in reaction centers from *Rhs. rubrum* at 77°K, when the molecule rotation is considerably decreased. Preliminary results of these experiments have been described earlier [9].

2. Materials and methods

Reaction centers were isolated from chromatophores of *Rhs. rubrum* by the method, described by Noël et al. [6], using dodecyldimethylamino-oxide for solubilization. Concentrated solution of reaction centers was diluted by 80% glycerol and frozen at 77°K.

Difference (light minus dark) absorption spectra

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were measured using the set-up, described earlier [14]. To polarize the actinic and the measuring light, Roshon and Frank-Ritter prisms were used. Linear dichroism (LD) of absorbance changes was measured as:

$$\frac{|\Delta A_{\parallel}| - |\Delta A_{\perp}|}{|\Delta A_{\parallel}| + |\Delta A_{\perp}|}$$

where indexes $\parallel$ and $\perp$ indicate the measurement of absorbance changes by light, polarized in parallel and perpendicularly to the actinic light, respectively. The angle between the actinic and the measuring light directed at the object was 90°.

3. Results

Figure 1 shows that photooxidation of BChl-870 at 77°K is accompanied by bleaching of the BChl bands at 600 and 890 nm, a blue-shift of the BChl band at 802 nm and a red-shift of the Bph band at 750 nm. The action of light at 890 nm, polarized in parallel and perpendicularly to the measuring light, induces linear dichroism of absorbance changes (fig.2). The values of LD for the difference spectrum bands bleached at 890 nm, 802 nm, 740 nm and 600 nm are +0.22, +0.12, –0.32 and –0.1, respectively (fig.1). In similar experiments with Polaroid filters, lower LD-values for these bands have been found [9]. The LD-value of 0.25 for the 890 nm band has been reported by Mar and Gingras [18]. The LD-value for the absorbance increase at 790 nm is +0.18. In the region of the absorbance decrease from 800–850 nm, changes of the LD-value, minimal at 812 nm (+0.08) and maximal at 830 nm (+0.31), are observed (fig.1).

In the region from 802–799 nm a positive LD-value is changed to a negative one, while the transition of the absorbance decrease to the absorbance increase near the isobestic point at 797 nm is accompanied by the transition of a negative LD-value to a positive one (fig.1).

The sharp changes of LD-value in the region of 790–850 nm are explained well enough by the presence of an additional red-shift of a slight band at 810 nm, the transition dipole moment of which is approx. perpendicular to that of the band at 890 nm. This agrees with results reported by Penna et al. for *Rps. sphaeroides* reaction centers [19]. Figure 1A shows the resolution of the difference absorption spectrum of *Rhs. rubrum* reaction centers on a number of components: bleaching of the BChl band at 600 nm (LD –0.1), a red-shift of the Bph band at 750 nm (LD –0.11), a blue-shift of the BChl band at 800 nm (LD +0.16), a red-shift of the BChl band at 810 nm (LD –0.11), bleaching of the BChl band at 890 nm (LD +0.22) and the development of a broad band in

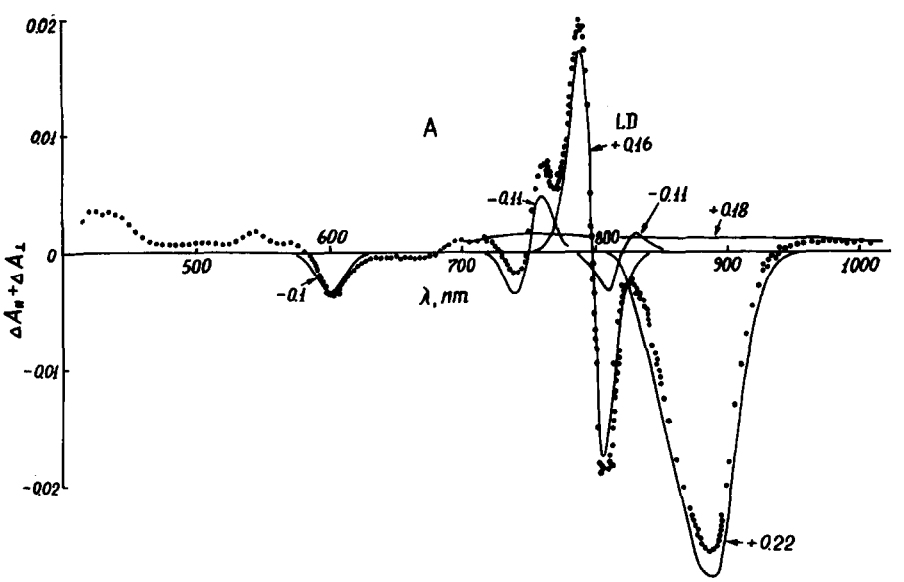


Fig.1A

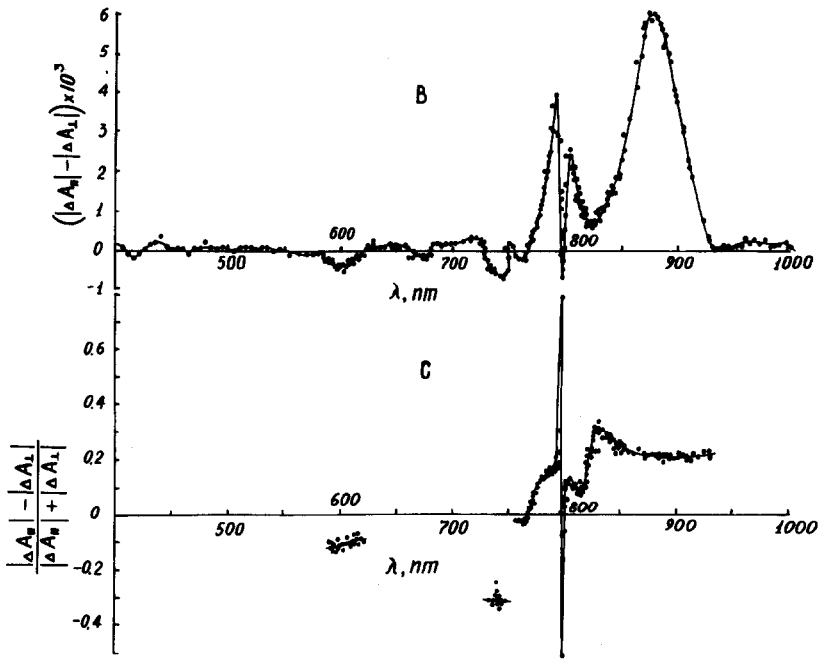


Fig.1 B and C

Fig.1. (A) (•) Spectrum of absorbance changes ($\Delta A_{\parallel} + \Delta A_{\perp}$) of *Rhs. rubrum* reaction centers at 77°K, induced by the actinic light at 890 nm, which was polarized in parallel ($\Delta A_{\parallel}$) or perpendicularly ($\Delta A_{\perp}$) to the measuring light. Absorbance at 890 nm of the sample was 0.15. The actinic light induced the oxidation of about 15% of reaction centers in the sample. Solid curves indicate the components of the difference absorption spectrum resolution deduced from an analysis of absorption, difference-absorption and LD-spectra of reaction centers. (B) Spectrum of absorbance changes ($|\Delta A_{\parallel}| - |\Delta A_{\perp}|$), induced by the actinic light at 890 nm, and (C) spectrum of LD = ($|\Delta A_{\parallel}| - |\Delta A_{\perp}|$)/($|\Delta A_{\parallel}| + |\Delta A_{\perp}|$) of *Rhs. rubrum* reaction centers at 77°K.

the region of 700–1000 nm (LD +0.18). This resolution corresponds to the spectrum of the LD-values.

Upon illumination at 800 nm the changes of the LD-sign near the isobestic point of the difference spectrum at 797 nm are similar to that described in the case of the action of light at 890 nm, while upon illumination at 745 nm (Q_y transition-moment for Bph) and at 600 nm (Q_x transition-moment for BChl) the absorbance decrease has a positive LD-value and the absorbance increase has a negative one near the isobestic point at 797 nm (fig.2). The action of light at 890 nm and 600 nm induces the absorbance decrease at 740 nm (Q_y transition-moment of Bph) with the negative (–0.3) and positive (+0.2) LD-value, respectively.

4. Discussion

Figure 1 shows that the transition dipole vectors of the bands at 800 and 810 nm are approximately parallel and perpendicular to the dipole vector of the transition at 890 nm, respectively. It is possible that the local electric field of P^+ shifts the bands at 800 nm and 810 nm to the blue and to the red, respectively. The similarity in the half-width of the bands at 800 nm and 810 nm can indicate splitting of dimer absorption band into two allowed exciton transitions at 800 nm and 810 nm, rather than at 810 nm and 870 nm, as it has been assumed by Vermeglio and Clayton for *Rps. sphaeroides* reaction centers [20]. The bands at 800 nm and 810 nm are observed in absorption [5,20] and linear dichroism [19] spectra of *Rps. sphaeroides* reaction centers at 35–77°K and correspond to the bands at 795 nm and 810 nm, respectively, in CD-spectra of reaction centers of *Rhs. rubrum* and *Rps. sphaeroides* [4].

To apply the exciton theory to dimers [21] we have used the dipole strengths of 66 debye^2, 60 debye^2.

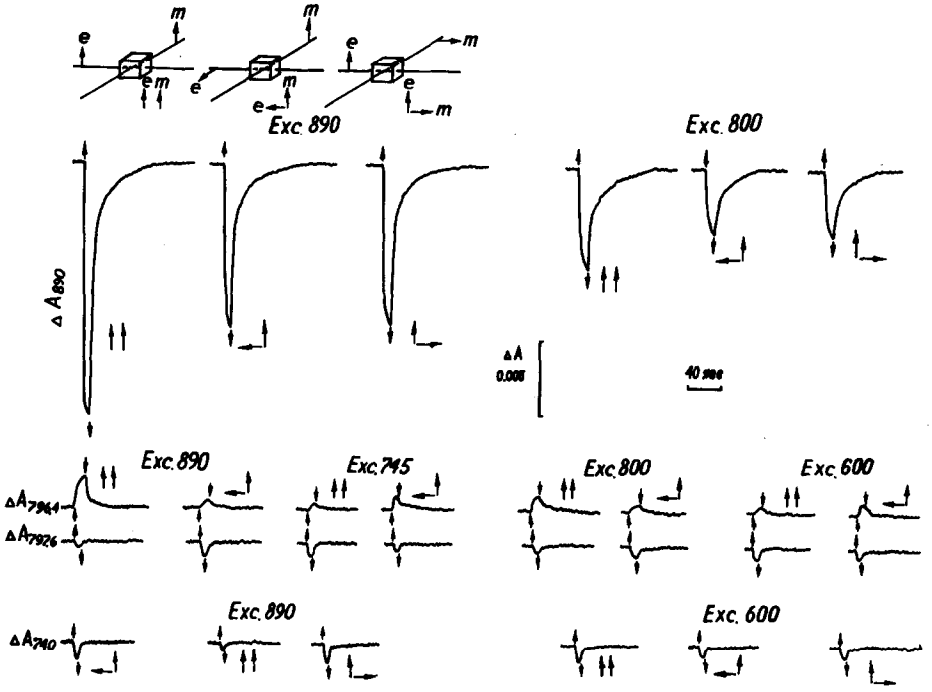
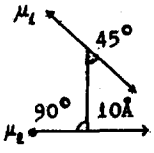
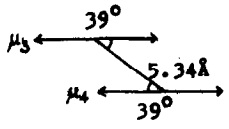


Fig.2. Kinetics of absorbance changes (ΔA) of *Rhs. rubrum* reaction centers, induced by the exciting (e) light, which was polarized in parallel (↑ ↑) or perpendicularly (← ↑) to the measuring (m) light, 77°K.

Table 1
The properties of BChl dimers of *Rhs. rubrum* reaction centers found from the experiments and from application of the exciton theory to dimers [21]

Dimer	Exciton transition bands (nm)		Absorption band of monomer in dimer (nm)	Ratio of extinction coefficients (ϵ)	Energy of splitting (cm^{-1})	Mutual orientation of Q_y transition dipole vectors (μ) of monomers in dimer
	Allowed	Forbidden				
BChl-800	800 810		805[a]	$\frac{\epsilon_{800}}{\epsilon_{810}} \simeq 5$	+77	
BChl-870	870 (20°C)	765	815[b]	$\frac{\epsilon_{870}}{\epsilon_{765}} > 100$	−780	

[a] Environmental-shift [22] of 480 cm^{-1}
[b] Environmental-shift of 620 cm^{-1}

26 debye^2 and 12 debye^2 for the transitions at 870 nm, 800 nm, 760 nm and 810 nm, respectively, in reaction centers. The energy of the exciton splitting in the BChl-800 dimer is about +77 cm^{-1} and the ratio of $\epsilon_{800}/\epsilon_{810} \simeq 5$ corresponds to an angle of 45° between the dipole-vectors of monomers. If the dipole strength of the Q_y (0.0) vibronic component of BChl-monomer is 36 debye^2, the distance between the centers of the monomers is 10–11 Å (table 1). This distance allows a coplanar orientation of the macrocycle-planes of the monomers with a slight rotational strength of the dimer exciton transitions.

The band at 870 nm seems to come from the absorption of a BChl-870 dimer, which is formed by two acetyl C=O. . .H–O- - -Mg interactions [22]. In this structure if the distance between the molecule centers is about 5.34 Å and the separation between the macrocycle planes is about 3.4 Å, the exciton splitting energy is −780 cm^{-1}. In this case there is an allowed exciton-transition at 870 nm and a forbidden one at 765 nm with almost no rotational strength for both the transitions (table 1).

The negative polarization of the 750 nm band which shifts to the red, shows that the Q_y transition vector of photoactive, monomeric [14,23] Bph is approximately perpendicular to that of BChl-870. This agrees with results reported for reaction centers of *Rhs. rubrum* and *Rps. sphaeroides* [9,19,20]. The data of fig.2 show that the Q_x transition dipole-vectors of BChl molecules are approx. parallel to the Q_y vector of Bph and to the dipole-vector of the transition at 810 nm; the latter two vectors are approximately parallel. It is important that the Q_x transition dipole-vector of Bph (545 nm) is approximately parallel to the Q_y vector of the transition at 870 nm [19]. Thus, the macrocycle-planes of all the pigment molecules in the reaction center are approximately parallel, and the macrocycle planes of BChl-800 molecules appear to be approximately coplanar.

Probably, weak exciton-interaction between the nonplanar and nonparallel dipole-vectors [21] of the transitions at 870 nm and 810 nm gives two approximately equal CD-bands [4,17]: a positive band at 870 nm and a negative one at 810 nm, since in the near-infrared only these CD-bands disappear under oxidation of BChl-870 [4,17]. On the other hand, weak exciton-interaction between the nonplanar and nonparallel dipole-vectors of the transitions at 800 nm and 760 nm can give two CD-bands approximately equal at 77°K [17]: a positive band at 795 nm and a negative one at 760 nm, since only similar CD-bands disappear under photoreduction of Bph in *Rps. viridis* reaction centers [9]. In *Rps. sphaeroides* reaction centers the rotational strengths of ~3 debye magneton for the CD-band at 810 nm and of ~2.5 debye magneton for the CD-band at 800 nm [17] can indicate [21] that the distances of the macrocycle-planes of BChl-870 and Bph molecules from the plane of BChl-800 dimer which is probably intercalated between P and Bph [9], are about 5 ± 1.7 Å and 3.5 Å, respectively. (The distances between the centers of BChl-870, BChl-800 and Bph are not estimated.) Lower rotational strengths found for corresponding CD-bands of *Rhs. rubrum* reaction centers [4] appear to reflect shorter distances between the macrocycle-planes of the pigment molecules in these reaction centers.

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