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PHOTOOXIDATION OF *P*-960 AND PHOTOREDUCTION OF *P*-800 (BACTERIOPHEOPHYTIN *b*-800) IN REACTION CENTERS FROM *RHODOPSEUDOMONAS VIRIDIS*

EXCITON INTERACTION BETWEEN THE PIGMENT MOLECULES

V.A. SHUVALOV, I.N. KRAKHMALEVA and V.V. KLIMOV

Institute of Photosynthesis, U.S.S.R. Academy of Sciences, Pushchino, Moscow Region (U.S.S.R.)

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Summary

The light excitation of *P*-960 results in the oxidation of *P*-960 and the reduction of *P*-800 (bacteriophytin *b*-800) in the reaction centers from *Rhodopseudomonas viridis*. A negative 847 nm band of the circular dichroism spectrum disappears under *P*-960 photooxidation, while a positive 827 nm band disappears under *P*-800 photoreduction. Exciton interaction of the pigment molecules in the reaction center is discussed.

Photochemical activity of bacteriochlorophyll and bacteriopheophytin has been demonstrated in the model systems [1, 2]. In *Rhodopseudomonas spheroides* reaction centers, when the "primary" electron acceptor X (ubiquinone [3]) is in the reduced form, the state P^F with a lifetime of 10 ns is observed [4, 5]. This state is probably a pigment anion-cation biradical of the reaction center [6], in which the cation is *P*-870$^+$ [7]. Oxidized X accepts an electron from the pigment biradical with the formation of *P*-870$^+$ in approx 200 ps [6]. In *Chromatium minutissimum*, when X is in the reduced form (E_h = −200 to 620 mV), the pigment biradical accepts an electron from ferrocytochrome with the formation of an anionic radical (EPR signal with g = 2.0025 ± 0.0005 and $\Delta H \simeq$ 12.5 G) of the pigment complex *P*-760, which involves bacteriopheophytin *a*-760 (Bph *a*-760) and bacteriochlorophyll *a*-800 (BChl *a*-800) [8]. A similar radical anion in the same conditions has later been demonstrated in *Chr. vinosum* [9]. In chromatophores of *Rps. viridis* the photoreduction of bacteriopheophytin *b*-800 is observed and accompanied by the increase in the quantum yield of the antenna bacteriochlorophyll *b* fluorescence [10].

The present work demonstrates that the photoreduction of a pigment complex *P*-800, involving bacteriopheophytin *b*-800 and bacteriochlorophyll *b*-830, is observed in the reaction center preparation from *Rps. viridis*, in which the pigment biradical can extract an electron from cytochrome. A pigment interaction in the reaction centers has been studied by the measurements of the difference absorption spectra, the spectra of circular dichroism (CD) and of linear dichroism of difference absorption spectra.

Reaction centers were isolated from *Rps. viridis* with use of sodium dodecyl sulfate for solubilization and of hydroxyapatite chromatography, as described by Thornber et al. [11]. For study, concentrated solutions of reaction centers were diluted with 50 mM Tris (pH 8.0). Reaction centers from *Rhodospirillum rubrum* were isolated as described by Nöel et al. [12]. Difference absorption spectra (light minus dark) in natural or polarized light were measured with a phosphoroscopic set-up described earlier [8]. CD spectra were measured with a set-up consisting of monochromator, polarizer, Pockel's cell, phosphoroscope, photomultiplier and an amplification and recording system. All measurements were made at room temperature.

Fig. 1 shows the absorption spectrum and difference spectra (light minus dark) of the reaction centers. At a redox potential of +250 mV the difference spectrum includes the bands of the photooxidation of cytochrome and *P*-960. At redox potential of −450 mV one can see the reversible photoreduction of the pigment complex *P*-800, accompanied by bleaching in bacteriopheophytin *b* bands at 540 and 800 nm and a blue shift in a bacteriochlorophyll *b* band at 830 nm. The development of a broad band at 680 nm indicates the formation

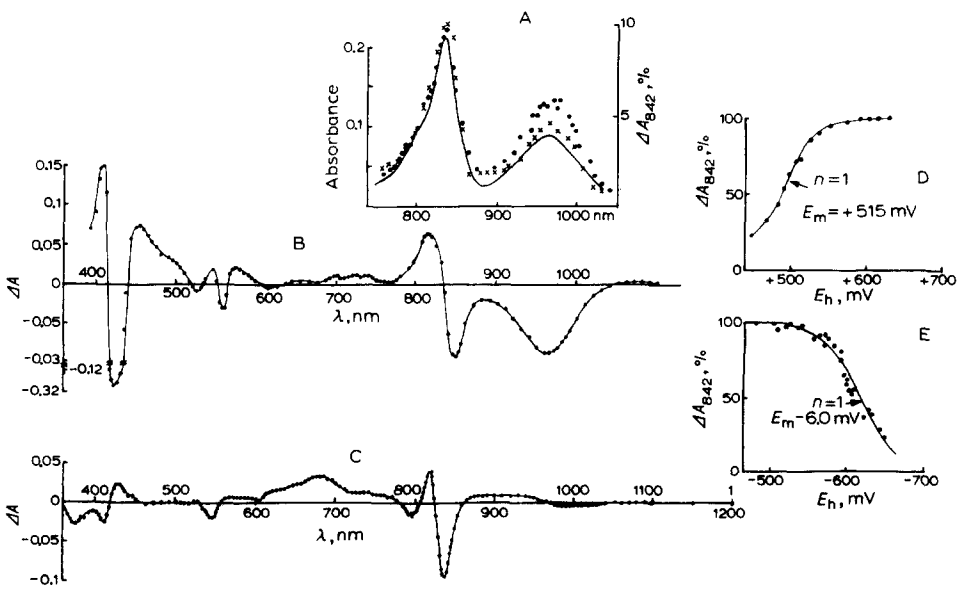

Fig. 1. (A) Absorption spectrum of the reaction centers prepared from *Rps. viridis* (—), action spectrum of *P*-960 photooxidation at E_h = +250 mV (•) and action spectrum of *P*-800 photoreduction at E_h = −450 mV (×) as measured by ΔA_{842} (actinic monochromatic light with spectral slit of 8 nm). (B) and (C), Light-minus-dark difference spectra of reaction centers at E_h = +250 mV (B) and at E_h = −450 mV (C) (actinic light with λ > 700 nm and intensity of 10^5 ergs·cm^{-2} s^{-1}). (D) and (E), The redox potential titration of *P*-960 photooxidation (D) and *P*-800 photoreduction (E), as measured by ΔA_{842} in chromatophores of *Rps. viridis* (data from ref. 10).

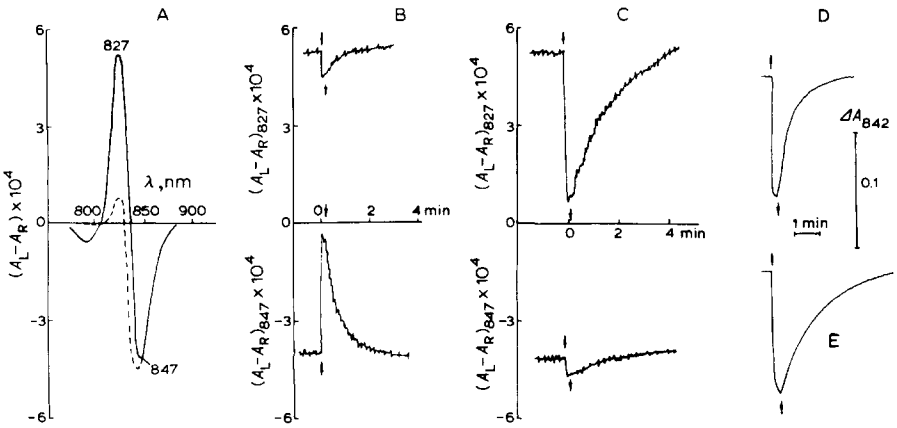

Fig. 2. (A) The CD spectra of the reaction centers, as measured without (——) or with (- - - -) continuous actinic illumination at E_h = −450 mV. (B) and (C), the changes of CD as spectrum bands at 827 (+) and 847 (−) nm under *P*-960 photooxidation at E_h = +250 mV in the presence of ubiquinone Q_2 (10^{-5} M) (B) and under *P*-800 photoreduction at E_h = −450 mV (C). Switching on (↑) and switching off (↓) actinic light, with λ > 700 nm and intensity of 10^5 ergs·cm^{-2} s^{-1}. (D) and (E) Kinetics of absorbance changes at 842 nm at E_h = +250 mV in the presence of ubiquinone Q_2 (10^{-5} M) (D) and at E_h = −450 mV (E).

of radical anion of bacteriopheophytin *b* by analogy with the data of bacteriopheophytin *a* photoreduction in *Chr. minutissimum* [8]. Midpoint potentials (E_m) of *P*-960 and *P*-800, as measured in chromatophores of *Rps. viridis* [10], are +515 and −620 mV, respectively (Fig. 1). Photooxidation of *P*-960 and photoreduction of *P*-800 are sensitized by light, absorbed by all the pigments of the reaction center (Fig. 1).

The CD spectrum of the reaction centers from *Rps. viridis* in the region of 770–890 nm is characterized by a negative band near the bacteriopheophytin *b* absorption band at 790 nm and by two bands of opposite sign in the region of the bacteriochlorophyll *b* absorption band at 830 nm (Fig. 2). Photooxidation of *P*-960 results in a decrease in the negative band at 847 nm with slight changes of the positive band at 827 nm (Fig. 2) and the negative band at 790 nm, that corresponds to the changes of CD spectrum under the chemical oxidation of *P*-960 [12]. In contrast, photoreduction of *P*-800 is accompanied by a decrease in the positive band at 827 nm and the negative band at 790 nm with slight changes of the 847 nm band (Fig. 2). The kinetics of these changes of CD spectrum upon illumination correspond to those of the photoconversion of *P*-960 and *P*-800, respectively.

To understand the data on CD spectra of reaction centers, the relative orientation of the transition electric dipole moments of the pigment molecules should be known. One of us (V.A.S) and A. Asadov have studied the linear dichroism of the absorbance changes ($D = (\Delta A_{\parallel} - \Delta A_{\perp})/(\Delta A_{\parallel} + \Delta A_{\perp})$) in the reaction centers from *Rps. rubrum* at 77 K under the photooxidation of *P*-870, induced by the vertically polarized light absorbed by BChl *a*-870 at 870 nm. The magnitudes of D for ΔA_{870}, ΔA_{812} and ΔA_{745} have been found to be +0.06, +0.051 and −0.13, respectively, i.e. the vectors of the Q_y transition dipole moments of BChl *a*-800 and Bph *a*-760 are approximately parallel and perpendicular to that of BChl *a*-870, respectively. However, near the isosbestic point of the

difference spectrum at 807 nm (BChl *a*-800) the bleaching of the band is observed, the transition dipole moment of which is perpendicular to that of BChl *a*-870, but also parallel to the transition dipole moment of Bph *b*-800. (A linear dichroism study of Q_x transition moments will be described in detail in a later communication (Shuvalov, V.A., Asadov, A.A. and Krakhmaleva, I.N. (1976), in preparation).)

One can assume that light absorption at 870 nm arises in the bacteriochlorophyll *a* dimer with approximately colinear orientation of the transition electric dipole moments of monomers (considerable splitting with an allowed long wavelength component [14]), while absorption at 800 nm arises in the dimer with approximately coplanar and mutually perpendicular orientation of the transition electric dipole moments (slight splitting into two mutually perpendicular and allowed components with slight own rotational strength). If the reaction centers of *Rsp. rubrum* and *Rps. viridis* are built analogously (the absorption and CD spectra of these centers are similar [12]), then the exciton interaction of the uncoplanar and mutually perpendicular Q_y transition moments in complexes BChl *b*-830 · BChl *b*-960 and BChl *b*-830 · Bph *b*-800 can give the negative band at 847 nm and the positive band at 827 nm in CD spectrum, respectively (Fig. 3A). Oxidation of BChl *b*-960 or reduction of Bph *b*-800 result only in the disappearance of their respective bands in the CD spectrum. As BChl *b*-830 participates in the formation of both 827 and 847 nm bands in the CD spectrum, an exciton interaction of all the pigments of reaction centers is possibly mediated by BChl *b*-830. Direct interaction between BChl *b*-960 and Bph *b*-800 is not revealed (Figs. 1 and 2).

The similarity in blue shift of the 830 nm band in difference absorption spectra under the photooxidation of BChl *b*-960 and the photoreduction of Bph *b*-800 is probably related to an influence of local electric fields of $BChl^+$ *b*-960 and Bph^- *b*-800 on the spectrum of BChl *b*-830. In this case one of the transition electric dipole moments for light absorption at 830 nm must be located between BChl *b*-960 and Bph *b*-800 (Fig. 3A).

In Fig. 3B we represent E_m values of the ground and excited levels of the pigment molecules in the reaction center, using E_m values of –620 mV for

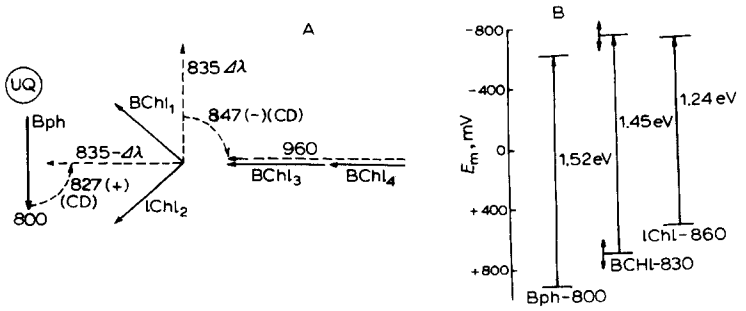

Fig. 3. (A) The schematic representation of the possible orientation of the Q_y transition electric dipole moments of the bacteriochlorophyll *b* and bacteriopheophytin *b* molecules in the reaction center of *Rps. viridis*. The transition dipole moments of $BChl_1$ and $BChl_2$ are approximately coplanar and mutually perpendicular, that of $BChl_3$ and $BChl_4$ are approximately colinear. The transition dipole moments for light absorption at 800, 830 and 960 nm are not coplanar. The digits indicate the correspondent bands of absorption and CD spectra. (B) Scheme of E_m values of the ground and excited levels of the pigments in the reaction center of *Rps. viridis*.

Bph *b*-800 and of +515 mV for BChl *b*-960. As BChl *b*-830 is a less oxidable pigment than BChl *b*-960, E_m for BChl *b*-830 appears to be in the region of 600—800 mV. Fig. 3B shows that the E_m of the excited levels of all the pigments lie in the same region as the redox potentials, forming the allowed zone for electron transfer between the pigment molecules which mutually interact by exciton mechanism, from BChl *b*-960 to Bph *b*-800. The recombination of separated charges is probably delayed by sterical separation of BChl *b*-960 and Bph *b*-800.

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