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Sub-picosecond dynamics of excited state of primary electron donor in reaction centers of *Rhodopseudomonas viridis* as revealed by hole burning at 1.7K broad and narrow holes

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Within the Q_Y band of the primary electron donor (P), the spectra of absorbance changes due to the formation of a state $P^+Q_A^-$ (Q_A is the primary quinone) at 1.7K in *Rhodopseudomonas viridis* reaction centers excited at 1014 nm have been shown to involve two spectral features characterized by: (i) a progression of broad (170–190 cm^{-1}) Gaussian vibronic bands (*S*-factor = 1.4) separated by 150 cm^{-1} and (ii) a 'narrow' structure near 1014 nm, characterized by 0–0 transition at 1014 nm with a width of ~ 50 cm^{-1} and 0–1 transition at 1000 nm with the width of ~ 100 cm^{-1}, and *S*-factor = 0.9. The width of 50 cm^{-1} can be related to either zero-phonon hole (ZPH) width or the structure involving phonon wings and ZPH being unresolved. Since dichroic value (~ 0.37) is unvarying over the P band, the vibrations involved are totally symmetric. The ZPH (width of ~ 3 cm^{-1}) and phonon wings (frequency of ~ 30 cm^{-1}) are resolved within the P band near 1014 nm when the spectrum of ΔA due to the formation of bacteriopheophytin$_L^-$ is measured at 1.7K.

Reaction center; Primary electron donor; Hole burning; *Rhodopseudomonas viridis*

1. INTRODUCTION

Primary processes of the energy conversion in bacterial photosynthesis take place in reaction centers (RCs) which comprise the primary electron donor P (a 'special pair' dimer of bacteriochlorophyll molecules) and electron acceptors such as bacteriochlorophyll monomers B, bacteriopheophytin molecules H, primary quinone Q_A and secondary quinone Q_B embedded into protein subunits L and M; these processes involve conversion of the excited state P* into the charge transfer (CT) states P^+P^-, $P^+B_L^-$, P^+Q^- and occur on (sub)picosecond time scale (see [3,4] for reviews and references).

The basic complementary approaches used to study the ultra fast energy conversion are (1) femtosecond spectroscopy, which reveals the transients in time domain; and (ii) low temperature selective absorption and fluorescence spectroscopies including hole-burning experiments which reveal the rates of relaxation of P* in frequency domain (see [5]). Measured widths of zero-phonon holes (ZPH) may give information about the rates of relaxation of P*. Direct measurements of femtosecond transients due to depopulation of P* at 8K yielded relaxation times 1.2 ± 0.1 ps for RCs of *Rhodobacter (Rb.) sphaeroides* and 0.7 ± 0.1 ps for RCs of *Rhodopseudomonas (R.) viridis* [6] which would correspond to ZPH widths of 9 ± 1 cm^{-1} and 15 ± 2 cm^{-1}, respectively.

Early hole burning studies of RCs from *R. viridis* and *Rb. sphaeroides* have shown broad holes burned at 4K within the Q_Y band of P, which are similar to the Q_Y absorption band itself, their widths would correspond to relaxation rates of tens of femtoseconds [7–9]. A narrow hole with a width which would correspond to a relaxation time of 1.4 ± 0.7 ps was burned within the P band of RCs from *Rb. sphaeroides* R-26 [10], however its relation with broad holes was not clear.

At low temperatures, the P band has a main peak at 1000 nm in RCs of *R. viridis* and at 900 nm in RCs of *R. sphaeroides*, accompanied in both cases by shoulders at longer wavelengths; in earlier studies these shoulders were assumed to reveal transitions to specific excited states which could possess a CT character [11–15]. However, the Stark effect measurements at 77K did not resolve any features in the P band which could have different dipole strengths [16,17].

Analysis of the fine structure of the long-wavelength absorption band of P has shown [1,2] that the shape of that band can be simulated by a progression of one vibronic mode with a frequency of 148 cm^{-1} and a

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Abbreviations: B_L and B_M, bacteriochlorophyll monomers located in L and M protein subunits, respectively; H_L and H_M, bacteriopheophytins in L and M subunits, respectively; Q_A and Q_B, primary and secondary quinones, respectively; Q_Y transition, long-wavelength transition of pigments; *S*-factor, Pekar-Huang-Rhys factor; ZPH, zero-phonon hole.

Pekar–Huang–Rhys factor $S = 1.2$ (for RCs of *R. viridis*) and also a contribution of a low-frequency phonon mode of 30–40 cm^{-1} with S equal to 0.87. Temperature dependence of the band shape has shown that the purely electronic 0–0 transition is located within the shoulder on the long-wavelength side of the P band at 1015 nm for RCs of *R. viridis* and at 910 nm for RCs of *Rb. sphaeroides* [2]. The same 150-cm^{-1} vibronic mode has been revealed in the shape of the spectral hole burned within the P band [1,14,15,18,19].

Here we report the results of burning studies of RCs from *R. viridis* obtained for reversible photochemical reactions and compare the ΔA spectra observed under selective excitation at 1014 nm and under illumination with red light.

2. MATERIALS AND METHODS

Reaction centers (RCs) were obtained from *Rhodopseudomonas viridis* by treatment of chromatophores with lauryldimethylamine oxide and DEAE-cellulose chromatography as described earlier [20]. RCs lost practically all the secondary quinone Q_B during the isolation procedure [21]. For low temperature measurements RCs suspended in 10 mM Tris-HCl (pH 8.0)/0.1% LDAO/150 mM NaCl, were diluted with 60% (v/v) glycerol. Four redox conditions were used for measurements. First, RCs were frozen in the presence of 1 mM ferricyanide. All haems were oxidized without the oxidation of P (Fig. 1). The illumination of RCs at 1.7K induced the formation of $P^+Q_A^-$ with subsequent recombination with time constant of 7–8 ms [9,18]. Second, without any redox additions, high potential haem c_{380} [21] was reduced completely, c_{310} partially. Third, in the presence of 0.4 mM ascorbate, both high potential haems were reduced. Fourth, in the presence of 50 mM ascorbate, two high potential and one low potential c_{20} haems [21] were reduced (Fig. 1).

Low temperature measurements were carried out in a home-made cryostat [1,2] with a sample placed in a 1.5-mm cuvette, cooled in the cryostat down to 95K overnight and immersed in liquid helium afterwards. The temperature was monitored by a carbon resistance thermometer above 4.2K and by the measurements of the vapour pressure below lambda point.

Low temperature absorbance and light-minus-dark difference absorbance spectra were measured using an MDR-23 grating monochromator (LOMO, Leningrad) as a polychromator after modification and OMA-2 optical multichannel analyzer (EG&G PARC, Princeton) as a detector. Spectral holes were burned with continuous light of a mercury lamp and the line at 1014 nm was isolated with band-pass filters. The line had a width of 8.3 cm^{-1} and the intensity of 6 mW · cm^{-2}. For the illumination with a red light the incandescent lamp light was passed through red filter ($\lambda \geq 700$ nm, $I = 2$ W · cm^{-2}). A phosphoroscope was used to avoid the scattered excitation light in the measurements of reversible absorbance changes (ΔA). The illumination and measurements were separated by a 0.3-ms dark period. The ΔA's with a relaxation time ≥ 1 ms were detected by this set up.

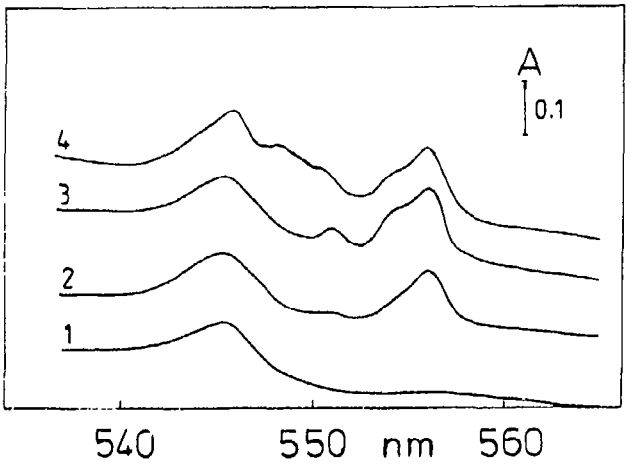

Fig. 1. Absorption spectra of *Rhodopseudomonas viridis* reaction centers (RCs) in the cytochrome c α-band region at 1.7K. 10 μM RCs suspended in 10 mM Tris-HCl (pH 8.0), 0.1% LDAO, 150 mM NaCl were diluted with 60% (v/v) glycerol and frozen with the following redox additions: (1) 1 mM potassium ferricyanide; (2) without any additions; (3) 0.4 mM sodium ascorbate; (4) 50 mM sodium ascorbate, 200 μM duroquinone, 18 μM ubiquinone-30, 100 μM phenazine ethosulphate ($E_h \approx 28$ mV).

3. RESULTS AND DISCUSSION

In RCs of *R. viridis* poised in the redox state with the electron transfer chain open from the primary donor P to the primary quinone acceptor Q_A, and all haems of the cytochrome oxidized, reversible ΔA's are observed which correspond to the formation of the state $P^+Q_A^-$ (Fig. 2). The spectrum of ΔA measured with the phosphoroscopic set up reflects the formation of $P^+Q_A^-$ recombining mostly in the ms time domain (lifetime of 7–8 ms [9,18]). In this case, excitation with the 1014-nm line at 1.7K leads to bleaching of a band whose structure is very similar to that of the initial absorption band (curve 1). However, comparison between spectra of ΔA induced by 1014-nm light and by red light (curve 2) reveals contributions of at least two components with different bandwidths. A broad component (we will call it component 1) dominates (accounts for about 95% of the integral intensity) in the both spectra, while a narrower component 2 can be discerned upon selective excitation of RCs.

Contribution of inhomogeneous broadening into the width of component 1 seems negligible because its shape is essentially the same under selective 1014 nm and red light excitation. This result is in remarkable contradiction with the simulations of spectral curves using inhomogeneous broadening of 180 cm^{-1} [2] and 120 cm^{-1} [19]. The structure of the bleaching band is very similar to that of the absorption band of P [2] and is presumably determined by vibronic and electron-phonon interactions. Since the dichroic value is unvarying over the band (Fig. 2, curve 3), the vibrations involved are totally symmetric. The 0–0 line of component 1 at 1015 nm may represent either a ZPH which might correspond to a very fast ($\sim$30 fs) dephasing process within P, or, more realistic, a phonon wing for a transition with a very strong electron–phonon coupling and the ZPH being unresolved (see [24]). The structure of component 1 is simulated by a progression of Gaussian vibronic bands separated by 150 cm^{-1} with FWHM (full width at the half maximum) of 170–190 cm^{-1} and $S = 1.4$ (not shown).

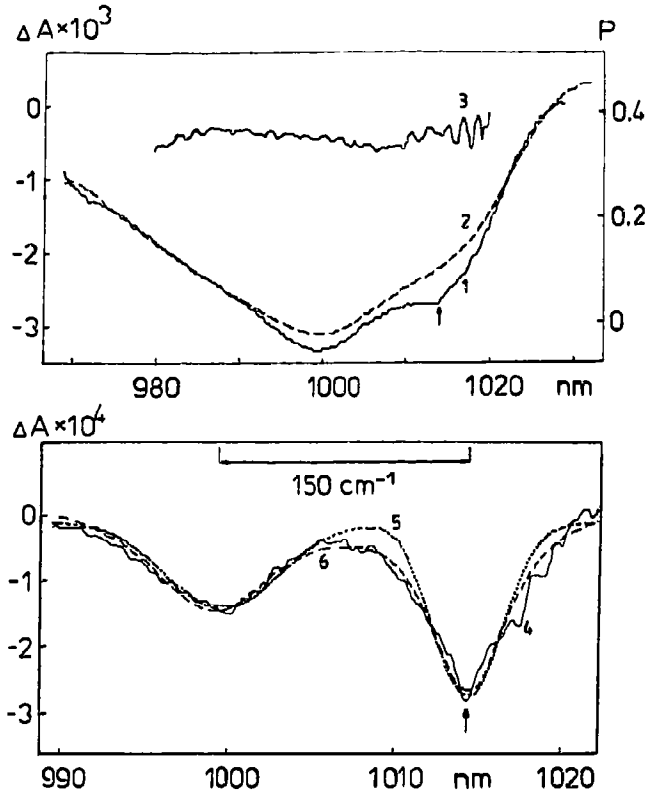

Fig. 2. Reversible absorbance changes (ΔA) measured with a phosphoroscopic set up for RCs of *R. viridis* ($A_{1000} = 0.35$) frozen in the presence of 1 mM ferricyanide at 1.7K and excited with the 1014-nm line of a mercury lamp (curve 1) and with red light (curve 2; normalized). The electron transfer chain open from P to Q_A, all haems of cytochrome oxidized. Curve 3 shows the dichroic value $P = (\Delta A\parallel - \Delta A\perp)/(\Delta A\parallel + \Delta A\perp)$ measured upon selective excitation with linearly polarized light at 1014 nm. $\Delta A\parallel$ and $\Delta A\perp$ are absorbance changes measured with linearly polarized measuring light whose electric vector was parallel ($\Delta A\parallel$) or perpendicular ($\Delta A\perp$) to that of the exciting light. Curve 4 shows the difference between the spectra presented by curves 1 and 2. Curve 5, a model sum of two Gaussian bands with FWHM = 47 cm^{-1} at 1014 nm and FWHM = 79 cm^{-1} at 1000 nm. Curve 6, a model sum of two Lorentian bands with FWHM = 57 cm^{-1} at 1014 nm and 95 cm^{-1} at 1000 nm ($S = 0.9$).

The two curves 1 and 2 in Fig. 2 are normalized in the regions where contribution of the latter component seems negligible. The difference between the two curves is shown enlarged in curve 4. It reveals the narrower component 2 whose shape is reasonably modelled by two Lorentian bands with FWHM of ~50 cm^{-1} at 1014 nm (0-0) and ~100 cm^{-1} at 1000 nm (0-1 vibronic transition) separated by ~150 cm^{-1} (Fig. 2).

If the band at 1014 nm of component 2 represents ZPH the value of 50 cm^{-1} for ZPH width corresponds to ~200 fs for relaxation of P*. This is shorter than the lifetime of P* measured in time domain, by a factor of 3.5. However, the band at 1014 nm might comprise narrower ZPH (15-20 cm^{-1}) and phonon and pseudo-phonon wings. Then the corresponding relaxation time would agree with the lifetime of P* (see [22]).

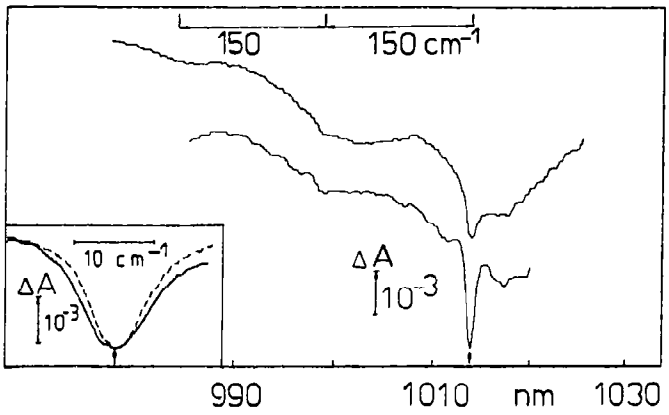

Fig. 3. Irreversible absorbance changes in RCs of *R. viridis* ($A_{1000} = 0.35$) with at least three haems of cytochrome and the primary quinone acceptor Q_A reduced in the dark in the presence of 50 mM sodium ascorbate and mediators indicated for curve 4 of Fig. 1. Excitation at 1014 nm at 1.7K leads to accumulation of radical-anion H_L^-. The lower curve is the spectrum measured after the first 5 min of illumination, the upper one is that measured after subsequent illumination. The inset shows the narrow bleaching band (solid curve) and the shape of the excitation line at 1014 nm (dashed curve) measured under the same optical conditions.

In RCs with two high-potential haems reduced in the dark (no additions or 0.4 mM ascorbate) selective excitation at 1014 nm leads to bleaching of a small narrow band, FWHM ~5 cm^{-1}, together with bleaching of broad bands (not shown, see [22]).

In RCs from *R. viridis* with the three haems of cytochrome and the primary quinone acceptor Q_A reduced in the dark, excitation at 1014 nm at 1.7K leads to irreversible accumulation of H_L^-. Spectra of irreversible ΔA reveal a blue shift of a broad band (which can belong to component 1) and 'narrow' structure which comprises bleaching of a narrow (FWHM ≈ 3 cm^{-1}) hole, probably ZPH, which is more clearly seen at the early stages of burning. ZPH is accompanied by small bleaching bands separated from the narrow hole by ~ +30 and ~ −30 cm^{-1} and probably representing phonon and pseudo-phonon wings, respectively, and a structureless feature separated from the narrow hole by +150 cm^{-1} and reflecting the 0-1 vibronic component (Fig. 3). The FWHM of ~3 cm^{-1} for ZPH corresponds to relaxation time of ~3 ps. This is longer than the lifetime of P* (~700 ps [6]) for RCs with oxidized Q_A. However this increase of the relaxation time may be related to the influence of Q_A^- on the lifetime of P* in agreement with fs measurements (compare [6] and [23]). Even narrower ZPH with FWHM of ≤1 cm^{-1} and essentially the same phonon structure was observed for RCs upon photoreduction of H_M when H_L was prereduced [1].

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