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Dynamics of the excited state of the primary electron donor in reaction centers of *Rhodopseudomonas viridis* as revealed by hole burning at 1.7K

Different conformational states

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The spectra of absorbance changes (ΔA) due to the formation of P^+Q^- (P, primary electron donor, Q, primary quinone acceptor) at 1.7K in *Rhodopseudomonas viridis* reaction centers (RCs) excited at 1014 nm has been shown to include, besides a progression of broad (170–190 cm^{-1}) Gaussian vibronic bands separated by 150 cm^{-1}, a 'narrow' structure near 1014 nm which can be simulated by a Lorentian zero-phonon hole (ZPH) and Lorentian one-mode (26.8 cm^{-1}) phonon wings. The widths of ZPH of ≈ 17 cm^{-1} for ΔA reflecting the formation of P^+Q^- decaying in the ms time domain and of 6.8 ± 0.4 cm^{-1} for P^+Q^- decaying in the min time domain at 1.7K, seems to correspond to different conformations of RCs with a relaxation time of P* of ≈ 0.6 ps (in agreement with measurements in this time domain) and 1.6 ± 0.1 ps, respectively. The comparison of the spectra of ΔA in the region of the B_L band for slow (min) and fast (ms) decaying components suggests a different mutual arrangement of P and B_L for different conformations of RCs. It is assumed that the broad and narrow structures of the P band reflect the transitions to two configurations with different P-protein interactions. 'Narrow' structure of ΔA spectrum with essentially the same phonon wings and ZPH (width of 3.8 ± 0.4 cm^{-1}) was observed within the P band when H_L was photoreduced at 1.7K.

Reaction center; Primary electron donor; Hole burning

1. INTRODUCTION

In recent years several groups (see [1] for references and discussion) have carried out hole-burning studies on reaction centers (RCs) of purple bacteria by measuring the spectra of absorbance changes (ΔA) due to the dormation of P^+Q^-, H_L^- and H_M^- induced by the selective excitation within the long-wavelength Q_y band of the primary electron donor P at 2–4K (Q, primary quinone; H_L and H_M, bacteriopheophytins located in L and M protein subunits, respectively [2,3]. These studies are directed to measure the relaxation time of the excited state P* in the frequency domain and to reveal the vibronic and phonon structure within the P band. Theoretical simulations were carried out as in [4].

In a preceding report [1] we have found that in *Rhodopseudomonas viridis* RCs ΔA spectra due to the formation of P^+Q^- decaying in the ms time domain, include at 1.7K two main spectral features: (i) a broad structure simulated by a progression of Gaussian vibronic bands separated by 150 cm^{-1} with FWHM (full width at half maximum) of 170–190 cm^{-1} and (ii) a 'narrow' structure with bands at 1014 nm (FWHM $\cong$ 50 cm^{-1}) with an unresolved zero-phonon hole (ZPH) and at 1000 nm (FWHM $\cong$ 100 cm^{-1}) representing 0–0 and 0–1 vibronic transitions, respectively.

This paper presents the results of a study of the 'narrow' structure within the P band for a different component of ΔA related to the formation of P^+Q^- and H_L^- at 1.7K in *R. viridis* RCs excited by a narrow line at 1014 nm.

Abbreviations: ΔA, light-minus-dark absorbance changes; B_L, bacteriochlorophyll located in L protein subunit; H_L and H_M, bacteriophenophytins located in L and M protein subunits, respectively; P, primary electron donor, bacteriochlorophyll dimer; Q, primary quinone acceptor Q_A; S, Pekar–Huang–Rhys factor equal to the ratio of integral intensity of 0–1 to that of 0–0 vibronic transition; ZPH, zero-phonon hole.

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2. MATERIALS AND METHODS

The isolation procedure of *R. viridis* RCs, redox additions, low-temperature absorption spectra of the samples in the cytochrome region, the apparatus for measuring absorbance changes at 1.7K were described in a preceding paper [1]. The spectra of ΔA associated with P^+Q^- decaying in the ms time domain were measured using a phosphoroscopic set up [1]. Spectra of ΔA decaying in the min time domain as well as ΔA caused by the photoformation of H_L^- were registered by means of sequential measurements of absorbance spectra before and after excitation.

3. RESULTS

Light-minus-dark absorbance changes (ΔA) at 1.7K induced by the selective excitation at 1014 nm of *R. viridis* RCs in the presence of 1.2 mM ferricyanide or in the absence of any redox additions are due to the for-

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mation of the state P^+Q^- and have two main components decaying in the min and ms time domain.

The spectra of ΔA of the min-decaying component of *R. viridis* RCs without any redox additions measured at 1.7K after 2 min (curve 1), 4 min (curve 2) and 8 min (curve 3) of illumination at 1014 nm, are shown in Fig. 1. The subsequent darkness allows the recovery of the absorbance to the original level. The dashed curves 4 and 5 show this recovery after 6 min (4) and 12 min (5) of the darkness, respectively. One can see that half of the absorbance is recovered within ≈ 6 min.

Curve 1 in Fig. 2A (analogous to curve 3 in Fig. 1 but obtained with the increasing of the averaging) shows that the excitation of RCs at 1014 nm for 8 min leads to absorbance changes which have two main spectral features: (i) a narrow structure near 1014 nm, and (ii) a broad structure underlying the narrow one. The comparison of this spectrum with the spectrum of ΔA ob-

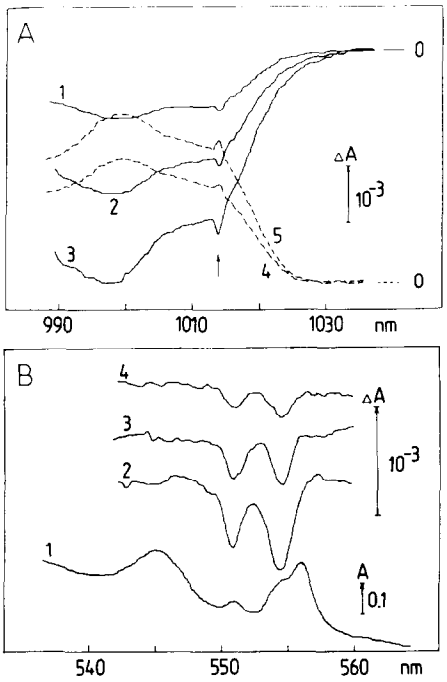

Fig. 1. (A) The spectra of light-minus-dark absorbance changes (ΔA) associated with the min decaying component of P^+Q^- in *R. viridis* Rcs frozen in the absence of any redox additions. ΔA induced by the excitation at 1014 nm (6 mW·cm^{-2}) at 1.7K for 2 min (curve 1), 4 min (curve 2) and 8 min (curve 3) The measuring light did not produce measurable absorbance changes. The dashed curves 4 and 5 show the recovery of the absorbance after 6 min (curve 4) and 12 min (curve 5) of the darkness. Here and below 12 μM RCs were frozen down to 1.7K as described in [1] (the absorbance of RCs at 1000 nm was ≈ 0.35); each spectrum is an average of 100–1000 measurements. Arrows show the excitation wavelength at 1014 nm; horizontal lines with index 0 show zero lines for ΔA. (B) Curve 1 shows the absorption spectrum of the sample in the 535–565 nm region at 1.7K. Curves 2–4 show the measurements of ΔA spectra related to the oxidation of the cytochrome c_{310} induced at 1.7K by the light in the region of 520–580 nm (intensity of 2.2 mW·cm^{-2}) for 40 s: first measurement (curve 2), second measurement after preliminary excitation of RCs at 1014 nm for 7 min (curve 3), and third measurement following the second one after 10 min of darkness (curve 4).

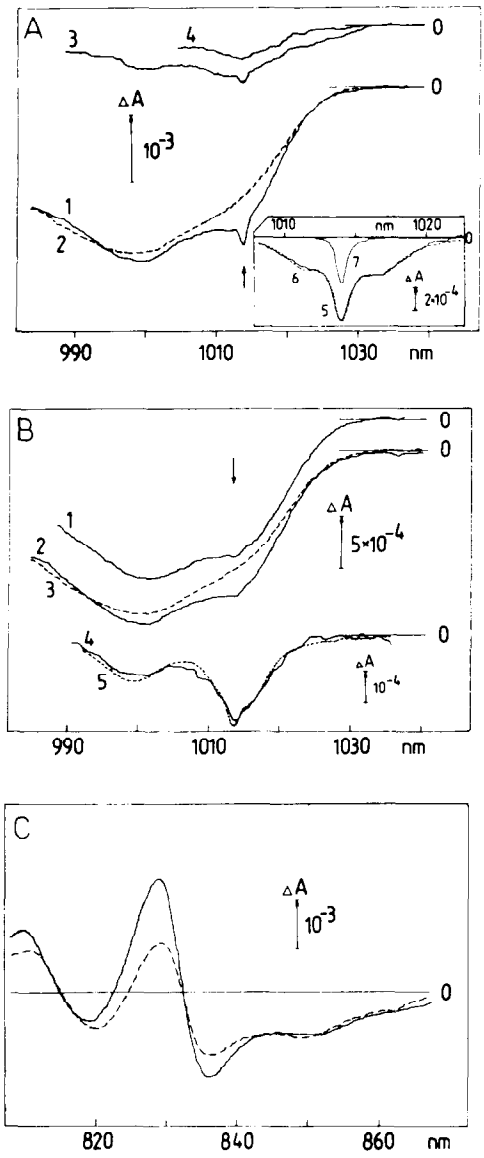

Fig. 2. The comparison of ΔA spectra of *R. viridis* RCs at 1.7K for slow (min time domain) and fast (ms time domain) decaying components of $P^+Q_A^-$ under different conditions. (A) Slow decaying component. RCs in the absence of any redox additions; the excitation at 1014 nm for 8 min (curve 1); the excitation by red light ($\gamma \geqslant 700$ nm, 2 W·cm^{-2}) for 5 min (curve 2 normalized at 985 nm with curve 1). RCs in the presence of 1.2 mM ferricyanide; the excitation at 1014 nm for first 2 min (curve 3) and second 2 min (curve 4). The bar refers to spectra 1, 3 and 4. The inset shows the result of the subtraction of spectrum 2 from, spectrum 1 (curve 5) and the reading of the Hg line at 1014 nm (curve 7). Curve 6 is a modelling curve with parameters indicated in the text. (B) Fast decaying component measured with the phosphoroscopic setup (see section 2). RCs in the absence of any redox additions (curve 1) and in the presence of 1.2 mM ferricyanide (curve 2 and 3), excited either at 1014 nm (curve 1 and 2) or by red light (curve 3 normalized at 985 nm with curve 2). The upper bar refers to spectra 1 and 2. Curve 4 shows the result of the subtraction of the spectrum 3 from the spectrum 2. Curve 5 is a modelling curve with parameters indicated in the text. The lower bar refers to spectrum 4. (C) The comparison of the spectra of ΔA in the range of 810–870 nm induced by the illumination at 1014 nm for fast (solid) and slow (dashed) decaying components of RCs in the absence of any redox additions after normalization of ΔA at 1000 nm.

tained with red light excitation (λ700 nm) and shown by curve 2 (dashed) demonstrates that the broad feature is almost independent of the excitation frequency (see [1]). Note that the amplitudes of the narrow and broad bands are changed in parallel in Fig. 1A.

Curve 5 of Fig. 2A shows the result of the subtraction of spectrum 2 from spectrum 1 (after normalization at 985 nm, where the contribution of the narrow component would be negligible [1]. This ΔA-minus-ΔA spectrum reveals the contribution of the narrow structure into the spectrum of ΔA. The narrow hole, possibly ZPH, with two side bands, reflecting phonon and pseudo-phonon wings, are clearly seen in the spectrum. Curve 7 shows the reading of Hg line at 1014 nm with Lorentian shape and FWHM of 8.3 cm^{-1}. Dashed curve 6 is a modelling curve with the following parameters (apparatus broadening was included separately): (i) Lorentian ZPH with FWHM of 6.8 cm^{-1} centered at 1014 nm, (ii) one-mode Lorentian phonon wing with FWHM of 27.8 cm^{-1}, Pekar-Huang-Rhys factor $S=0.69$ and centered at 26.8 cm^{-1} higher than ZPH, and (iii) a one-mode Lorentian pseudo-phonon wing with FWHM of 27.8 cm^{-1}, $S=0.74$ and centered at 26.8 cm^{-1} lower than ZPH.

Curves 3 and 4 of Fig. 2A show that in the presence of ferricyanide the spectra of ΔA relaxing in the min time domain, also have narrow and broad components. However the narrow component is suppressed by 2 min of illumination at 1014 nm in contrast with the broad one.

Fig. 1B shows that in the absence of any redox additions the photo-oxidation of the high-potential haem c_{310} [5] (peaks at 554 and 551 nm) occurs at 1.7K (in agreement with [6] without any measurable relaxation in the dark. Thus there is no evidence for a correlation of the slow decaying ΔA within the P band and ΔA in the cytochrome region.

Fig. 2B shows that the spectra of ΔA associated with $P^+Q_A^-$ decaying in the ms time domain at 1.7K upon selective excitation at 1014 nm have the same features as found earlier [1,7]. i.e. (i) a narrow structure near 1014 nm and (ii) a broad structure independent of the excitation frequency. There are no considerable differences between the spectra measured for RCs in the presence or absence of ferricyanide (curves 2 and 1, respectively).

The result of the subtraction of the spectrum of ΔA obtained with red light and normalized at 985 nm (dashed curve 3) from that obtained with the excitation at 1014 nm (curve 2), is shown by curve 4 in Fig. 2B. It has two bands at 1014 nm and at 1000 nm. The structure of the 1014 nm band with FWHM of ≈ 50 cm^{-1} can be simulated by ZPH surrounded by phonon wings with the same parameters as found for the slow decaying component (Fig. 2A) except for some broadening. Modelling curve 5 in Fig. 2B has the following parameters (apparatus broadening was separately included): (i) Lorentian ZPH with FWNM of 17.3 cm^{-1} centered at 1014 nm, (ii) one-mode Lorentian phonon wing with FWHM of 38.5 cm^{-1}, $S=0.69$ and centered at 26.8 cm^{-1} higher than ZPH, and (iii) one-mode Lorentian pseudo-phonon wing with FWHM of 48.5 cm^{-1}, $S=1.5$ and centered at 26.8 cm^{-1} lower than ZPH.

The band at 1000 nm with FWHM of ≈ 100 cm^{-1} represents 0–1 vibronic transition with $S=0.9$ (see [1]).

The slow decaying ΔA have a smaller (by factor of ≈ 2) amplitude in the region of a blue shift of the B_L band at 830 nm than the fast decaying ΔA (Fig. 2C). At 850 and 817 nm ΔA are similar for fast and slow decaying components.

In the presence of 50 mM ascorbate no photoinduced ΔA due to the formation of P^+Q^- are observed at 1.7K as a result of the accumulation of RCs with Q_A prereduced before illumination. Several minutes of illumination at 1014 nm induces ΔA related to the formation of H_L^- at 1.7K (Fig. 3). The formation of H_L^- is accompanied by blue shifts of the P and B_L bands and the bleaching of the H_L band at 808 nm (curves 1 and 3). The difference spectrum of the P band shift includes narrow ZPH (FWHM $=3.8\pm0.4$ cm^{-1}) at 1014 nm with essentially the same electron-phonon structure as mentioned above. The width of ZPH corresponds to the relaxation time of P* of 2.8 ± 0.3 ps. The vibronic

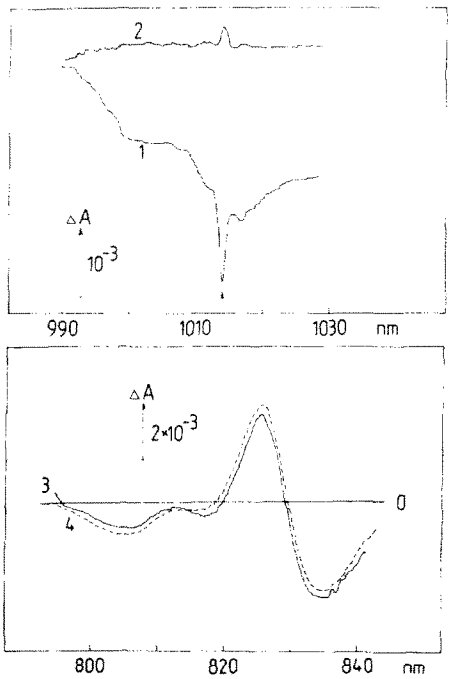

Fig. 3. The difference (light-minus-dark) absorbance spectrum due to the formation of H_L^- in *R. viridis* RCs frozen in the presence of 50 mM ascorbate, 100 μM phenazine ethosulphate and 18 μM ubiquinone-30 ($E_h=28$ mV), and induced by excitation at 1014 nm at 1.7K for 15 min (curve 1). Curve 2 shows the partial recovery of the absorbance after 20 min of the darkness. Curves 3 and 4 show the spectra of ΔA in the 795–840 nm region induced by excitation at 1014 nm for 28 min (curve 3) and by red light for 5 min (curve 4). The bar refers to curve 3.

repetition of the structure with a smaller amplitude of ΔA is seen at 1000 nm (see [1]). The partial relaxation ($\approx 25\%$) of ΔA is observed after 20 min of darkness (Fig. 3, curve 2).

4. DISCUSSION

The results presented here show that there are two main components of ΔA related to the formation of P^+Q^- in *R. viridis* RCs at 1.7K and decaying in the ms (fast) and min (slow) time domain. This suggests that there are at least two conformations of RCs with different time constants for P^+Q^- charge recombination. In the region of the Q_y band of P, the spectra of both components have a narrow structure near the excitation wavelength at 1014 nm with an underlaying broader one. The similar structure has been described earlier [1]. The shape of the broad structure is similar to that of the Q_y absorbance band of P at 1.7K [8] and has small ($\leqslant 6$ cm^{-1} [1]) inhomogeneous broadening. It can be simulated by a progression of broad Gaussian vibronic bands with FWHM of 170–190 cm^{-1} and *S*-factor of 1.2–1.4 (see [1]). The broad structure dominates (90–95% of integral intensity) in spectra of both components of ΔA and can be related to strong multiphonon-electron coupling.

The 'narrow' structure in the spectrum of ΔA of the slow decaying component can be reasonably interpreted as ZPH with a Lorentian shape and FWHM of 6.8 ± 0.4 cm^{-1} accompanied by a one-mode (26.8 cm^{-1}) phonon wing with $S = 0.69$. An analogous feature is not directly resolved in the spectrum of ΔA of ms-decaying component. In this case the band at 1014 nm may be due to ZPH with FWHM of ≈ 50 cm^{-1}. It would correspond to a relaxation time of P* of ≈ 200 fs and disagrees with the lifetime of P* at 8K (700 fs [9]). The relaxation time of ≈ 100 fs was estimated earlier [11]. However, the narrow structure of the 1014 nm band for the ms component of ΔA can be simulated with essentially the same parameters of electron–phonon coupling found for the min decaying component except for the broadening of ZPH from 6.8 to 17.3 cm^{-1} and corresponding broadening of phonon and pseudo-phonon wings (some increase of the intensity of pseudo-phonon wing is observed, see Fig. 2). ZPH broadening can be related to the decrease of the relaxation time of P* from 1.6 to 0.1 ps (min component) to ~0.6 ps (ms component). The latter time corresponds to the lifetime of P* measured at 8K [9].

Another difference between the slow and fast components of ΔA is revealed in the 800–870 nm region where the amplitude of the blue shift of the B_L band at 830 nm is relatively decreased for the slow decaying component. One can suggest that the latter component belongs to some conformation of RCs with a mutual arrangement of P and B_L different from that of RCs with the fast decaying ΔA. This arrangement might be responsible at least partially for an increase of the relaxation time of P* (from 0.6 to 1.6 ps), which is mainly determined by forward electron transfer rates.

It is possible that two different nuclear configurations of RCs with different P-protein interaction exist (both configurations are present in RCs with fast and slow decaying ΔA): (i) the broad spectral structure belongs to the 'first' configuration of RCs in which the Q_y transition of P has a strong electron–phonon coupling and (ii) the narrow structure belongs to a 'second' configuration of RCs which is characterized by a weak one-mode electron–phonon coupling. The 'second' configuration can be related to a small fraction of partially distorted RCs. However two points should be considered: (i) ZPH width found for the narrow structure of ΔA decaying in the ms time domain corresponds to a relaxation time of ≈ 600 fs for P*, which agrees with fs measurements of a lifetime of bulk of P* at 8K [9]; (ii) the fluorescence spectrum of RCs at 4K [10] is probably symmetric to the transition within the 'second' configuration, since both have relatively small vibronic coupling. Therefore it would be interesting to suggest that the main fraction of the excited state of P* in the 'first' configuration is converted in the sub-ps time domain into the 'second' configuration which participates in fluorescence and electron transfer. The 'second' configuration is normally converted back to the 'first' one when a ground state of P is formed, except for a small fraction which can be frozen in the 'second' configuration.

The ZPH, phonon and vibronic structure of spectra of ΔA for the photoreduction of H_L (Fig. 3) and H_M [11] at 1.7K is similar to the 'narrow' structure of ΔA spectra observed for the formation of P^+Q^-. The widths of ZPH correspond to the relaxation time of P* of 2.8 ± 0.3 ps for the reduction of H_L and >10 ps for the reduction of H_M [11].

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