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Burning of a narrow spectral hole at 1.7 K in the absorbance band of the primary electron donor of *Rhodopseudomonas viridis* reaction centers with blocked electron transfer

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Burning of spectral holes within the absorption band of the primary electron donor P in reaction centers of *Rhodopseudomonas viridis* is studied using selective excitation at 1014 nm and 1.7 K. A narrow (≤ 1.0 cm^{-1}) spectral hole with relatively weak electron-phonon and vibronic coupling is burned if the intermediary electron acceptor I is pre-reduced. The hole is broadened up to ~90–150 cm^{-1} if I is neutral and the electron-transfer chain is open. Broadening of the hole is discussed in terms of the fast (~70–110 fs) electron hopping between P* and P^+BL^- (BL, bacteriochlorophyll monomer in L protein subunit).

Hole burning; Reaction center; Electron transfer; Primary electron donor; (*Rhodopseudomonas viridis*)

1. INTRODUCTION

Reaction centers (RCs) of phototrophic bacteria convert light energy into chemical energy via electron transfer initiated by oxidation of the excited bacteriochlorophyll (BChl) dimer, P. Recent hole-burning studies failed to reveal narrow holes in the Q_y band of P upon formation of the states PI^-Q^- and P^+Q^- under selective excitation of RCs from *Rhodopseudomonas viridis*, *Rhodobacter sphaeroides*, and *Chloroflexus aurantiacus* [1–6] [I (BLHL) denotes the intermediary electron acceptor comprising the BChl monomer B and bacteriopheophytin molecule H in L protein subunit; Q, primary quinone acceptor Q_a]. Therefore, it was impossible to resolve the zero-phonon line (ZPL) and phonon wing (PW) and to discern vibronic structure. It was suggested that the observed bandwidth (300–500 cm^{-1}) might be due either to strong electron-phonon coupling or to fast charge separation beyond or within P. In all these studies, the RCs were in active states, and the electron-transfer chain was open. Hence, the question remains as to whether the spectral holes were broad because of fast electron transfer from P*, or due to processes in P itself.

In the accompanying paper [7] we report that the long-wavelength Q_y absorbance band of P comprises contributions of 0-0, 0-1 and 0-2 transitions, etc. separated by ~150 cm^{-1} in Stokes and anti-Stokes regions as revealed by derivative spectroscopy at 1.7 K and by the difference absorption spectra measured on heating to 60 K.

Here, we report that a narrow (≤ 1 cm^{-1}) spectral hole with relatively weak electron-phonon and vibronic couplings is burned at 1.7 K in the 0-0 transition region of the Q_y band of P under selective excitation at 1014 nm of *Rps. viridis* RCs with reduced I. This hole is broadened up to ~90–150 cm^{-1} (homogeneous ZPL width ~45–75 cm^{-1}) in RCs with an open electron-transfer chain. This broadening is discussed in terms of the fast (~70–110 fs) electron hopping between excited state P* and P^+BL^-.

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2. MATERIALS AND METHODS

RCs of *Rps. viridis* were isolated as described [11] and their absorption, derivative and difference spectra at 1.7–60 K were measured using an OMA-2 optical multichannel analyzer (EG&G, Parc, NJ) (see [7]). For hole-burning, the narrow line at 1014 nm of a mercury arc lamp was isolated with interference and cut-off filters. The line bandwidth was 10 cm^{-1}. The intensity of the burning light was 0.11 mW/cm^2.

Samples of RCs were prepared in the dark and poised at redox potentials which ensured light-induced electron-transfer reactions that are irreversible at 1.7 K:

$$CytPIQ \rightarrow Cyt^+PIQ^- \quad (1)$$

$$CytPIQ^- \rightarrow Cyt^+PI^-Q^- \text{ [8,9]} \quad (2)$$

$$CytHMPI^-Q^- \rightarrow Cyt^+HM^-PI^-Q^- \text{ [10,11]} \quad (3)$$

Here, Cyt denotes RC-bound *c*-type cytochrome of *Rps. viridis*, HM is the bacteriopheophytin molecule in the M protein subunit of RCs. The state $CytHMPI^-Q^-$ was obtained after illumination at 293 K of RCs prepared in the state $CytHMPIQ^-$. The absorbance of samples in the Q_y band of P was ~0.5.

3. RESULTS

Fig.1A and B shows changes in the RCs' absorption spectra caused by photochemical reactions 1 and 2, respectively, excited at 1014 nm and 1.7 K.

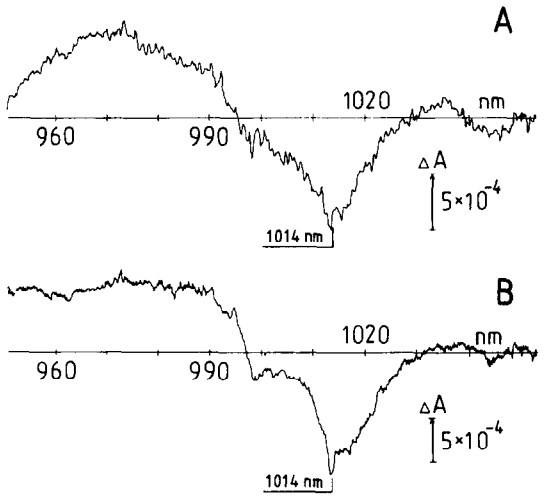

Fig.1. (A) Changes in the absorption spectrum of RCs from *Rps. viridis* caused by photochemical reaction 1: $CytPIQ \rightarrow Cyt^+PIQ^-$ excited at 1014 nm and at 1.7 K. Spectra before and after illumination were measured at 1.7 K. Illumination time, 15 min. (B) Same, for reaction 2: $CytPIQ^- \rightarrow Cyt^+PI^-Q^-$. Illumination time, 10 min. Here and in figs 2,3 horizontal lines with wavelength scales show zero lines for ΔA and $d^2A/d\lambda^2$.

In both cases, the Q_y band of P is shifted to shorter wavelengths below 1000 nm, presumably due to the electric field of Q^- or I^-, respectively. The difference spectrum in fig.1A comprises only a broad (~150 cm^{-1}) component around 1014 nm, no narrow bands being observed. In fig.1B the broad bands also dominate, yet a narrow hole at the excitation wavelength 1014 nm is discerned along with its satellite spaced ~150 cm^{-1} to shorter wavelengths. A weak, narrow hole has been previously observed in RCs of *Rb. sphaeroides* selectively excited at 865 nm and 2 K [12].

In samples of RCs prepared in state $CytHMPI^-Q^-$, photochemical reaction 3 was excited at 1014 nm. The hole-burning spectrum is quite different in this case (see fig.2B). Here, a narrow hole at the burning wavelength dominates (presumably, ZPL) and is accompanied by wings with maxima shifted by ~30 cm^{-1} to shorter and longer wavelengths. The bandwidth of the ZPL was estimated using better spectral resolution (fig.2B, inset) and found to be $\leqslant 1$ cm^{-1}. Moreover, a similar structure is discerned, shifted by ~148 cm^{-1} to shorter wavelengths, namely a narrow line accompanied by shoulders at ~30 and ~60 cm^{-1}. A weaker, almost structureless band shifted to shorter wavelengths by ~296 cm^{-1} from ZPL is also observed. For comparison, the second derivative of the absorption spectrum of RCs at 1.7 K in the state CytPIQ is depicted by the unbroken line in fig.2A in which the separation between negative extrema is similar.

Fig.2C shows changes in the absorption spectrum of RCs in state $CytPI^-Q^-$ at 1.7 K after additional illumination at 1.7 K with white light (reaction 3). This difference spectrum has the same negative maxima (with a similar ratio of integral intensities) as that shown in fig.2B. However, the excitation with white light yields much greater bandwidths (~120 cm^{-1}) near 1014 nm.

Fig.3A shows the second derivative of the absorption spectrum of RCs after hole-burning via reaction 3 excited at 1014 nm and 1.7 K. The narrow hole with complex accompanying structure is clearly seen here.

Fig.3B shows differences between the absorption spectra measured at 6.5 and 13 K after hole-burning at 1014 nm via reaction 3 in RCs of *Rps. viridis* at 1.7 K. A narrow hole is discerned due to its decrease on increase in temperature (see [7]).

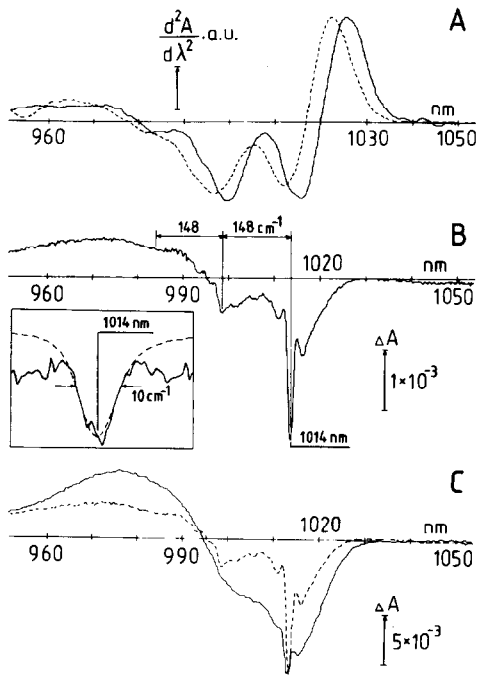

Fig.2. (A) Second derivative of the absorption spectrum of *Rps. viridis* RCs in the normal (CytPIQ) state (solid) and in state $CytPI^-Q^-$ (dashed) measured at 1.7 K. (B) Same as A but for reaction 3: $CytHMPI^-Q^- \rightarrow Cyt^+HM^-PI^-Q^-$. Illumination time, 15 min. (Inset) Shape of burned hole with greater spectral resolution (solid) and profile of Hg line at 1014 nm measured under the same optical conditions (dashed). (C) Same as B after subsequent illumination with white light (10 mW/cm^2) for 10 min at 1.7 K (solid). The bar refers to the solid line. Dashed line shows the spectrum of B, normalized with solid curve at 1014 nm.

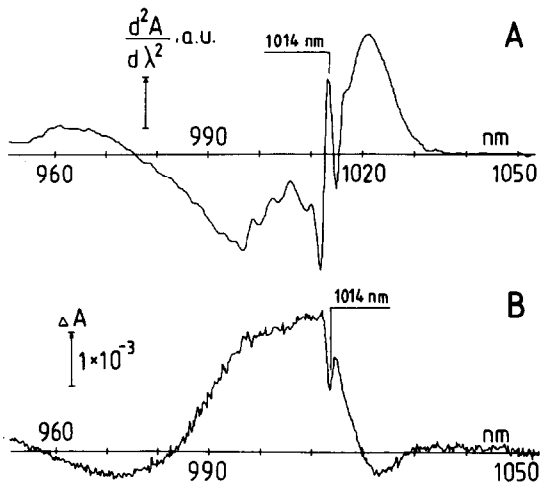

Fig.3. (A) Second derivative of the absorption spectrum of *Rps. viridis* RCs at 1.7 K after illumination for 1 h in state $CytHMPI^-Q^-$ (reaction 3) at 1014 nm and 1.7 K. (B) Difference between two absorption spectra of the same RCs measured at 6.5 and 13 K.

4. DISCUSSION

Let us first consider the spectrum of a hole burned at 1014 nm via reaction 3 in *Rps. viridis* RCs at 1.7 K (fig.2B). The narrow hole at 1014 nm with a $\leqslant 1$ cm^{-1} bandwidth is evidently a ZPL which corresponds to a relaxation time of $\geqslant 10$ ps, in accordance with picosecond measurements [13] for the same state. The band shape of the hole implies weak electron-phonon coupling with Pekar-Huang-Rhys factor S [14] about 0.7 for the PW whose maximum is shifted by ~ 30 cm^{-1} to the blue from ZPL at 1014 nm. A pseudo-PW of width ~ 65 cm^{-1} is observed at the longer wavelength side due to excitation of RCs via the phonon wing. A structure similar to that accompanying the central line is repeated at ~ 148 cm^{-1} to the blue from 1014 nm and accompanied by a PW with shoulders shifted by ~ 30 and ~ 60 cm^{-1}. A similar weaker and poorly resolved band is observed shifted by ~ 296 cm^{-1} to the blue from 1014 nm. Such repetition is characteristic of vibronic structure [15] with a frequency of 148 cm^{-1} and relatively weak vibronic coupling. In other words, the structure around 1014 nm represents the 0-0 transition region. The bands shifted to the blue by 148 and 256 cm^{-1} reflect the 0-1 and 0-2 transitions, respectively (further discussion [7]).

Comparison of the hole-burning spectrum (fig.2B) with the second derivative of the absorption spectrum of RCs in the state CytPIQ (fig.2A, unbroken line) shows coincidence of the negative extrema in both spectra and a correlation of relative integral intensities of their bands. Hence, we conclude that the hole-burning spectrum reflects the absorption spectrum of RCs in the normal state which is very similar to the spectrum of RCs in the state $CytPI^-Q^-$ (fig.2A).

Comparison between the hole-burning spectrum (fig.2B) with the difference absorption spectrum of photochemical reaction 3 excited with white light (fig.2C) implies that the inhomogeneous width of the 0-0 transition of P is 120 cm^{-1} which includes the ZPL and PW.

The narrow hole (fig.2B) discussed above is absent from the spectrum of reaction 1, excited at 1014 nm (fig.1A), where the 0-0 transition region has an approximately Lorentzian shape and bandwidth of $\sim 150\ cm^{-1}$ including nonresolved ZPL, PW and pseudo-PW. This broadening may be due either to a change in ZPL width or to a drastic increase in electron-phonon coupling which may suppress a narrow ZPL (the parameter S should increase to 5-6 [14]). In the latter case, the fine structure of the absorption spectrum should change upon reduction of I which does not agree with experiments (fig.2A). Furthermore, the absorption spectrum of RCs in the normal state includes broad ($\sim 60\ cm^{-1}$) ZPL and PW in the 0-0 transition region with low $S \simeq 0.8$ [7].

Therefore, the most plausible origin of the broad hole in fig.1A is a broadening of ZPL itself. To simulate the hole shape with $\sim 150\ cm^{-1}$ width (fig.1A) the amplitude of ZPL was taken to be equal to that of PW (see fig.2B). If the integral intensity of ZPL remains constant (S is constant), its width should increase to $\sim 90\ cm^{-1}$ (homogeneous width $\sim 45\ cm^{-1}$). We cannot rule out an alternative possibility that the width of the hole ($\sim 150\ cm^{-1}$) in the 0-0 transition region is mostly due to that of ZPL itself (homogeneous width $\sim 75\ cm^{-1}$). Assignment of the width of the total hole to ZPL alone [2-10] is misleading, since this hole includes ZPL, PW, pseudo-PW and vibronic components. The homogeneous width of ~ 45-$75\ cm^{-1}$ of ZPL is consistent with that found from absorption spectra of RCs [7] and can be due to faster relaxation of P* in the state CytP*IQ. This may result from either a 'new' process within P or faster electron transfer beyond P. However, the structure of the absorption spectrum is the same in states CytPIQ and $CytPI^-Q^-$ (fig.2A) and the fluorescence spectrum measured in state CytP*IQ has a mirror symmetry with respect to absorption in the 0-0 transition region [16]. In other words, no new states within P have been discerned.

Hence, the most probable reason for broadening of the ZPL is electron transfer from P* to the acceptor complex I (BLHL) in open RCs. Reduction of HL and decay of P* occur within 3-10 ps at 293 K [17,18] and 0.7 ps at 8 K [19] which corresponds to a ZPL width of ~ 0.6-$7\ cm^{-1}$ in contrast with the estimated value of ~ 45-$75\ cm^{-1}$. We assume that a very fast (~ 70-110 fs) noncoherent electron hopping between P* and P^+BL^- in open RCs leads to broadening of the ZPL. This assumption agrees with picosecond [17,20] and femtosecond [21] measurements which show the formation of the complex state $P^*[P^+BL^-]$ before P^+HL^-.

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