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Electron-phonon and vibronic structure of absorption spectra of the primary electron donor in reaction centers of *Rhodopseudomonas viridis*, *Rhodobacter sphaeroides* and *Chloroflexus aurantiacus* at 1.7–70 K

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The fine structure of the absorption band of P in reaction centers of various phototrophic bacteria has been studied at 1.7–70 K. The vibronic structure with a characteristic frequency of ~140 cm^{-1}, zero-phonon line and phonon wing for the 0–0 transition with relatively weak coupling parameters have been resolved.

Reaction center; Primary electron donor; Electron-phonon coupling; Vibronic coupling; (*Rps. viridis*, *Rb. sphaeroides* R-26, *C. aurantiacus*)

1. INTRODUCTION

In reaction centers (RCs) of phototrophic bacteria, the primary steps in conversion of solar energy into chemical energy involve the excited state P* of the primary electron donor P (a 'special pair' of bacteriochlorophyll molecules) which interacts with two other bacteriochlorophyll (B) and two bacteriopheophytin (H) molecules within RCs [1]. At low temperature the long wavelength (Q_y) absorption band of P is at least 2-fold wider than those of Bs and Hs [2]. In RCs of *Rhodopseudomonas viridis* [2–5] and *Rhodobacter sphaeroides* (R-26) [5] two components have been resolved in the Q_y absorption band of P at 1.9–4.2 K. Stark effect measurements have shown that at 77 K the change of the static electric dipole is at least 2–4-fold greater within the band of P compared to bands of B and H in RCs [6]. Hole-burning studies have revealed bleaching of a broad (300–400 cm^{-1}) absorption band of P in RCs with an open electron-transport chain [7–9]. In an accompanying paper [10] we have reported burning of a narrow ($\leqslant 1$ cm^{-1}) spectral hole within the absorption band of P in RCs of *Rps. viridis* with reduced acceptor complex I (= BH) at 1.7 K. The spectral features of the hole include a zero-phonon line (ZPL) and phonon wing (PW) for the 0–0, 0–1, 0–2, etc. vibronic transitions.

Here we report the fine structure of Q_y absorption band of P in various RCs with an open electron-transport chain studied by second derivative spectroscopy, temperature difference spectra, and by the dependence of various spectral components on temperature in the 1.7–70 K range.

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2. MATERIALS AND METHODS

RCs from *Rps. viridis*, *Rb. sphaeroides* (R-26) and *Chloroflexus aurantiacus* were isolated as described [9,11] and suspended in buffer solution containing 10 or 50 mM Tris-HCl (pH 8.0), 0.1% lauryldimethylamine oxide (LDAO) or Triton X-100, and 60% (v/v) glycerol. Samples for low temperature studies were frozen in 2-mm cuvettes in the dark.

A cuvette with a sample of RCs and an empty reference cuvette were fixed on the same sample holder of a home-made optical cryostat and immersed into liquid He at 1.7–4.2 K or He vapor at 5–70 K. Absorption spectra were measured, and their difference and derivatives were calculated with an Oma-2 optical multichannel analyzer (EG&G, PARC, Princeton).

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3. RESULTS

Absorption characteristics of the Q_y band of P in RCs from *Rps. viridis, Rb. sphaeroides* (R-26) and *C. aurantiacus* in the state PIQ are shown in fig.1: absorption spectra at 1.7 K in (A-C), their second derivative in (D-F), and the difference between spectra measured at various temperatures in (G-I). The dashed curve in fig.1A shows the calculated absorption spectrum (see below). From fig.1A,B one can see that the main absorption maximum is accompanied by a distinct long-wavelength shoulder in RCs of *Rps. viridis* and *Rb. sphaeroides* in accordance with earlier reports [2-5]. The maximum and the shoulder correspond to two negative extrema b and a in the second derivative, and a third component appears as negative extremum c (fig.1D,E). The energy difference between extrema a and b is approximately equal to that between b and c, and is equal to ~160 cm^{-1} in *Rps. viridis* and ~145 cm^{-1} in *Rb. sphaeroides*. No fine structure is seen in the case of *C. aurantiacus* (fig.1C,F). Each curve in fig.1G-I is the result of subtraction of the absorption spectrum at $T > 10$ K from that at $T_0 = 7$ K. In *Rps. viridis* and *Rb. sphaeroides* two positive maxima are resolved coincident in frequency with components a and b, and two negative extrema are

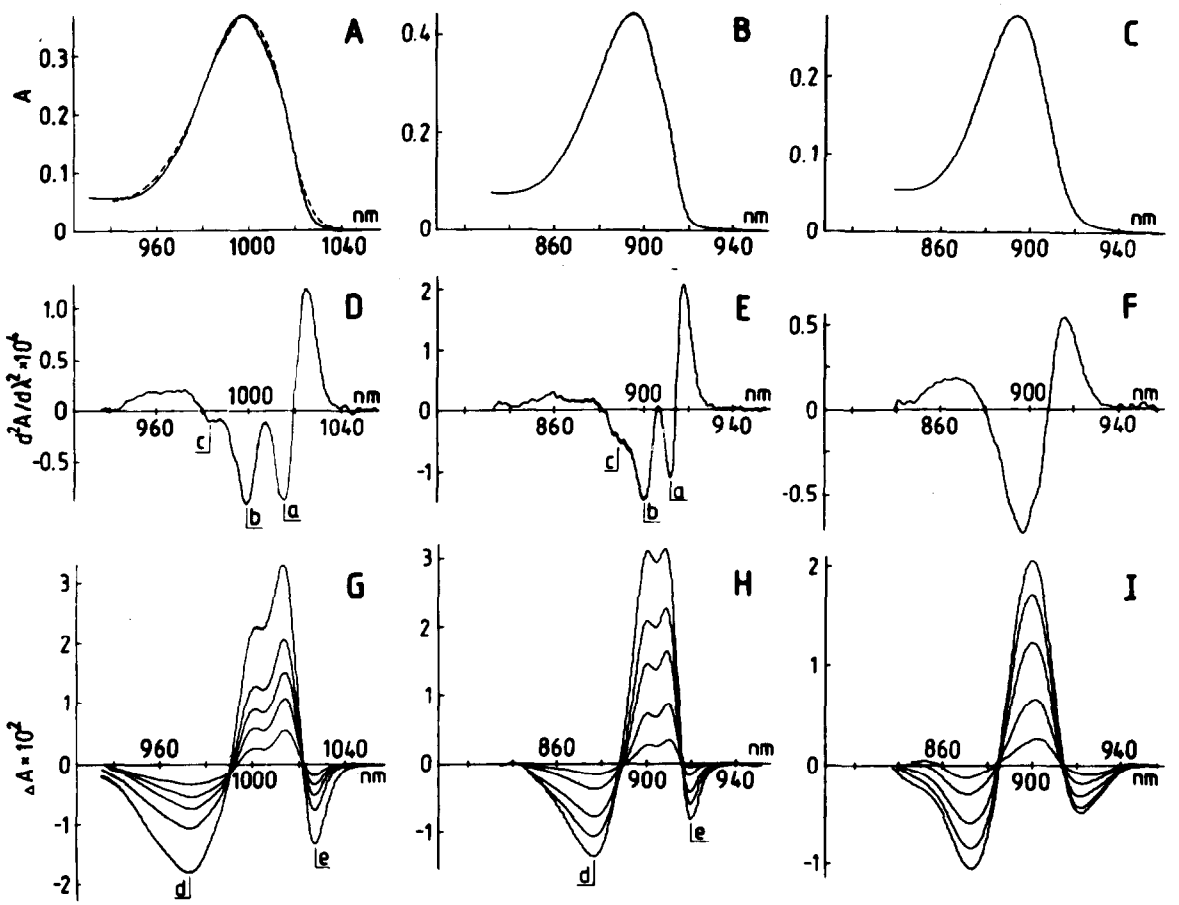

Fig.1. Spectral features of RCs from *Rps. viridis* (left column), *Rb. sphaeroides* R-26 (middle column) and *C. aurantiacus* (right column): absorption spectra at 1.7 K (top row), their second derivative (middle row), difference between absorption spectra measured at various temperatures (bottom row). Dashed line in (A) shows the spectrum calculated with parameters $\omega_{vib} = 140$ cm^{-1}, $\omega_{ph} = 38.5$ cm^{-1}, $S_{vib} = 1.2$, $S_{ph} = 0.87$. Each curve in (G-I) was calculated as a differente between the absorption spectrum measured at temperature $T_0 = 7$ K and that at $T > T_0$. Amplitudes of the difference spectra increase monotonically as $(T-T_0)$ increases. T values 15, 24, 33, 39, 51 K (G); 16, 24, 33, 38, 46 K (H); 21, 30, 39, 45, 51 K (I).

observed at shorter (d) and longer (e) wavelengths. The amplitudes of components d and e are plotted vs temperature in fig.2A.

At 10–15 K negative band a in the second derivative of the absorption spectrum of RCs from *Rps. viridis* splits into two components, a_1 and a_2 separated by 30 cm^{-1} (see fig.2B, inset). The corresponding band in *Rb. sphaeroides* does not split but shifts to the blue upon heating (not shown). The temperature dependence of the amplitudes of a_1, a_2 and b for *Rps. viridis*, and of components a and b for *Rb. sphaeroides* is demonstrated in fig.2B. Noteworthy is that the curves for a_1 and a_2 differ drastically.

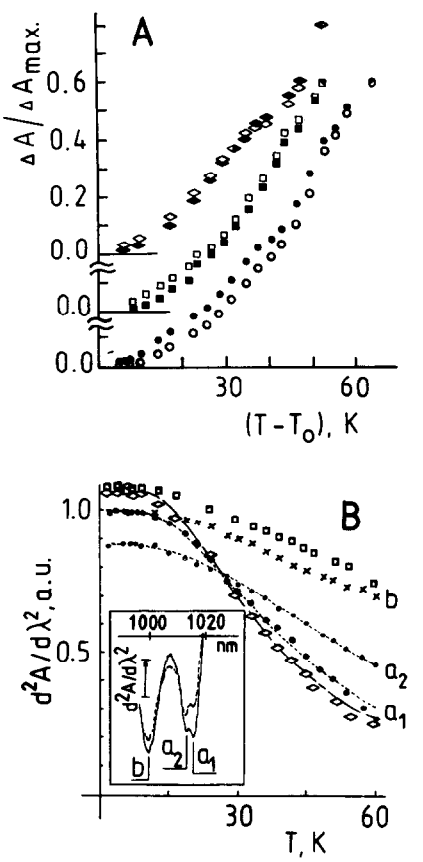

Fig.2. (A) Amplitudes of the Stokes (d, open symbols) and anti-Stokes (e, closed symbols) components of the difference absorption spectra (fig.1G–I) plotted vs temperature difference (T–T_0) for RCs from *Rps. viridis* (middle), *Rb. sphaeroides* R-26 (top) and *C. aurantiacus* (bottom). $T_0 = 7$ K. (B) Temperature dependence of amplitudes of peaks a_1 (●), a_2 (○), and b (×) in the second derivative of absorption spectra of *Rps. viridis* RCs shown in the inset for 21 K (——) and 36 K (– – –), and for peaks a (◇) and b (□) in the second derivative of the absorption spectrum of *Rb. sphaeroides* RCs.

4. DISCUSSION

Earlier reports [2,3,5,9] suggested that the presence of two components a and b in absorption spectra may be associated with a mixture of states P*B and P^+B^- in RCs of *Rps. viridis*. However, the results reported here and in [10] offer an alternative explanation of the fine structure. Namely, components b, c, etc. are vibronic repetitions of the purely electronic transition (band a) characterized by vibronic frequency, ω_{vib} = 130–160 cm^{-1}, and Pekar-Huang-Rhys factor S = 1.12–1.28. In other words, band a corresponds to the 0–0 transition, band b to 0–1, band c to 0–2, etc. Vibrations with characteristic frequencies of 130–180 cm^{-1} have been found in quasi-line fluorescence excitation spectra of bacteriochlorophyll and bacteriopheophytin [12], but their intensities are low. Supposedly, these vibrations are not totally symmetric and their intensities may increase due to mixing of excitonic and charge-resonance states of P, which leads to a change of symmetry of P's molecular orbitals.

It can be shown (see [13]) that for linear vibronic (or electron-pronon) coupling the shape of a homogeneous absorption band can be described as:

$$\mathscr{L}(\omega) = e^{-S(1+2n)} \sum_{m=0}^{\infty} \sum_{r=0}^{m} S^m \frac{(n+1)^{m-r}}{(m-r)!} \cdot \frac{n^r}{r!} \cdot \frac{\gamma^2/4}{[\omega_0 + (m-2r)\omega_{vib} - \omega]^2 + \gamma^2/4} \qquad (1)$$

where ω_0 is the frequency of the zero-phonon transition, ω_{vib} is the frequency of a vibration, γ is the width of the ZPL and $n = 1/[\exp(\hbar\omega_{vib}/kT)^{-1}]$. Each summand in eqn 1 corresponds to a transition in which $(m-r)$ vibrons are born and r vibrons disappear.

The difference absorption spectra shown in fig.1G,H imply that heating leads to a decrease in intensity of the 0–0 and 0–1 transitions and to an increase of the 0–3, 0–4, etc. transitions in the Stokes region, and to an increase of 0–1, 0–2, etc. transitions in the anti-Stokes region in agreement with eqn 1. Fig.2A demonstrates that at $T > 50$ K, $\Delta A(T)$ values are predominantly determined by

high frequency modes ($\omega_{vib} = 148$ cm^{-1} [10]) which probably belong to intramolecular vibrations within P. The contribution of low frequency modes (< 100 cm^{-1}) dominates at lower temperatures.

Fig.1A (dashed line) shows absorption spectra of P in *Rps. viridis* RCs calculated for 1.7 K using an equation that is similar to eqn 1 and comprises contributions from the phonon states. It is assumed that the width of the nonhomogeneous Gaussian distribution of ZPL frequencies for the 0–0 transition is 180 cm^{-1}, and 70% of the absorbance at 940 nm is assigned to light scattering. One can observe a good agreement between the two curves.

Hole-burning experiments on *Rps. viridis* RCs in the state $CytPI^-Q^-$ show [10] that each band (a, b, c, etc.) comprises a ZPL and its PW. Accordingly, the splitting of band a into two components in second derivative spectra may imply that a_1 belongs to the ZPL, while a_2 belongs to the PW. If one assumes that the bandshape of each component does not change within the 1.7–60 K region, then the amplitudes of peaks a_1 and a_2 in the second derivative (fig.2B) relate to amplitudes of absorption peaks. The dashed lines in fig.2B show the amplitudes of the ZPL and one-phonon peak vs temperature calculated for electron-phonon coupling for *Rps. viridis* RCs using $\omega_{ph} = 38$ cm^{-1} and $S = 0.87$, which agree with the results of hole-burning experiments [10], and for *Rb. sphaeroides* RCs using $\omega_{ph} = 40$ cm^{-1} and $S = 1.3$ (assuming that band a mainly corresponds to ZPL).

The lack of resolution of bands a and b in the spectra of RCs from *C. aurantiacus* (fig.1C,F,I) might result from stronger electron-phonon coupling of P in these RCs ($S \geqslant 2$), which may be caused by the presence of an additional negative charge of Asp-281 (absent in RCs of purple bacteria) near P [14].

Comparison of RCs of the three bacteria implies that the increase in the electron-phonon interaction reported here correlates with the decrease in the rate of electron transfer from P* to 1 (0.7 ps for RCs of *Rps. viridis* at 8 K [15], 1.2 ps for RCs of *Rb. sphaeroides* at 8 K [15] and 9 ps for RCs of *C. aurantiacus* at 293 K [16]).

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