Dynamic hole burning within special pair absorption band of *Rhodobacter sphaeroides* (R-26) reaction center at room temperature

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Abstract The absorbance spectrum of reaction centers of *Rhodobacter sphaeroides* at room temperature consists of relatively narrow spectral components which are moving in the femtosecond time scale and can be bleached by femtosecond laser pulses in the short wavelength region with a subsequent broadening and red shift of the bleaching (time constant ~250 fs). These data are discussed in terms of the population of the vibronic wave packets in the ground state by the interaction with phonons at 293K. The motion of these packets is probably responsible for the absorbance spectrum of the primary electron donor P at 293K with enhanced short wavelength components and with suppressed Stokes components.

Key words: Reaction center; Primary electron donor P; Wave packet

1. Introduction

The electron transfer reaction in bacterial hexachromic reaction center protein (RC) occurs from the excited state of the bacteriochlorophyll special pair, primary electron donor P. This reaction takes place within ~3 ps at 300K and is accompanied by the reduction of bacteriopheophytin (HL) via an intermediary electron acceptor, monomeric bacteriochlorophyll (BL) (where L indicates chromophore location in the L protein subunit) (for a review see [1,2]).

The absorption and hole burning measurements at 2–60K on bacterial RCs have revealed the progression of the enhanced (S = 1.2–2) around 150 cm^{-1} (73, 110 and 148 cm^{-1}) frequency modes which are accompanied by a ~30 cm^{-1} protein mode seen clearly for hole burning in RCs with prereduced HL [3] and found later for neutral acceptor state (with much less spectral resolution) [4,5]. The frequencies found in hole burning experiments in the range of 30–150 cm^{-1} were established in Raman spectra as well [6].

The important contribution to the understanding of the primary step was made by a visualization of coherent nuclear motion in RCs by femtosecond spectroscopy in a wide range of temperatures (10–290K) [7]. The presence of the phase coherence of low frequency motions on the time scale of electron transfer was demonstrated. The vibration motion of the excited state reveals the fundamental frequencies around 15 cm^{-1}, and at 69, 92, 122 and 153 cm^{-1} in native *Rhodobacter sphaeroides*

RCs [7] and at 15 and 77 cm^{-1} in the D_{LL} mutant of *Rhodobacter capsulatus* [8].

The most obscure behavior of the P absorbance spectrum is its temperature dependence. The spectrum peak is blue shifted from 900 nm at 2K to 860 nm at 293K [1] with suppression of the Stokes components, in contrast to the calculated overlapping of the vibration wave functions of the ground and excited states and to low temperature measurements (7–60K) which showed the coincidence with calculations (see [9]).

This work shows that the absorbance spectrum of RCs at room temperature (but probably not at 10K [7]) consists of the relatively narrow spectral components which are moving in the femtosecond time scale and can be bleached by femtosecond laser pulses in the short wavelength region with a subsequent broadening and red shift with a time constant of ~250 fs. These data are discussed in terms of the population of the vibronic wave packets in the ground state by the interaction with phonons at 293K. The motion of these packets is probably responsible for the absorbance spectrum of P at 293K with enhanced short wavelength components and suppressed Stokes components.

2. Materials and methods

RCs from *R. sphaeroides* (R-26) were isolated as described earlier [9]. The concentration of the sample was adjusted to an optical density of 0.45 at 860 nm and at 293K (optical path length of 1 mm). The 2 mM ascorbate was added to keep P in the neutral state before the arrival of each pump pulse (1 Hz repetition rate).

The femtosecond spectrometer consisted of cpm dye laser (~50 fs pulse duration) with rhodamine 590 (Exciton) jet pumped by a continuous argon laser (Spectra Physics) and DODCI (Exciton) jet. Fs pulses were amplified in a 6 pass dye jet amplifier and in a dye cell, both pumped by a YAG laser emitting 10 ns pulses at 538 nm. The amplified pulses were focused in a water cell (H_2O + D_2O) to produce a fs continuum. Its near-IR part (760–900 nm) was amplified in a Styryl 9M (LDS 821, Exciton) cell pumped by the same YAG laser. The spectrally filtered emission centered at 840 nm was used for excitation and a small fraction (~4%) of the whole NIR emission as measuring and reference beams. The measuring and exciting pulses propagated through delay lines and the sample. Then the measuring and reference pulses were passed through a polychromator and directed by the three-lens system to the photodiode array of OMA-2 (PARC, Princeton). The first half of the OMA-2 array (1024 pixels) was used for the measuring and the second for reference spectra. Spectra of absorbance changes (ΔA) at different delays with respect to the excitation were obtained by taking a logarithm of the ratio of the reference and measuring spectra with and without excitation. Cross-correlation function of the exciting and measuring pulses showed ~90 fs pulse duration. The relative position of the zero time delay within a 810–890 nm range differed by less than 50 fs.

3. Results

Fig. 1 shows the 293K transient difference absorbance spec-

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Abbreviations: ΔA, light-minus-dark absorbance changes; P, primary electron donor; RC, reaction center; S, Pekar-Huang-Rhys factor.
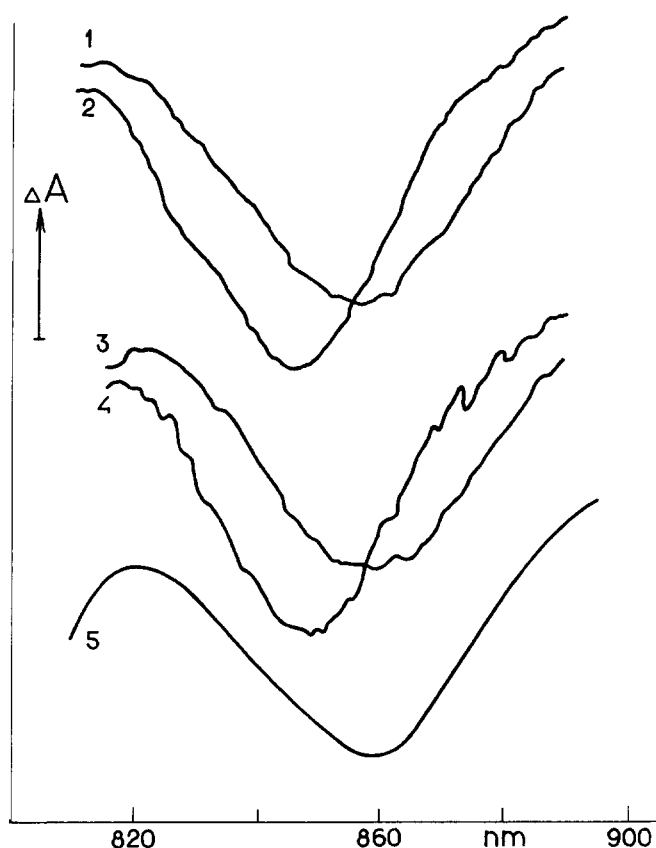
Fig. 1. The spectra of absorbable changes (ΔA) of *R. sphaeroides* RCs at 293K excited by 90 fs pulses at 840 nm. The ΔA spectra measured at 760 fs (1) and 100 fs (2) delays using the excitation which bleaches ~30% of RCs. The ΔA spectra measured at 17 ps (3) and 70 fs (4) delays using the excitation which bleaches ~12% of RCs. The ΔA bar corresponds to 0.1 for curves 1 and 2 and to 0.04 for curves 3 and 4. Curve 5 shows the reversed absorption spectrum of RCs.

tra within $Q_Y(-)$ transition of P in *R. sphaeroides* RCs excited at 840 nm at different delays. At 70–100 fs delay using stronger (curve 2) and weaker (curve 4) excitation the ΔA spectra centered at 847 nm are relatively narrow and blue shifted with respect to the absorbance spectrum (curve 5). In contrast to that, ΔA spectra measured at 760 fs (P*) (curve 1) or 17 ps

(P^+HL^-) (curve 3) coincide very well with the absorbance spectrum of P in its major part (820–890 nm) at 293K. The increase of the delay from 50 fs to 1 ps shows the gradual red shift of the center of the bleaching and its broadening for both intensities of the excitation.

Fig. 2 shows the kinetics of the center of the ΔA spectra upon stronger (O) and weaker (●) excitation. The center is shifted from 847 to 860 nm by monoexponential kinetics with a time constant of 250 ± 50 fs in both cases.

The described effects with some longer excitation pulses were also observed by the Woodbury group (private communication by Su Lin).

4. Discussion

The data presented here can be interpreted in two different ways. The bleaching of the short wavelength side of the P band and subsequent red shift and broadening of the bleaching measured from 50 fs to 1 ps in the P band excited at 840 nm can be related to (i) the Stokes shift of the stimulated emission from 840 to 900 nm during the measured kinetics (Fig. 2) or to (ii) the real red shift and broadening of the bleached P band.

According to the measurements at 10K [7] the amplitude of the stimulated emission is not more than ~0.5 of that of the band bleaching. At 293K this emission amplitude is even less [10]. It means that the small amplitude of the bleaching centered at 865–875 nm at 70–100 fs delay is not due to the presence of the stimulated emission at 840 nm. Then the spectrum of ΔA measured at 17 ps (Fig. 1) when no stimulated emission is present, shows that the bleaching spectrum coincides with the absorbable spectrum. Thus, dynamic hole burning, i.e. the dynamic broadening and red shift of the P band bleaching, is probably real. Furthermore the direct measurements of the stimulated emission excited at short wavelengths demonstrated that the stimulated emission appears at 50 fs at 910 nm and it's Stokes shift approaches the maximal value (960 nm) at 150 fs at 10K and at <100 fs at 290K [7]. This time scale is much shorter than the kinetics of the band shift presented in Fig. 2. Therefore we believe that the major part of the kinetics presented in Fig. 2 reflects the dynamic hole burning of the P band.

Excitation at the short wavelength side of the P band at 10K shows the simultaneous (<30 fs) bleaching of the total band centered at 895 nm [7]. It means that at 10K the P band is
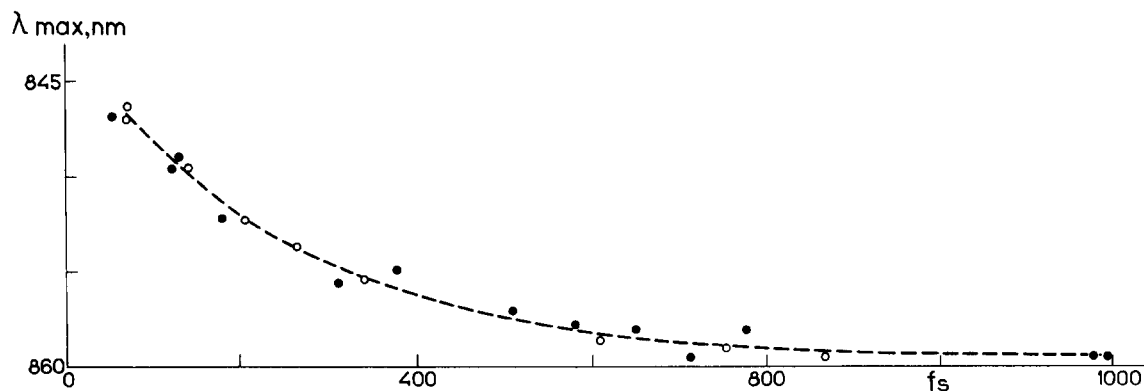

Fig. 2. Kinetics of the shift of the ΔA peak for *R. sphaeroides* RCs at 293K excited by 90 fs pulses at 840 nm. The data set for 30% bleaching (O) and 12% bleaching (●). Dashed curve shows the exponential decay with a time constant of 250 fs.
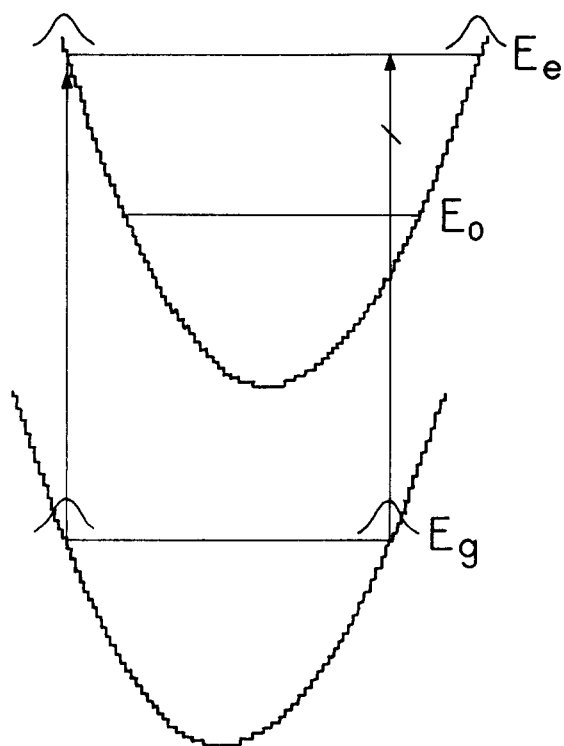
Fig. 3. Scheme of the optical transitions from the ground state wave packet (eq. 2) formed due to interaction with phonons. The $|C_{n+j}|^2$ values ($n = 0$) were estimated using Boltzman factor $\exp(-\hbar\omega/kT)$ where ω is an oscillation frequency. Since the optical transition should occur with the conservation of the nuclear oscillation momentum, the overlapping is high for the left part of the potential curves. Bold lines are potential curves for ground (lower one) and excited (upper one) states. E_g is a non-zero energy level of the ground state; E_o, E_e are zero and some non-zero energy levels of the excited state.

bleached as a whole and there is no dynamic hole burning of the P band in the fs time scale. One can suggest that at 10K the optical transition takes place from the zero vibronic level of the ground state. In contrast to that at 293K the optical transition probably occurs from the vibronic wave packet of the ground state formed due to the thermal activation by phonons of vibronic levels with $n \geq 1$.

Since the vibronic wave packet ($\Psi_e(x,t)$) is a result of the superposition of the wave functions for several ($v + 1$) vibronic levels (see [11]) we have

$$\Psi_n(x) = 1/(1.77\ 2^n\ 2!\ x_o)^{0.5}\ \exp((-0.5\ x/x_o)^2)\ H_n(x/x_o) \qquad (1)$$

where

$x_o^2 = h/(m\omega)$,

$H_n(x/x_o) = (-1)^n \exp((x/x_o)^2)\ d^n \exp((-x/x_o)^2)/d(x/x_o)^n$,

ω is a frequency of the oscillation. Then

$$\Psi(x,t) = \sum_{j=0}^{v} C_{n+j}\ \Psi_{n+j}\ \exp(-i\omega(n+j+0.5)t) \qquad (2)$$

From eq. 2 one can find the time dependence of the optical transition from the ground state wave packet which is proportional to the overlapping of the vibronic wave functions of the ground and excited states. Since the optical transition should occur with the conservation of the nuclear oscillation momentum, the overlapping is high for the left part of the potential curve (short wavelength transitions) and small for its right part (long wavelength transitions) due to displacement of the potential curves (Fig. 3). This explains enhancement of short wavelength components and suppression of Stokes components in the P absorbance spectrum at 293K. This is not the case in the temperature range of 7–60K where the phonon energy is probably not enough to produce a vibronic wave packet and where the Stokes components are comparable with anti-Stokes ones [9].

Thus the data presented in Figs. 1 and 2 can be interpreted in the following way. The fs excitation at 840 nm bleaches RCs which have the wave packet at the left side of the potential curve. These RCs are not recovered for several ms. Then in an assembly of unbleached RCs the redistribution of the spectral components occurs over the whole range of the P band and the ΔA spectrum becomes close to the P absorbance spectrum. The computer simulation of this effect shows that the time constant for the band shift roughly corresponds to half of the period of oscillation of the wave packet if this shift directly corresponds to the motion of the wave packet in unbleached RCs. Since the time constant is equal to ~250 fs (Fig. 2) the period of 500 fs would correspond to the frequency oscillation of 67 cm⁻¹. A similar frequency was found in hole burning experiments [3], Raman spectra [6] and oscillation dynamics of the wave packet of the excited state [7].

References

[1] Shuvalov, V.A. (1990) Primary Light Energy Conversion at Photosynthesis, Nauka, Moscow.

[2] Arlt, T., Schmidt, S., Kaiser, W., Lauterwasser, C., Meyer, M., Scheer, H. and Zinth, W. (1993) Proc. Natl. Acad. Sci. USA 90, 11757–11760.

[3] Shuvalov, V.A., Klevanik, A.V., Ganago, A.O., Shkuropatov, A.Ya. and Gubanov, V.S. (1988) FEBS Lett. 237, 57–60.

[4] Johnson, S.G., Tang, D., Jankowiak, R., Hayes, J.M., Small, G.J. and Tiede, D.M. (1990) J. Phys. Chem. 94, 5849–5855.

[5] Shkuropatov, A.Ya., Ganago, A.O. and Shuvalov, V.A. (1991) FEBS Lett. 287, 142–145.

[6] Cherepy, N.J., Shreve, A.P., Moore, L.J., Franzen, S., Boxer, S.G. and Mathies, R.A. (1994) J. Phys. Chem. 98, 6023–6029.

[7] Vos, M.H., Jones, M.R., Hunter, C.N., Breton, J., Lambry, J.-C. and Martin, J.-L. (1994) Biochemistry 33, 6750–6757.

[8] Vos, M.H., Rappaport, F., Lambry, J.-C., Breton, J. and Martin, J.-L. (1993) Nature 363, 320–325.

[9] Klevanik, A.V., Ganago, A.O., Shkuropatov, A.Ya. and Shuvalov, V.A. (1988) FEBS Lett. 237, 61–64.

[10] Woodbury, N.W., Becker, M., Middendorf, D. and Parson, W.W. (1985) Biochemistry 24, 7516–7521.

[11] Sokolov, A.A., Loskutov, Yu.M. and Ternov, I.M. (1962) Quantum Mechanics, State Education Publisher, Moscow.