B_A and B_B Absorbance Perturbations Induced by Coherent Nuclear Motions in Reaction Centers from *Rhodobacter sphaeroides* upon 30-fs Excitation of the Primary Donor

A. M. Streltsov,[†,‡] S. I. E. Vulto,[†] A. Ya. Shkuropatov,[§] A. J. Hoff,[†] T. J. Aartsma,[†] and V. A. Shuvalov*[,‡]

Biophysics Department, Huygens Laboratory, Leiden University, P.O. Box 9504, 2300 RA Leiden, The Netherlands, Laboratory of Photobiophysics, Belozersky Institute of Chemical and Physical Biology, Moscow State University, Moscow, 119899 Russia, and Institute of Soil Science and Photosynthesis, Russian Academy of Sciences, Pushino, Moscow regoin, 142292, Russia

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Subpicosecond oscillations in the B (accessory bacteriochlorophylls) band were found in native and B_B-modified reaction centers from *Rhodobacter sphaeroides* R26 and of the mutant YM210W at 293 K using 30 fs excitation pulses at 865 nm. The (30 ± 3) and (12 ± 2) cm^{-1} modes were found to be enhanced with respect to the broad structure around 150 cm^{-1} in all reaction centers (RC). The maximal amplitude of both modes is observed in native and B_B-modified RCs, while their minimal amplitude is registered in the YM210W mutant, in which the rate of ET is significantly decreased. In the presence of Q_A^{2-} new modes at 50 and 95 cm^{-1} are observed, concomitant with a decrease of the 30 cm^{-1} mode. These results are discussed in terms of the influence of protein motions, induced by the excitation of P, on the Q_Y transition of B_A at 800 nm and their relation to the primary electron transfer.

Introduction

The primary electron transfer (ET) in reaction centers (RCs) of photosynthetic bacteria results in the oxidation of the primary donor, P, and reduction of the bacteriopheophytin acceptor H_A with a ~3 ps time constant at room temperature (see refs 1 and 2). The participation of the bacteriochlorophyll monomer B_A (active chain) in ET was suggested by picosecond measurements as early as 1978.[3] This concept was subject to many further studies.[4−14] Recent measurements in pheophytin *a* reconstituted RCs[6−8,12−14] and in the so-called β-mutant[10] have shown that the energy of the $P^+B_A^-$ state in these modified RCs is below that of P*. These observations support the model in which B_A is a distinct intermediate.

Another important aspect in the primary ET is related to the close position of tyrosine M210 with respect to P and B_A in RCs.[15] The replacement of the tyrosine by leucine or tryptophan leads to a considerable decrease of the ET rate.[16−18] This decrease is explained by an upward relative change of the energy of $P^+B_A^-$ with respect to P*. However, in *Chloroflexus aurantiacus*, where the M210 residue is a leucine, the ET rate is decreased by a factor of 3−4 at room temperature[19] although the redox potential of P (+386 mV) is lower than that in *Rhodobacter* (*Rb.) sphaeroides* (+454 mV)[20] presumably because of the altered surrounding of P.[21] Consequently, the tyrosine may be involved in the ET by some mechanism other than shifting the energy of $P^+B_A^-$.

In a number of papers Vos et al.[18,22−25] have shown that excitation of P by short femtosecond laser pulses leads to coherent nuclear motions in the P* state, revealed by the stimulated emission kinetics. Similar results were obtained with spontaneous emission measurements.[26] The oscillations in the kinetics were Fourier transformed, and low-frequency spectra in the range 10−400 cm^{-1} were established.[18,22−26] These spectra are very similar to those produced by hole-burning measurements in RCs with prereduced H_A (which served to decrease the ET rate and to improve spectral resolution).[27] Special interest is attached to the 30 cm^{-1} mode, which appears to be strongly coupled to the P → P* transition.[28] Low-frequency modes are also observed in resonance Raman spectra,[29−32] and vibrational bands have been reported at 34, 71, 95, and 128 cm^{-1}, which are characteristic of P.[30]

Recent work has indicated that direct excitation of B_A and B_B around 800 nm in isolated RCs is followed by ultrafast relaxation by which the energy is transferred to P.[33−37] The extremely fast rate of energy transfer in the RC implies a strong coupling between the pigments involved. In recent papers[38,39] femtosecond oscillations were reported in the kinetics of the absorbance changes around 800 nm (B_A band) upon excitation of P at 870 nm. The frequencies of the oscillations in the 800 nm band are similar to those found in the stimulated emission of P*, but the 15 and 30 cm^{-1} modes in particular are highly pronounced. From spectral and polarization measurements it was concluded that the femtosecond oscillations near 800 nm are related to the B_A band and are induced either by reversible electron transfer to B_A or by protein motions perturbing the B_A band.

In the present work the femtosecond oscillations in the 800 nm band of B_A were studied in native as well as B_B-modified RCs of *Rb. sphaeroides* R26 in the presence of either reduced Q_B or doubly reduced Q_A, and in RCs of the YM210W mutant of the wild type. The B_B-modified RCs were chosen because around 800 nm only the B_A pigment absorbs.[20,40] When compared with oscillations in stimulated emission, the (30 ± 3) and (12 ± 2) cm^{-1} modes in the 800 nm kinetics were found

* Corresponding author. Fax: 7-96-7790532. E-mail: shuvalov@issp.serpukhov.su.
† Leiden University.
‡ Moscow State University.
§ Russian Academy of Sciences.

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to be enhanced with respect to the broad structure around 150 cm^{-1} in all RCs. The maximal amplitude of both modes is observed in native RCs, while their minimal amplitude is registered in the YM210W mutant in which the rate of ET is significantly decreased. In the presence of Q_A^{2-} new modes at 50 and 95 cm^{-1} are observed accompanied by the decrease of the 30 cm^{-1} mode. The present results are interpreted in terms of the influence of the protein motions, induced by the excitation of P, on the Q_Y transition of B_A at 800 nm and their relation to the ET.

Experimental Section

Materials. Reaction centers of *Rb. sphaeroides* R26 were isolated and purified as described in ref 20. B_B-modified reaction centers were prepared by treatment of isolated reaction centers of *Rb. sphaeroides* R26 with sodium borohydride in a procedure described previously.[20] This treatment results in a loss of the B_B contribution to the 800 nm absorption band of RCs probably because of a change in protein environment around B_B.[40] More precisely, at low temperatures the 812 nm band responsible for the B_B absorption fully disappears[41] because of a shift to shorter wavelength (760−800 nm). The double photoreduction of Q_A was performed as described earlier[42] in the presence of 5 mM dithionite and cytochrome *c* (5/1 Cyt/RC). The isolation of RCs from YM210W mutant was performed as described in ref 17. The absorbance of the samples at 860 nm was 0.4 in a cell of 1 mm optical path length. The reaction centers were kept open (i.e., P was not oxidized) by adding sodium ascorbate (5 mM) and DAD (0.2 mM) or dithionite and cytochrome *c* to obtain RCs with Q_A^{2-} (see above). The sample was contained in a rotating cell (sample thickness of 1.5 mm) spinning at about 3000 rpm, which corresponds to a linear velocity of 16 m/s at the focus of the laser beam.

Laser System. A mode-locked Ti:sapphire laser was home-built according to the design of Asaki et al.[43] The laser was pumped by an argon ion laser (Coherent-Innova). A cavity dumper was installed to reduce the repetition rate of the system to 1 MHz (see ref 44). The experimental setup is a pump−probe configuration with a parallel orientation of polarizations of pump and probe beams. The pump beam, modulated at 1 kHz by a chopper, was filtered by a RG850 (Melles Griot) filter to select the spectral band of 850−880 nm, thus avoiding direct excitation of B. Both beams were precompensated for dispersion in the optical components by double-passing of two fused-silica prisms. This arrangement resulted in an instrumental response function of 45 fs (fwhm) measured at the sample position, which corresponds, assuming a $sech^2$ pulse shape, to near-transform-limited pulses of 30 fs. The chirp within the probe beam was less than 10 fs. The energy of the pump pulse used for the experiments was 4 nJ/pulse. The diameter of the spot at the sample position was 30 μm. At most, 5% of RCs in the sample were excited. The intensity of the probe beam was measured by a photodiode followed by a phase-sensitive detection scheme referenced to the 1 kHz signal of the chopper. Part of the probe beam spectrum was wavelength-selected by a monochromator installed in front of the photodiode. Data were collected over a 10 ps time window. All measurements were performed at room temperature.

Results

General Oscillatory Features. Previously, we reported on the observation of oscillations in the 800 nm absorption band of B_B-modified RCs upon excitation of the primary donor.[39]

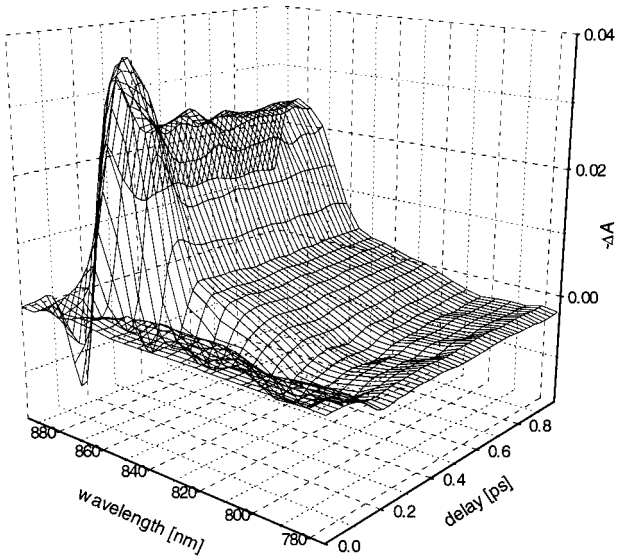

Figure 1. Femtosecond time-resolved three-dimensional smoothed spectra of B_B-modified RCs of *Rb. sphaeroides* (R26) at 293 K upon 30 fs excitation centered at 865 nm with parallel polarization of pump and probe beams.

We have extended these measurements to isolated RCs from *Rb. sphaeroides* R26 and from the YM210W mutant in order to make a more detailed study of this effect under different conditions.

Figure 1 shows a femtosecond time-resolved three-dimensional transient absorbance spectrum of B_B-modified *Rb. sphaeroides* (R26) RCs at 293 K upon 30 fs excitation centered at 865 nm with parallel polarization of pump and probe beams. A prominent feature in the kinetics is an apparent oscillation with a period of ∼210 fs within the absorption band of B_A and at the long-wavelength side of the P band, but other frequencies are also present (see below). Note that oscillations in the P band itself are due to the P* stimulated emission on the short-wavelength side. These oscillations at 805 nm (Figure 2) and 875 nm are very similar to those observed earlier for the stimulated emission of P*[18,22−25] and display at 805 nm an absorbance decrease near 220 fs delay, an absorbance increase near 320 fs delay, an absorbance decrease near 430 fs delay, etc., corresponding to a frequency of ∼150 cm^{-1}. The amplitude of this oscillation is about 10% of the total bleaching of the 805 nm band at 10 ps when ET is complete (ΔA(10 ps) = −0.01). At 875 nm (or 890 nm) the phase of the oscillations is opposite that at 805 nm (Figure 1), seen also from the spectrum of the amplitude of the oscillations measured at delays between 220 and 420 fs.[39] The phases of the high-frequency (∼150 cm^{-1}) oscillations around 805 nm are the same on the short- and long-wavelength side of the 805 nm bleaching (Figure 1), showing that these oscillations are due neither to the wave packet motion in the ground state of B_B nor to the $S_2 \leftarrow S_1$ transition of P (P* → P**). Otherwise, in both cases these two phases should be shifted by 180° with respect to each other.

It is known that the stimulated emission from P* at low temperature[24] and room temperature[25] has oscillations with almost the same period (∼250 fs, 145 cm^{-1}) as reported here and with a phase at the short-wavelength side shifted by ∼180° with respect to the long-wavelength side of the emission band, reflecting the wave packet motion in the excited state of P. In our case the oscillations in the ΔA at 805 nm are 180° out of phase with those observed at the short-wavelength side of the stimulated emission of P* (Figure 1) or, consequently, in phase with those at its long-wavelength side.

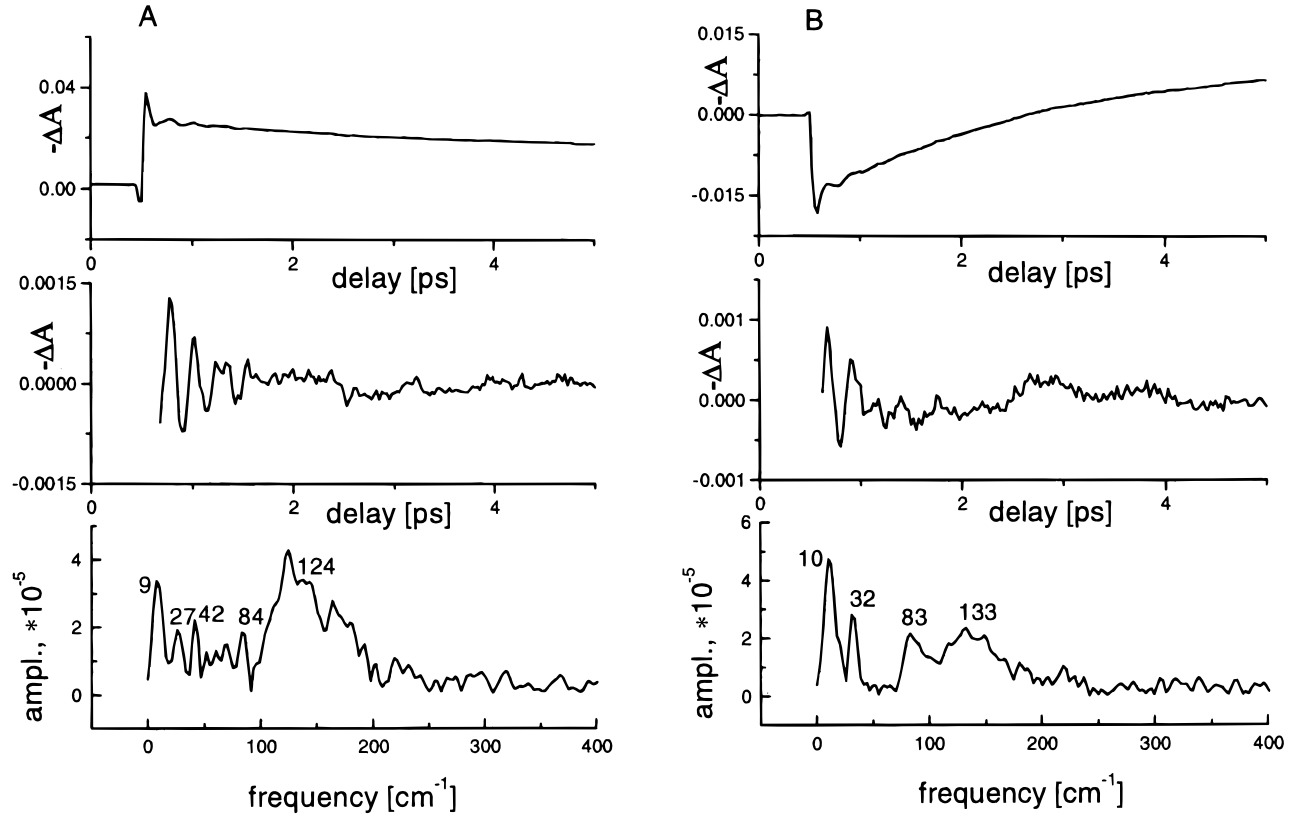

Figure 2. Kinetics of ΔA at 890 nm (A) and 805 nm (B) in R26 RCs (top). The oscillatory part is obtained by fitting the data to two-exponential decay kinetics (middle). Fourier transformation yields the frequency spectrum (bottom).

Frequency Analysis of the Oscillations. The frequency components of the oscillations in the kinetics were analyzed by Fourier transformation. The oscillatory parts were obtained by subtracting a two-exponential decay with appropriate time constants from the kinetics. The time constants were determined by fitting the average of typically 70−80 kinetic traces.

Figure 2 shows the femtosecond kinetics, the oscillatory residuals, and the Fourier transform (FT) spectrum for the absorbance changes at 890 nm (A) and 805 nm (B) in native R26 RCs. The initial absorbance decrease at 890 nm and the absorbance increase at 805 nm are due to the formation of P*, which decays within 2.5 ps under formation of P^+H^-. The decay of the absorbance increase at 805 nm is accompanied by the decay of the stimulated emission at 890 nm (∼40% of the total bleaching at 890 nm) and by a bleaching around 805 nm. For the oscillatory residual of the kinetics at 890 nm the FT spectrum is similar to that measured earlier[25] and includes a broad band around ∼150 cm^{-1} with some structure and discrete modes at 9, 27, 42, 84, 124 cm^{-1}, etc. The FT spectrum for the 805 nm kinetics includes also a broad 150 cm^{-1} band and a mode at 83 cm^{-1}. In the 0−70 cm^{-1} region the most pronounced modes at (12 ± 2) and (30 ± 3) cm^{-1} are characteristic of the 805 nm kinetics. It was suggested earlier[38,39] that these modes are related to protein motions along the ET coordinate. To further study this purported relation, the femtosecond kinetics at 890 and 805 nm in the RC of the YM210W mutant of *Rb. sphaeroides* were measured and analyzed (Figure 3) because in this RC the primary ET rate is considerably decreased with respect to the wild type (about 6 times at room temperature). The FT spectrum of the 890 nm kinetics in the mutant is very similar to that in R26 RCs, showing that the motions inside P are not changed in the mutant, as earlier found for low temperature.[18] The FT spectrum for the 805 nm kinetics has similar modes as for R26 RCs. However, the amplitudes of the 30 and 12 cm^{-1} modes are decreased by a factor of 2−4, close to that of the decrease of the ET rate. We suggest, therefore, that the weak amplitude of the low-frequency modes is related to the change of the ET rate in the mutant.

Another interesting question is whether the oscillations in the 805 nm region can be assigned either to one or to both of the bacteriochlorophyll monomers B_A and B_B. Comparison of the FT spectra of the 805 nm kinetics for native and B_B-modified R26 RCs shows that these spectra are similar to each other (Figures 2 and 4) with respect to the frequencies and amplitudes. Some decrease of the amplitudes of the 12 and 83 cm^{-1} modes and an increase of the broad band around 150 cm^{-1} are observed. These results indicate that the 33 cm^{-1} mode may be associated with B_A, the 12 cm^{-1} mode with both B_A and B_B, and the 83 cm^{-1} mode mostly with B_B. The broad 150 cm^{-1} structure may be due to both B_A and B_B with shifted phases, since in B_B-modified RCs this mode is more pronounced.

The possible influence of the reduction of the acceptor side on the femtosecond oscillation was studied in B_B-modified R26 RCs in which Q_A was doubly reduced (see Experimental Section). The FT spectrum for the 805 nm kinetics displays the 32 cm^{-1} mode, which is smaller in amplitude, and new modes at 51 and 94 cm^{-1} (Figure 5). These new modes are also seen in the 890 nm FT spectrum.

Discussion

To interpret the obtained results, it should be clarified to which state the measured absorbance changes at 805 nm and, consequently, to which state the oscillations can be attributed. At this wavelength, according to present knowledge, the induced absorption of the P excited state around 800 nm and a bleaching

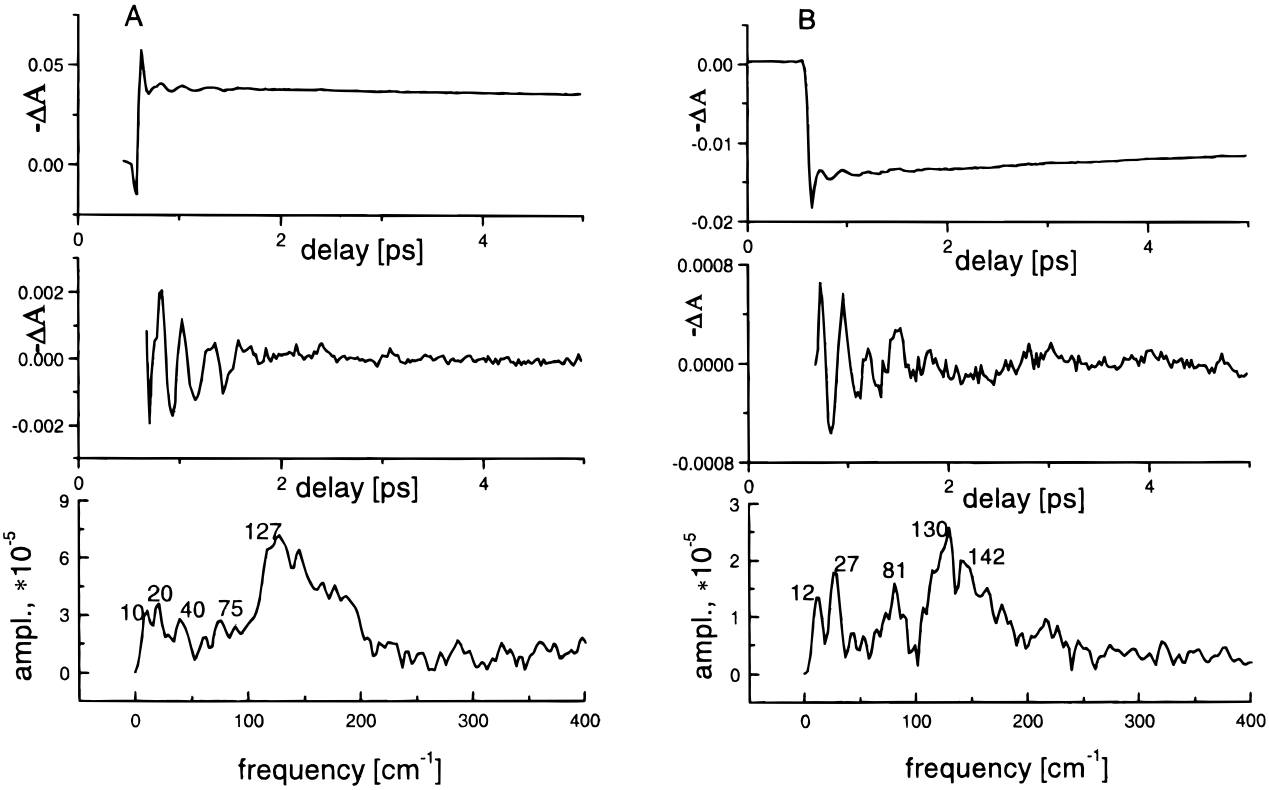

Figure 3. Kinetics of ΔA at 890 nm (A) and 805 nm (B) in the YM210W mutant of *Rb. sphaeroides* RCs (top). The oscillatory part is obtained by fitting the data to two-exponential kinetics (middle). Fourier transformation yields the frequency spectrum (bottom).

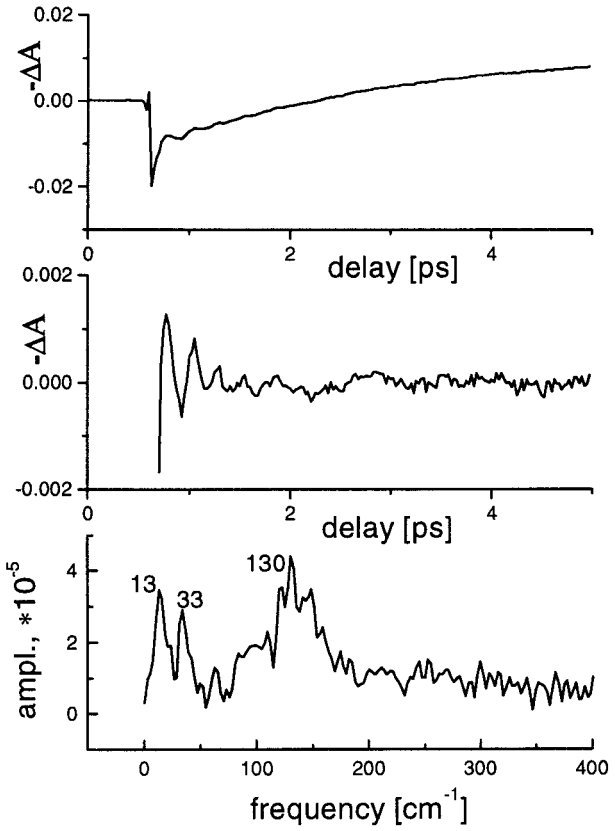

Figure 4. Kinetics of ΔA at 805 nm in B_B-modified R26 RCs (top). The oscillatory part is obtained by fitting the data to two-exponential decay kinetics (middle). Fourier transformation yields the frequency spectrum (bottom).

of the ground-state absorption band of the accessory bacteriochlorophyll B_A are observed. The bleaching of the upper excitonic state of the primary donor does not contribute significantly in our experiments because its transition moment is almost perpendicular to those of B and P* around 800 nm. In fact, the amplitude of the oscillations is much larger for parallel than for perpendicular polarization of the pump−probe beams (see also ref 39).

Concerning the oscillations around 800 nm, they could in principle also be due to the P* → P** transition when wave packet motions on the P* excited state potential surface occur. Another possibility might be a wave packet motion in the ground state of B generated indirectly through vibrational coupling in the P^*/P^- transition, possibly involving an admixture of P^+B^- in P*. This wave packet motion should be characterized by oscillations on the short- and long-wavelength sides of the B absorption band that are phase-shifted by 180°, since the bacteriochlorophyll molecule has an experimentally observable displacement of the excited state along the nuclear coordinate with respect to the ground state (without displacement, the oscillations are not observable). Figure 1, however, shows that, at least for the 150 cm^{-1} mode, these oscillations are in-phase. It follows that the observed oscillations do not correspond to ground-state oscillations of B. The same conclusion holds for the P* → P** transition. This means that we should attribute the oscillations in the kinetics at 805 nm to a change either of the B electron configuration due to reversible ET between P* and B or of the 800 nm dipole transition moment of B due to a strong coupling to the wave packet motion in the excited state of P.

To distinguish these two possibilities, we consider the oscillation frequencies for different wavelengths. The similarity of the oscillation spectra for the 890 and 805 nm kinetics, especially around the 150 cm^{-1} broad structure for the various RCs considered here, demonstrates the coupling of the wave packet motions in P* with the changes in dipole strength around 800 nm. It is possible that these changes are related to the

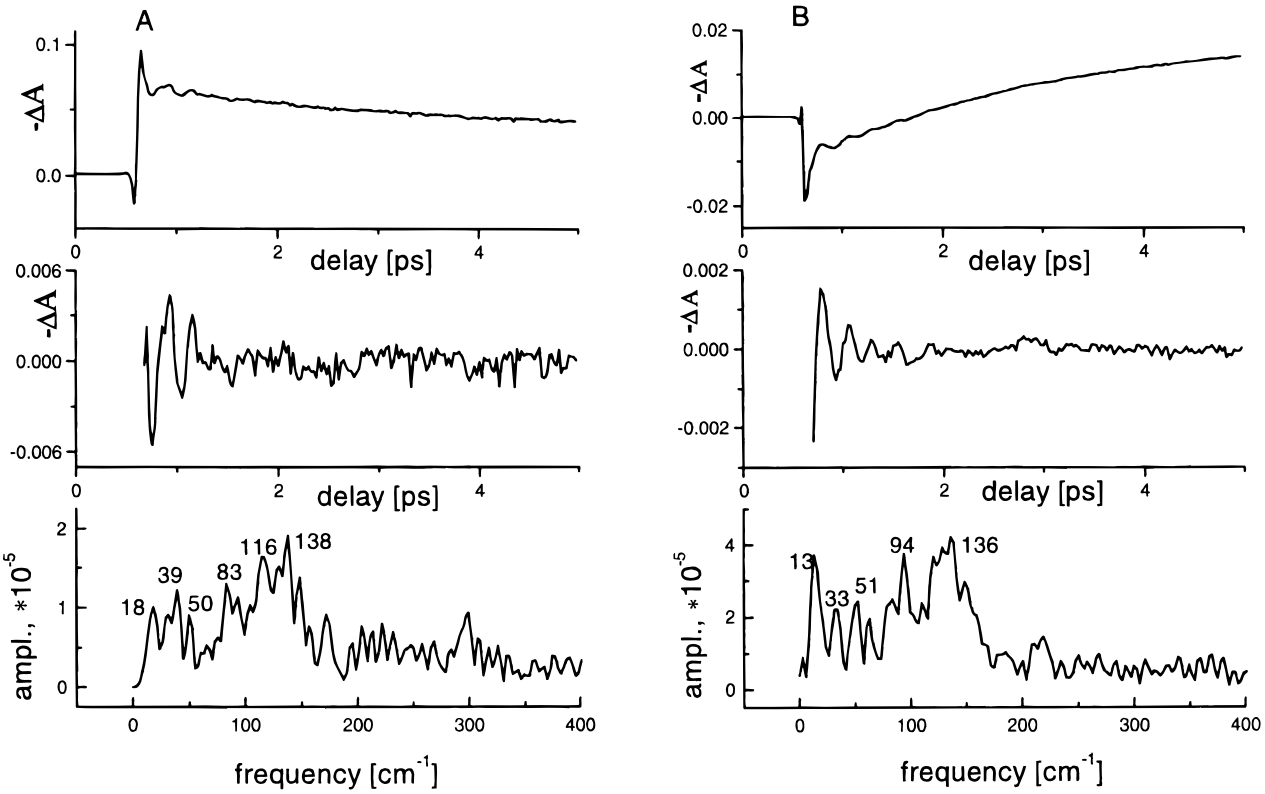

Figure 5. Kinetics of ΔA at 890 nm (A) and at 805 nm (B) in B_B-modified R26 RCs in the presence of Q_A^{2-} (top). The oscillatory part is obtained by fitting the data to two-exponential decay kinetics (middle). Fourier transformation yields the frequency spectrum (bottom).

formation of the charge-transfer character in the P* state (see ref 45), the degree of which might depend on nuclear coordinates and hence is modulated by vibrational motions. Possibly, the charge transfer inside P* includes also a small admixture of $P^+B_A^-$, thus serving as a starting point for the charge separation between P* and B_A.

The main differences between the oscillations spectra (Figure 2) measured around 890 nm (stimulated emission of P*) and around 805 nm (B band) are a relative enhancement of the 12 cm^{-1} mode, the shift of the 27 cm^{-1} mode to 32 cm^{-1}, and the enhancement of the latter mode relative to the broad structure around 150 cm^{-1} in the 805 nm spectrum.

The relative enhancement of the 12−30 cm^{-1} modes in the 805 nm kinetics with respect to the 890 nm kinetics (see also ref 39) suggests that these motions involve the formation of a greater fraction of $P^+B_A^-$ in the P* state. In other words these oscillations can be along the reaction coordinate. This suggestion is consistent with the considerable decrease of the amplitudes of these modes in the 805 nm kinetics in the YM210W mutant, in which the ET rate is concomitantly decreased. On the other hand the relative enhancement of the 12−30 cm^{-1} modes in the 805 nm kinetics with respect to the 890 nm kinetics is consistent with the molecular dynamics calculations,[46] which show the presence of 17 cm^{-1} oscillations in the pigment−protein complex of RC that modulate the distance between P and B. This motion involving the protein part could be important for ET. Therefore, it is realistic to suggest that the 12−30 cm^{-1} modes in the 805 nm kinetics are related to reversible electron transfer between P* and B_A, which modulates the dipole strength of B_A.

In B_B-modified RCs in the presence of doubly reduced Q_A, the decrease of the 32 cm^{-1} mode is accompanied by the appearance of a new mode at 50 cm^{-1} and its second harmonic at 95 cm^{-1}, seen both in the 890 and 805 nm kinetics (Figure 5). One might suggest that in RC with the doubly reduced Q_A a shift of electron density occurs from Q_A^{2-} to Fe^{2+}, leading to a dilatation of the coordination bonds of the Fe^{2+} ion with its ligands (histidines in the D and E α-helices), similar to observations in PSII reaction centers.[47] This dilatation can be responsible for a partial loss of the connection between the two D transmembrane α-helices, which also contain two histidines liganding the Mg atoms of the bacteriochlorophyll dimer P. Such a change of interaction between different parts of the protein may give rise to the change of vibrational frequencies in the presence of doubly reduced Q_A.

Conclusions

The observed oscillations in the femtosecond kinetics of the stimulated emission from P* (Figures 1−5) in native, B_B-modified, and mutant (YM210W) RCs at 293 K excited by 30 fs pulses at 865 nm show oscillation frequencies similar to those found earlier for native RCs at low temperature[24] and room temperature[25] and to those found from hole-burning experiments[27] and resonance Raman spectra.[29−32] The femtosecond oscillations are also observed in the kinetics near 805 nm. The high-frequency components (∼150 cm^{-1}) have a positive anisotropy and an amplitude spectrum (as a function of probing wavelength) with a maximum at 805 nm and a halfwidth of ∼25 nm,[39] similar to the features of the B_A absorption band. It was concluded earlier[39] that the oscillations at 805 nm are not due to an upper excitonic transition of P*, which would have a negative anisotropy. The absence of a phase shift of the oscillations at the short- and long-wavelength sides of the 805 band for high-frequency modes (∼150 cm^{-1}) modes shows that at least these oscillations are not due to wave packet motions in the ground state of B or to the P* → P** transition. The frequency spectrum of the femtosecond oscillations for the 805 nm kinetics shows frequencies similar to those for the 890 nm kinetics but with relatively enhanced amplitudes for two modes at 30 and 12 cm^{-1}.

This suggests a selective coupling of vibrational modes to the B molecules. The relative enhancement of the 12−30 cm^{-1} modes in the 805 nm kinetics is consistent with molecular dynamics calculations, which show[46] the presence of 17 cm^{-1} oscillations in the pigment−protein complex of RC. This motion may modulate the distance between P, which could be important for ET. We suggest that the suppresion of these modes in the YM210W mutant (Figure 3B) may be associated with the decrease of ET rate in this mutant, especially if these modes are along the reaction coordinate.

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