Subpicosecond Excited-State Relaxation of the Accessory Bacteriochlorophylls in Native and Modified Reaction Centers of *Rb. sphaeroides* R26

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Energy transfer within native and borohydride treated (chemically modified) reaction centers (RCs) isolated from *Rhodobacter sphaeroides* R26 was investigated, in particular from the excited accessory bacteriochlorophylls (B*) to the primary donor (P). The decay kinetics of chemically modified and native RCs show some similarities as well as distinct differences. In reduced RCs, B* decays with a time constant of 100 fs, both in native and in modified RCs, followed by a partial recovery of the bleaching with a time constant of about 3 ps due to charge separation. In native RCs, however, an induced absorption is observed with a maximum at a delay of 500 fs, which is absent in chemically modified RCs. The initial bleaching in modified and in native RCs is characterized by an anisotropy of 0.4. After the excitation is transferred from B* to P, the anisotropy in modified RCs is decreased to a value of about 0.2. In native RCs the time-resolved anisotropy varies and depends strongly on the wavelength of detection. These observations are analyzed and discussed in terms of the individual contributions of B_A and B_B to the absorbance kinetics at different wavelengths. In oxidized RCs, in addition to a fast relaxation of about 100 fs in the decay of B*, we observed, a decay component with a time constant of ∼400 fs.

Introduction

Light energy is converted into the energy of charge separation in the photosynthetic reaction center (RC). The RC of the purple bacterium *Rhodobacter (Rb.) sphaeroides* R26 has an approximately 2-fold symmetry with two groups of chromophores in addition to the primary electron donor P, located in the A- and B-branch. Each branch contains an accessory bacteriochlorophyll ($B_{A,B}$), a bacteriopheophytin ($H_{A,B}$), and a quinone ($Q_{A,B}$). It has been established that only the A-branch is active in electron transfer.

It has been shown that, upon excitation of the primary donor P, an electron is transferred from P to H_A in about 3 ps.[1] The role of B_A in this process is still a matter of contention,[2] but experimental evidence has been obtained which suggests that stepwise electron transfer takes place, first from P* to B_A, forming a $P^+B_A^-$ state with a time constant of 2.3 ps, and subsequently from B_A^- to H_A with a time constant of 0.9 ps.[3−5] The relative magnitude of the two time constants involved makes the observation of a $P^+B_A^-$ state difficult.[5]

The strong dipolar interactions between the chromophores in the RC imply that optical excitation of either the bacteriopheophytins or the accessory bacteriochlorophylls is followed by rapid downhill energy transfer to the primary donor. This process has been shown to be extremely fast, exceeding the theoretical estimates of the transfer rates involved.[6] Energy transfer in isolated RCs of *Rb. sphaeroides* R26 upon direct excitation of the accessory bacteriochlorophylls has been the subject of a number of recent investigations.[7−10] All these reports show that energy is transferred in about 100 fs from the accessory bacteriochlorophylls to the primary donor, but there is no consensus about the basic mechanism involved.

Haran et al.[8] have investigated several near- and mid-IR transitions of the primary donor by time-resolved anisotropy measurements with excitation at 800 nm. They suggest a mixing of the upper exciton state of P (P_{Y+}) and the excited states of the accessory bacteriochlorophylls to form a "supermolecular" delocalized state. The fast transients are thought to be associated with an internal conversion process of the mixed states to the lower exciton state of P (P_{Y-}).

Stanley et al.[10] studied the B* → P energy transfer process at different temperatures by time-resolved fluorescence up-conversion and observed time constants varying from 165 fs at 85 K to 120 fs at 200 K. Apparently, the energy transfer rate is rather insensitive to a change in temperature, contrary to what is expected on the basis of the Förster mechanism.[11] Accordingly, Stanley et al.[10] conclude that an exchange mechanism[12] should be considered to be active in this energy transfer process.

Jonas et al.[9] performed anisotropy measurements in the 800 nm band. They find a rapid decay of the anisotropy. To explain this behavior, they assume that the upper exciton level of the primary donor (P_{Y+}) is involved as a real intermediate in the energy transfer from B* to P_{Y-}. This results in a two-step model with energy transfer from B* to P_{Y+} in 75 fs as the first step, followed by an internal conversion from P_{Y+} to P_{Y-} in 165 fs.

In the experiments by Jonas et al.[9] both accessory bacteriochlorophylls, B_A and B_B, were excited, while in probing the 800 nm band the full spectral content of the probe pulse was monitored without additional wavelength selection. Therefore, the differences in the absorption spectra of B_B and B_A around 800 nm have not been taken into consideration, nor has the possibility of a different response of B_A and B_B to events following direct optical excitation. We note that B_A and B_B do

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not absorb exactly at the same wavelength. In fact, at low temperatures B_A has an absorption maximum at 802 nm and B_B at 812 nm. At room temperature these bands are broadened, but still a difference in absorbance is found.[13] Moreover, it has been reported that the absorption band of B_B exhibits a shift upon formation of P* which has to be taken into account in the interpretation of the absorbance kinetics of the 800 nm band.[14,15]

In this paper we report the results of experiments in which we reanalyzed the absorbance decay kinetics in isolated RCs of *Rb. sphaeroides* R26 around 800 nm, following excitation of the accessory bacteriochlorophylls, $B_{A,B}$, as a function of detection wavelength and of the relative polarization of pump and probe pulses. In particular, we have compared the differences between (1) "oxidized" RCs, where P is oxidized with ferricyanide, and (2) "reduced" RCs, where the secondary electron transport $P^+H_A^- \rightarrow P^+Q_A^-$ was blocked. This comparison was done for two types of RCs: native RCs and chemically modified RCs that were treated with borohydride.[16−20] The latter are characterized by a shift of the B_B absorption band to shorter wavelengths (around 765 nm).[16−20] These chemically modified RCs are of great interest for an assessment of the individual contributions of B_B and B_A to the decay kinetics because they offer the possibility to excite B_A selectively.

Experimental Procedures

Materials. RCs of *Rb. sphaeroides* R26 (carotenoidless mutant) were isolated and purified as described previously.[16] Chemically modified RCs were prepared by treatment of isolated RCs of *Rb. sphaeroides* R26 with sodium borohydride as described by Shuvalov and co-workers.[16] The absorbance at 800 nm of the samples used in the measurements was 0.56/mm for native and 0.58/mm for modified RCs. To keep the RCs in the reduced state, sodium ascorbate (50 mM) was added to the sample, together with DAD (2,3,5,6-tetramethyl-1,4-phenylenediamine) (1 mM) as a mediator. Sodium ascorbate reduces the special pair cation in photooxidized RCs, thus prohibiting the accumulation of P^+ and leaving reduced quinones Q_A^-.[21] In some of the experiments, "oxidized" RCs were used, in which P had been oxidized chemically by the addition of potassium ferricyanide to a concentration of 4 mM.

Laser System. A mode-locked Ti:sapphire laser was homebuilt according to the design of Asaki et al.[22] with the addition of a cavity dumper as described by Pshenichnikov et al.[23] to provide a variable pulse repetition rate and an increased pulse energy. The laser was pumped by an argon ion laser (Coherent-Innova) at about 5.5 W. The output of the Ti:sapphire laser was centered at a wavelength of 820 nm with a bandwidth of 90 nm (fwhm). The experimental setup consisted of a pump−probe configuration with either parallel or perpendicular polarization of pump and probe beams. The polarization was set with a dispersion compensated waveplate (CVI). The probe beam was obtained by splitting off a fraction of the excitation beam. A band-pass filter was inserted in the pump beam to limit the bandwidth to 24 nm (fwhm), with a maximum at 799 nm. This narrowed band covers the absorption band of the accessory bacteriochlorophylls, while at the same time the direct excitation of the primary donor and the pheophytins is limited to less than 10%. Both beams were precompensated for dispersion by double passing of two fused silica prisms in the optical path. This resulted in an instrumental response function of about 60 fs (fwhm), which corresponds, assuming a $sech^2$ pulse shape, to near transform-limited pulses of 38 fs. The pump pulse energy used for experiments was less than 2 nJ/pulse. The pulse repetition rate was set to 1 MHz, while the pump beam was modulated at 1 kHz by a mechanical chopper in the beam. The change in transmission of the probe beam was monitored by an integrating photodiode in combination with phase-sensitive detection referenced to the 1 kHz signal of the chopper. The probe wavelength was selected by a monochromator (bandwidth 1 nm). The sample was contained in a rotating optical cell spinning at about 3000 rpm with a sample thickness of 1.5 mm (sample volume of 2 mL), providing a linear velocity of 16 m/s of the sample. This velocity was sufficient to provide a fresh sample volume for each excitation pulse up to a pulse repetition rate of 1 MHz. The spot diameter in the focus of both beams was 30 μm. All measurements were performed at room temperature.

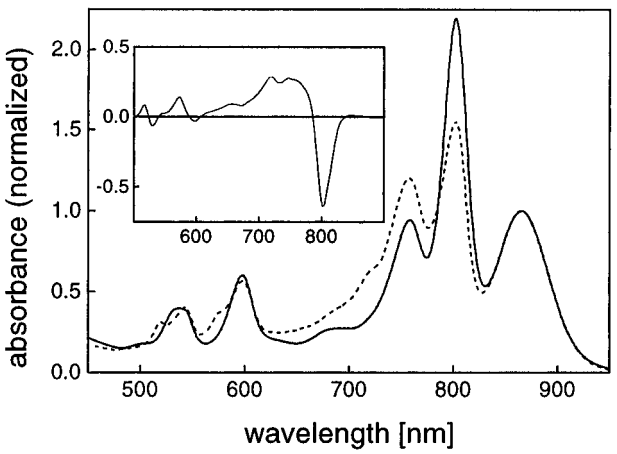

Figure 1. Absorbance spectrum of native (solid) and chemically modified (dashed) RCs of *Rb. sphaeroides* R26 at room temperature. The spectra are normalized at 865 nm. The inset shows the difference of the absorbance spectra of the chemically modified and native RC spectra.

Data Analysis. The data were fitted with a convolution of the instrument response function and a sum of exponentials. The errors were estimated from analysis of the data of several independent measurements. For calculation of the anisotropy the data for parallel and perpendicular polarization were fitted with the same set of time constants, taking into account the convolution with the instrument response function. Anisotropy values were calculated as $r(t) = (\Delta A_{\parallel} - \Delta A_{\perp})/(\Delta A_{\parallel} + 2\Delta A_{\perp})$. The time dependence of the anisotropy was calculated both from the raw and from the fitted data.

Results

It has been known for some time that treatment of isolated RCs with sodium borohydride leads to a partial loss of absorbance in the 800 nm absorption band.[16,18,19] The absorbance spectrum of such chemically modified RCs, together with that of native RCs, is shown in Figure 1 and is in agreement with earlier reports.[16,18,19] It has been shown by Struck et al.[20] that the borohydride treatment leaves the tetrapyrrole structures of pigments intact and that each RC still contains two bacteriopheophytins and four bacteriochlorophylls. Inspection of the difference spectrum of native and modified RCs (inset, Figure 1) shows that a loss of absorbance in the 800 nm band is accompanied by an absorbance increase in the range 700−780 nm, a feature which was also noted by Struck et al.[20] From low-temperature measurements[24] it has been concluded that in borohydride-treated RCs the contribution of B_B at 812 nm has fully disappeared. Taken together, these observations indicate that the Q_y absorption band of the accessory bacteriochlorophyll in the inactive branch, B_B, is shifted to a broad absorbance around 765 nm in such modified RCs.[16−19] It seems reasonable to conclude that the effect of the borohydride treatment is limited

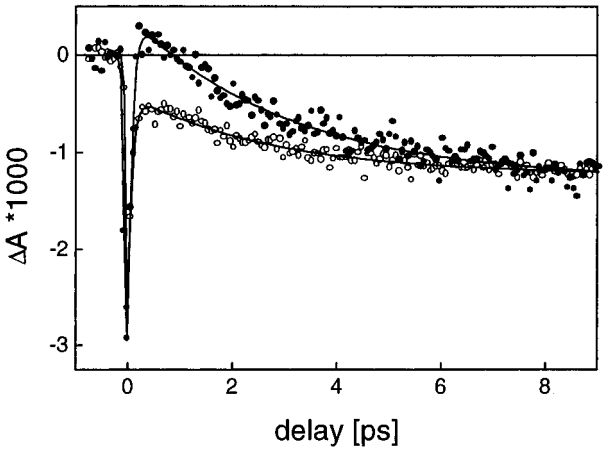

Figure 2. Transient change in absorbance for native (dots) and chemically modified (circles) RCs excited at 799 nm and probed at 808 nm at room temperature. The lines show the fits with parameters described in the text.

to the inactive branch. This is supported by the observation that the effect of the borohydride treatment is inhibited in RCs of wild-type *Rb. sphaeroides*, probably because the carotenoid is blocking access to the B_B site.[25] Moreover, the primary electron transport is essentially unaffected in these chemically modified RCs.[16,24,26] We also note that the shape and position of the P-absorption band at 870 nm are unchanged, which indicates that the structure of the primary donor remains intact. Thus, we conclude that the borohydride treatment induces changes in the M-subunit[17] in the immediate vicinity of the binding pocket of the chromophore, which results in the observed blue-shift of the B_B absorption. On the basis of a more comprehensive spectroscopic and chemical analysis, Struck et al.[20] have reached similar conclusions.

Figure 2 shows the kinetics of the absorbance changes of the reduced native and modified RCs at 808 nm, induced by optical excitation with 38 fs pulses centered at 799 nm. The signal is characterized in both cases by a bleaching that decays very rapidly with time constants of 95 ± 20 and 100 ± 20 fs for native and modified RCs, respectively (see also Figure 6). This rapid decay is followed by a partial recovery of the bleaching on a time scale of picoseconds. This recovery has a time constant of 2.6 ± 0.2 ps in the case of native RCs and of 2.3 ± 0.3 ps for chemically modified RCs. The recovery of the bleaching on this longer time scale is due to a bleaching and an electrochromic shift of the 800 nm band which accompanies the electron transfer from P* to H_A (see below). The observed time constants for native RCs are in good agreement with earlier reports.[1,7]

We also probed the bleaching of the Q_y band of the primary donor at 860 nm following excitation of the accessory bacteriochlorophylls at 799 nm (data not shown). The bleaching does not occur instantaneously but develops with a rise time of 100 ± 20 fs for modified RCs and 90 ± 30 fs for native RCs, confirming the estimates by Breton et al.[27] Within the experimental error these time constants are identical with those of the rapid recovery of the ground-state absorption of the Q_y bands of the accessory bacteriochlorophylls at 800 nm.

A distinct feature of the kinetics in Figure 2 is the induced absorption around a delay of 500 fs in native RCs, which is absent in chemically modified RCs. In the latter case only B_A is excited, and the absorbance changes reflect the energy transfer from B_A* to P and subsequent electron transfer. The different kinetics of native and modified RCs strongly suggest that B_A and B_B respond differently to the formation of the P* state.

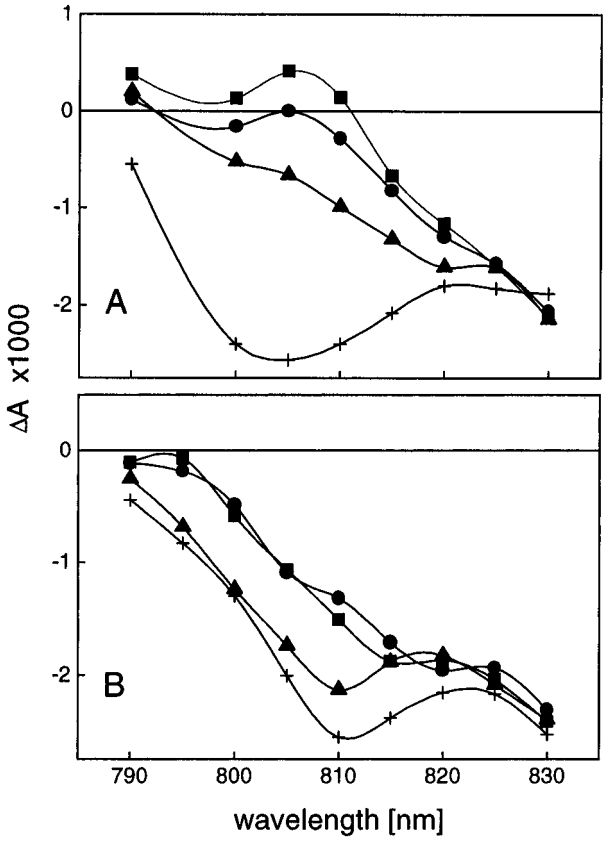

Figure 3. Time-resolved isotropic absorbance difference spectra for (A) native and (B) chemically modified RCs of *Rb. sphaeroides* R26 excited at 799 nm at a delay of 0.2 (cross), 0.4 (dot), 0.6 (square), and 1.5 ps (triangle).

To analyze the spectral distribution of the various kinetic components, absorbance changes were measured as a function of detection wavelength. The results for native and chemically modified RCs are presented in Figure 3 in the form of time-resolved absorbance difference spectra. The induced absorption is clearly present in the difference spectra of native RCs as a band with a maximum at 805 nm. The induced absorption is maximal at 0.5−0.6 ps delay and disappears at longer delays. In chemically modified RCs this feature is absent. These spectra clearly show that the 800 nm bleaching in native RCs is heterogeneous on the subpicosecond time scale and presumably reflect the different behavior of B_B and B_A. Since in chemically modified RCs the absorbance changes in the 800 nm region are only influenced by B_A, these results imply that the electrochromic band shift around 800 nm upon $P^+H_A^-$ formation is mainly due to B_A.

Significant differences between native and modified RCs are also observed in the time development of the anisotropy. Figure 4 shows the kinetics around 805 nm with parallel and perpendicular polarization of the pump and probe pulses for native RCs (Figure 4A) and chemically modified RCs (Figure 4B). From these polarization measurements, anisotropy values were calculated from the raw data and from the fitted data, as shown in the insets in Figure 4.

In modified RCs the initial anisotropy in the 800 nm band remains essentially constant at a value of 0.4 during the initial fast decay of B_A*. This value corresponds to the maximum anisotropy of a transition in an isolated molecule. After about 100 fs the anisotropy rapidly decays to a value of 0.24 ± 0.02 at 805 nm and then remains constant (inset, Figure 4B). At wavelengths longer than 825 nm the initial anisotropy is 0.20 ± 0.01 and does not change (not shown). At these longer

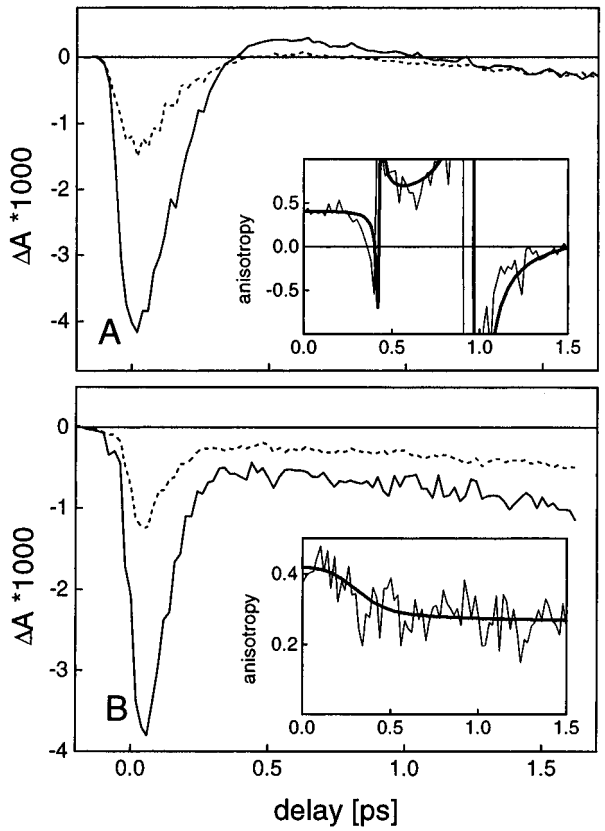

Figure 4. Time-resolved absorbance changes with parallel (solid line) and perpendicular (dashed line) polarizations of pump and probe pulses, excited at 799 nm and probed at 805 nm, for (A) native RCs and (B) chemically modified RCs. The insets show the raw anisotropy (thin line) and anisotropy values calculated from the fitted curves of the parallel and perpendicular data sets (thick line).

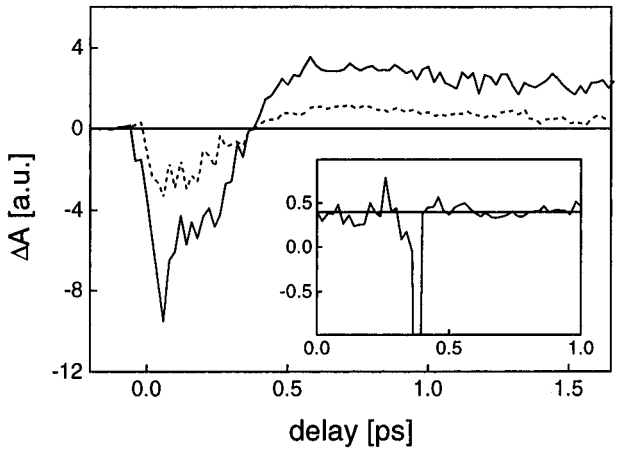

Figure 5. Time-resolved absorbance change of B_B only at parallel (solid line) and perpendicular (dashed line) polarization of pump and probe pulses, at 805 nm, calculated as described in the text. The inset shows the time-resolved anisotropy of the B_B contribution.

wavelengths the signal is dominated by the contribution of the primary donor.

At longer wavelengths, we also observe in native RCs an anisotropy of 0.2, which is constant on the time scale of the measurement (data not shown). At 805 nm the initial anisotropy of about 0.4 (inset, Figure 4A) is similar to that in modified RCs. However, after the initial fast decay, the bleaching observed with parallel as well as perpendicular polarization converts to an induced absorption. After this conversion point the anisotropy becomes high, indicating that the induced

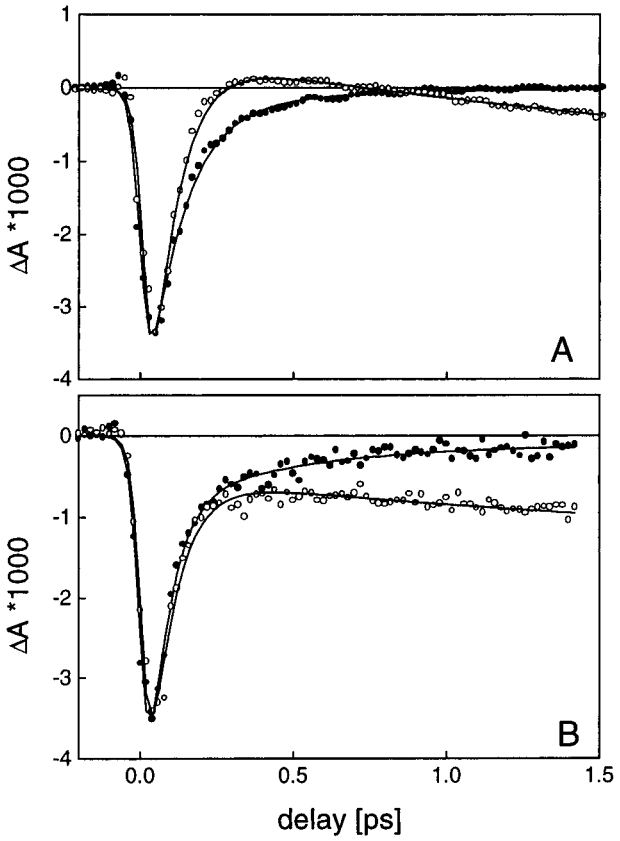

Figure 6. Transient change in absorption of reduced (circles) and oxidized (dots) RCs of *Rb sphaeroides* R26 probed at 808 nm and excited at 799 nm, (A) native RCs, (B) chemically modified RCs. The lines show the fits with parameters described in the text.

absorption is an effect of a molecule with the same orientation of the transition moment.

Since in borohydride-treated RCs B_A is selectively excited, the time-resolved anisotropy in chemically modified RCs can be assigned to effects associated with the excitation of B_A only. Therefore, we can use this information to determine the contribution of B_B to the anisotropy in native RCs. Given the bandwidth of excitation and the loss of the 800 nm band in chemically modified RCs, it is reasonable to assume that B_A and B_B are excited in a ratio 2:1. With this assumption, we can subtract the absorption changes of B_A, by subtracting two-thirds of the signal for modified RCs, where we assume that only B_A is excited, from that of native RCs. This results in the absorbance changes of B_B only, as shown in Figure 5. Here we clearly see that B_B is responsible for the induced absorption and that the anisotropy for the B_B contribution remains about 0.4 on the time scale of the measurement (inset).

Having calculated the B_B contribution for different wavelengths in the 800 nm band, we find a maximum for the induced absorption at 805 nm, which is consistent with time-resolved absorbance difference spectra. In these spectra an increase in absorption is present around 805 nm at a delay of 0.5 ps (Figure 3).[2,14,28−30]

Figure 6A shows the transient absorption signals at 808 nm for oxidized and for reduced native RCs of *Rb. sphaeroides* R26, following excitation with 38 fs pulses at 800 nm. In both cases a fast instantaneous bleaching is observed, which decays in about 100 fs. In comparison with reduced RCs (see also Figure 2), the partial recovery of the bleaching on a picosecond time scale is absent when the primary donor is oxidized by ferricyanide. This picosecond recovery of the bleaching in reduced RCs can be attributed to a shift and a bleaching within

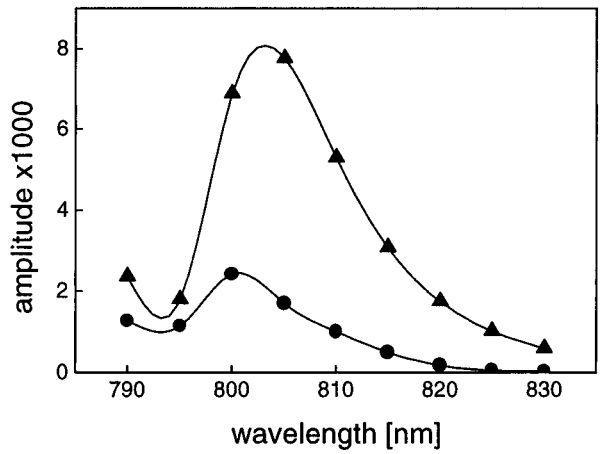

Figure 7. Wavelength dependence of the amplitudes of the 100 (triangle) and 400 fs (dot) time constants in oxidized native RCs.

the 800 nm band associated with the formation of the charge-separated state $P^+H_A^-$, a process which, of course, is inhibited in oxidized RCs. Fitting the data for oxidized native RCs resulted in a biexponential decay with time constants of $\tau_1 = 75 \pm 20$ fs ($A_1 = 85 \pm 4\%$) and $\tau_2 = 390 \pm 30$ fs ($A_2 = 15 \pm 4\%$). It should be pointed out that the dominant time constants τ_1 in oxidized and reduced RCs are very similar. It was found that the amplitude of the ~400 fs component for oxidized RCs is wavelength dependent, with a maximum amplitude at 800 nm (Figure 7). This spectrum differs from the spectrum of the 100 fs component, where the maximum is located at 805 nm. These results show that in oxidized RCs an additional component of ~400 fs is present, with a rather similar wavelength distribution as the absorption of the accessory bacteriochlorophylls. For oxidized modified RCs (Figure 6B) we obtained $\tau_1 = 95 \pm 20$ fs ($A_1 = 85 \pm 3\%$) and $\tau_2 = 435 \pm 30$ fs ($A_2 = 15 \pm 3\%$), results which are very similar to those for oxidized native RCs.

Polarization measurements (data not shown) on oxidized native RCs show that the anisotropy of the ~100 fs as well as that of the ~400 fs component has a value of 0.4, independent of the delay between pump and probe pulse. Thus, it is most likely that these components originate from different relaxation paths following excitation of B in ferricyanide oxidized RCs, due to the presence of P^+.

Discussion

Spectroscopic and chemical analyses support previous results which indicate that in borohydride-treated RCs the Q_y absorption of B_B has shifted to shorter wavelength and that in such chemically modified RCs the absorbance around 800 nm originates from B_A only.[16,18−20] Comparing the absorbance difference kinetics with B_A excitation only (chemically modified RCs) with those with simultaneous excitation of B_A and B_B (native RCs), it is possible to distinguish between processes involving B_A and B_B.

First, we have investigated the energy transfer from B_A* and B_B* to the primary donor. The time constants for the decay of the bleaching in modified (100 ± 20 fs) and native (95 ± 20 fs) RCs are identical. Assuming that in native RCs half of the excitations go to B_B, we conclude that the energy transfer rate from B_A* to P is the same as that from B_B* to P. This is in agreement with theoretical simulations of Haran et al.,[8] who have calculated times of 158 fs for $B_A^* \rightarrow P$ and 130 fs for $B_B^* \rightarrow P$, and with experimental results of Stanley et al.[10]

The primary charge separation in isolated RCs has been extensively investigated over the past decade by time-resolved spectroscopic methods. It has been suggested that the accessory bacteriochlorophyll B_A is a real intermediate for electron transfer from the primary donor P to the bacteriopheophytin H_A.[4,5,26] We refer to this mechanism as a sequential process. Alternatively, an electron may be transferred directly to H_A by the superexchange mechanism. If we now excite one or both of the accessory bacteriochlorophylls in the RC, the primary charge separation is preceded by an energy transfer step from B_A* or B_B* to P. Adding this step to the familiar kinetic schemes for electron transfer results in models (1) and (2) for sequential electron transfer and for the superexchange mechanism, respectively, which should describe the observed absorbance changes following excitation of B_A or B_B, we obtain

$$PB_{A,B}{}^*H_A \xrightarrow{k_A} P^*B_{A,B}H_A \xrightarrow{k_B} P^+B_A{}^-H_A \xrightarrow{k_C} P^+B_AH_A{}^- \quad (1)$$

$$PB_{A,B}{}^*H_A \xrightarrow{k_A} P^*B_{A,B}H_A \xrightarrow{k_D} P^+B_AH_A{}^- \quad (2)$$

The decay of $PB_{A,B}{}^*H_A$ and the formation of $P^+B_A{}^-H_A$ affect the bleaching of the B_A absorption band directly because of the involvement of B_A* and $B_A{}^-$, respectively, while the $P^+B_AH_A{}^-$ formation induces a bleaching and an electrochromic shift of the 800 nm band.[3−5] The rate constant for the energy transfer step (k_A) is determined from the rapid recovery from the bleaching of the 800 nm band shown in Figure 2.

In chemically modified RCs, with excitation of B_A only, the absorbance kinetics of the 800 nm band, as shown in Figure 2, can be simulated by the sequential electron transfer mechanism, if we assume that the first two steps only affect the degree of absorbance of B_A and that the third step gives rise to a shift and/or a bleaching of the B_A absorption band. The simulation for modified RCs is shown in Figure 8B,D. Rate constants of $(100 \text{ fs})^{-1}$, $(3.5 \text{ ps})^{-1}$, and $(0.9 \text{ ps})^{-1}$ were used for k_A, k_B, and k_C, respectively; the amplitudes of the bleaching of B_A and that associated with the electrochromic shift were determined by the absorbance changes at zero delay and at infinity, respectively. We have also assumed that 5−10% of the excitations are directly absorbed by P, based on an estimate of the small overlap between the absorption band P and the spectrum of the excitation. An equally good fit could be obtained by the superexchange mechanism, but only with a slightly higher contribution (10−15%) of processes related to direct excitation of P. In this model rate constants for chemically modified RCs of $(100 \text{ fs})^{-1}$ and $(2.2 \text{ ps})^{-1}$ were obtained for k_A and k_D, respectively.

If we now apply the same assumptions to the case of native RCs, when both B_A and B_B are excited, it is evident that the kinetic model predicts the same absorbance changes as in modified RCs where only B_A is excited. This is clearly in contradiction with the experimental observation that the initial bleaching converts to an induced absorption, followed by a slow (partial) recovery of the bleaching. Therefore, in native RCs we have to assume an effect of P* on B_B to account for the induced absorption. The possibility that P is also affected by the borohydride treatment, and contributes to the differences between native and modified RCs, can be ruled out when spectroscopic data of both types of RCs are compared.[17] We suggest that the absorbance kinetics of native RCs in the 800 nm band are governed by a shift of the B_B absorption band to slightly shorter wavelength upon formation of P*. This suggestion is corroborated by experimental observations: time-resolved absorbance difference spectra[14,28−30] of the 800 nm region show a band shift around 805 nm when P is excited. Since this band shift is absent in chemically modified RCs (see Figure 3), we conclude that the band involved in this shift has

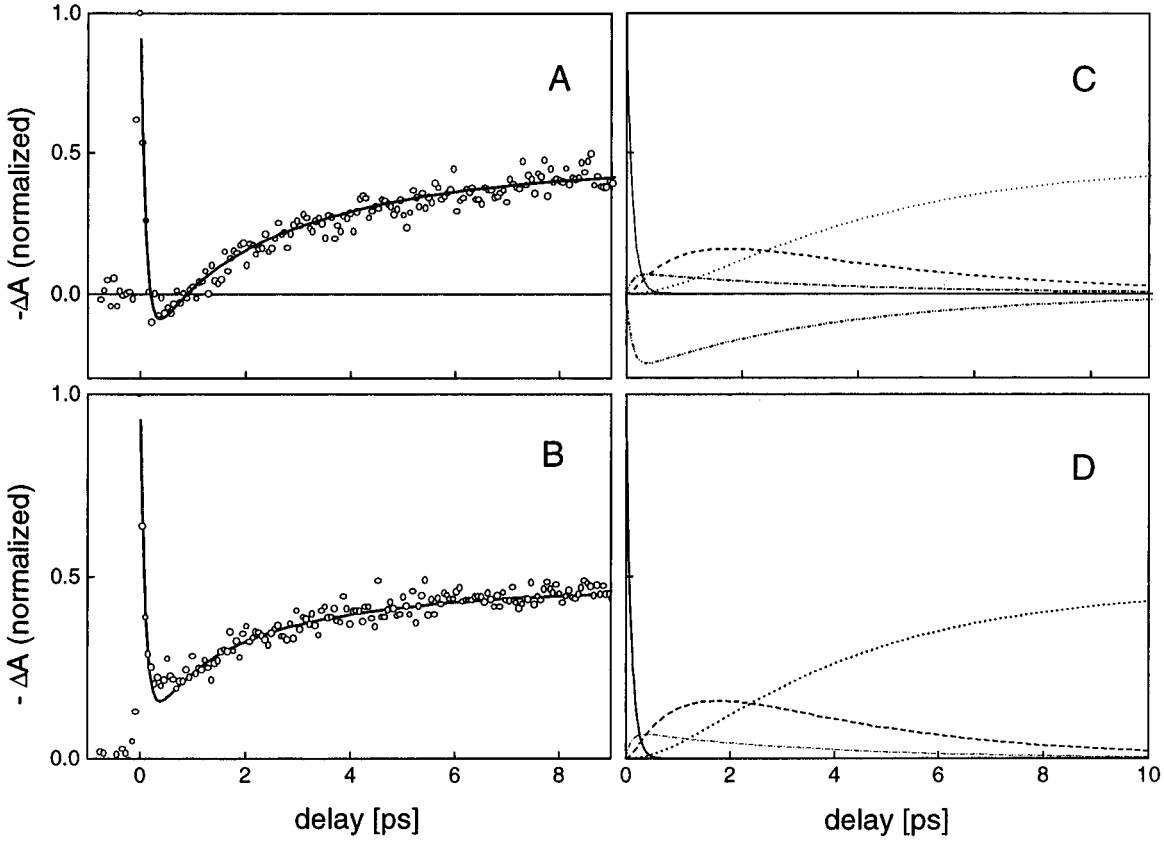

Figure 8. Simulations (solid line) of the kinetics (circles) at 808 nm for native (A) and chemically modified RCs (B) (parameters, see text). The simulated curves are the sum of the various contributions to the absorbance changes in the 800 nm region of native RCs (C) and of chemically modified RCs (D), in particular of B* (solid line) and $P^+B_A^-$ (dashed line); electrochromic shift upon $P^+H_A^-$ formation (dotted line); contribution of P*, by direct excitation (dot-dashed line); shift of the B_B absorption band upon P* formation (dot-dot-dash line).

to be attributed to B_B.[14,28,31] The band shift gives rise to the induced absorption with a maximum at 805 nm at a delay of a few hundred femtoseconds, followed by an electrochromic shift when an electron is transferred to H_A (Figure 3). The origin of the signal at 805 nm is not clear, but one possibility is that this band shift arises from altered electrostatic fields within the reaction center, due to the charge transfer character of the state P*. It is probably more likely that the excitonic interactions between the pigments change upon formation of P*.[14] In principle, the B_A absorption band is expected to shift as well, but so far it seems that predominantly the B_B absorption band is affected.[14,28] When we include this shift of the B_B absorption band in the kinetic model, we can accurately reproduce the observed kinetics (Figure 8A) in terms of the sequential model of native RCs, with rate constants of $(100\ \text{fs})^{-1}$, $(3.3\ \text{ps})^{-1}$, and $(0.9\ \text{ps})^{-1}$ for k_A, k_B, and k_C, respectively. The simulation is shown in Figure 8C.

To simulate the kinetics of native RCs and reproduce the induced absorption around a delay of 500 fs with the superexchange model, it is also necessary to take into account a shift of the B_B absorption band upon formation of P*. With this model rate constants of $(100\ \text{fs})^{-1}$ and $(2.6\ \text{ps})^{-1}$ are used for k_A and k_D, respectively. Irrespective of the model, it can be concluded that a contribution of a shift of the B_B absorption band should be taken into account in a model for the transient absorbance changes around 800 nm in native RCs.

A critical test for the validity of these kinetic models is provided by the time dependence of the anisotropy measurements. The experimental results at 805 nm for native and chemically modified RCs are shown in Figure 4, A and B, respectively. In chemically modified RCs the time dependence of the anisotropy is clearly different than in native RCs. The initial anisotropy in modified RCs at 805 nm is 0.4 and is constant during the lifetime of B_A*, after which it drops to 0.24 (Figure 4B, inset). This latter value is presumably determined by the state P^+H^-, because it is also observed (but then time independent) at wavelengths between 820 and 850 nm.

In native RCs the situation is more complicated. The anisotropy is initially equal to 0.4 and is also constant at early times, but at later times shows large variations due to the effect of the induced absorption (Figure 4A, inset). The curves for the two different polarization directions actually cross the zero line at a delay of about 400 fs; the polarization after this point remains high, however. Because these features are absent in modified RCs, we conclude that the anisotropy measurements for native RCs are perturbed by the blue-shift of the B_B absorption band upon P* formation. Assuming that in modified RCs we only excite B_A, we can separate out the B_B contribution (Figure 5) to the anisotropy in modified RCs. The fact that the anisotropy remains high, at a value of 0.4, is consistent with the assignment of this contribution to B_B.

A time dependence of the anisotropy in native RCs similar to that in Figure 4A has been observed earlier by Jones et al. [9] These authors explained their observations by assuming a sequential process involving energy transfer from B* to the upper exciton level of P (P_{Y+}), followed by internal conversion from the upper to the lower exciton level of P (P_{Y-}). In such a model the large variations can be attributed to the negative contribution of the P_{Y+} state to the anisotropy. If indeed P_{Y+} functions as an intermediate in the sequential energy transfer, as suggested by Jonas et al.,[9] we would have expected to observe such a contribution in modified RCs as well, with similar effects on the anisotropy as in native RCs. However, the contrary is true. Thus, the sequential energy transfer process involving the

upper exciton state P_{Y+} as a distinct intermediate [9] does not appear to be a satisfactory model.

Modeling the results of our experiments on native and modified RCs is improved if we assume that the B_B absorption band is shifted. In particular, this assumption provides a consistent explanation for the differences in the time dependence of the anisotropy observed in these systems. The extremely fast rate of energy transfer in the RC implies a strong coupling between the pigments involved. It is not unlikely that the B* state is strongly mixed with the P_{Y+} state and vibronic levels of P_{Y-}, possibly by exchange interactions,[10] thus forming a supermolecular excited state which can decay to the lowest P_{Y-} state by internal conversion. This relaxation process would correspond to the first step in the kinetic model discussed earlier and may be more appropriately referred to as energy dissipation rather than energy transfer. Also note that in this case no rapid drop of the anisotropy in the B-absorption band is expected, since the excited-state population is transferred to nonresonant, lower energy states.

We have also investigated the influence of the redox state of the primary donor on the energy transfer process following excitation of the accessory bacteriochlorophylls. In oxidized RCs (both native and chemically modified) we observe an additional 400 fs component which is absent in reduced RCs. The latter is in contrast to observations by Jia et al.[7] In a later publication[9] these authors themselves confirmed that in their earlier measurements P was partially photooxidized, presumably due to experimental conditions by which P^+ may have been accumulated. In our experiments, this effect has been avoided by the addition of ascorbate. The amplitude of the 400 fs component is wavelength dependent and has a distinct maximum at 800 nm. We note that the dominant decay component of B* in oxidized RCs still occurs with a time constant of 100 fs. Presumably, the 100 and 400 fs components correspond to two different relaxation paths following excitation of B. The 100 fs component may be associated with a strong coupling to the vibronic manifold of P^+ and internal conversion. The 400 fs component is probably a different process, but at present its origin is not clear.

It is remarkable that in all samples used in this work (native, chemically modified, reduced and/or oxidized RCs) the dominant time constant found is about 100 fs. Obviously, the presence of the upper exciton state P_{Y-} of the primary donor is not a prerequisite to achieve the extremely fast decay rate. Therefore, it seems unlikely that this time constant is related to specific interactions between two electronic states, considering that significant variations exist in the electronic structure of the systems we examined. This observation is in favor of the strong coupling model combined with rapid internal conversion of a supermolecular state.

Conclusions

In RCs modified by borohydride treatment the absorbance changes around 800 nm are only due to the absorbance changes of B_A induced by (1) energy transfer from B* to P and by (2) charge separation. However, in native RCs we also observe the development of an induced absorption at 805 nm due to a shift of the B_B absorption band upon P* formation. The absorbance changes and the anisotropy measurements can be adequately explained in terms of these effects. Our measurements on chemically modified and native RCs can be accurately described by a sequential model (scheme 1) or by a superexchange mechanism (scheme 2). In particular, the induced absorption around a delay of 500 fs at 805 nm can be accurately reproduced. In oxidized native and chemically modified RCs a fast component of 100 fs as well as an additional 400 fs component is present.

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References and Notes

(1) Martin, J. L.; Breton, J.; Hoff, A. J.; Migus, A.; Antonetti, A. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 957.

(2) Kirmaier, C.; Laporte, L.; Schenck, C. C.; Holten, D. *J. Phys. Chem.* **1995**, *99*, 8910.

(3) Holzapfel, W.; Finkele, U.; Kaiser, W.; Oesterhelt, D.; Scheer, H.; Stilz, H. U.; Zinth, W. *Proc. Natl. Acad Sci. U.S.A.* **1990**, *87*, 5168.

(4) Arlt, T.; Schmidt, S.; Kaiser, W.; Lauterwasser, C.; Meyer, M.; Scheer, H.; Zinth, W. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 275.

(5) Schmidt, S.; Arlt, T.; Hamm, P.; Huber, H.; Nagele, T.; Wachtveitl, J.; Meyer, M.; Zinth, W. *Chem. Phys. Lett.* **1994**, *223*, 116.

(6) Jean, J. M.; Chan, C. K.; Fleming, G. R. *Isr. J. Chem.* **1988**, *28*, 169.

(7) Jia, Y.; Jonas, D. M.; Joo, T.; Nagasawa, Y.; Lang, M. J.; Fleming, G. R. *J. Phys. Chem.* **1995**, *99*, 6263.

(8) Haran, G.; Wynne, K.; Moser, C. C.; Dutton, P. L.; Hochstrasser, R. M. *J. Phys. Chem.* **1996**, *100*, 5562.

(9) Jonas, D. M.; Lang, M. J.; Nagasawa, Y.; Joo, T.; Fleming, G. R. *J. Phys. Chem.* **1996**, *100*, 12660.

(10) Stanley, R. J.; King, B.; Boxer, S. G. *J. Phys. Chem.* **1996**, *100*, 12052.

(11) Förster, T. *Ann. Phys.* **1948**, *2*, 55.

(12) Dexter, D. L. *J. Chem. Phys.* **1952**, *21*, 836.

(13) Kirmaier, C.; Holten, D.; Parson, W. W. *Biochim. Biophys. Acta* **1985**, *810*, 49.

(14) Vos, M. H.; Lambry, J. C.; Robles, S. J.; Youvan, D. C.; Breton, J.; Martin, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 613.

(15) Chekalin, S. V.; Matveets, Yu. A.; Yartse, A. P. In *Ultrafast Phenomena V*; Fleming, G. R., Ed.; Springer-Verlag: Berlin, 1986; p 402.

(16) Shuvalov, V. A.; Shkuropatov, A.Ya.; Kulalova, S. M.; Ismailov, M. A.; Shkuropatova, V. A. *Biochim. Biophys. Acta* **1986**, *849*, 337.

(17) Beese, D.; Steiner, R.; Scheer, H.; Angerhofer, A.; Robert, B.; Lutz, M. *Photochem. Photobiol.* **1988**, *47*, 293.

(18) Ditson, S. L.; Davis, R. C.; Pearlstein, R. M. *Biochim. Biophys. Acta* **1984**, *766*, 623.

(19) Maroti, P.; Kirmaier, C.; Wraight, C.; Holten, D.; Pearlstein, R. M. *Biochim. Biophys. Acta* **1985**, *810*, 132.

(20) Struck, A.; Muller, A.; Scheer, H. *Biochim. Biophys. Acta* **1991**, *1060*, 262.

(21) Clayton, R. K. *Photosynthesis: Physical Mechanisms and Chemical Patterns*, Cambridge University Press: New York, 1980.

(22) Asaki, M. T.; Huang, C.−P.; Zhou, J., Kapteyn, H. C.; Murnane, M. M. *Opt. Lett.* **1993**, *18*, 977.

(23) Pshenichnikov, M. S.; De Boeij, W. P.; Wiersma, D. A. *Opt. Lett.* **1994**, *19*, 572.

(24) Holten, D.; Kirmaier, C.; Levine, L. In *Progress in Photosynthesis Research*; Biggens, J., Ed.; Martinus Nijhoff: Dordrecht, 1987; Vol. 1, p 169.

(25) Allen, J. P.; Feher, G.; Yeates, T. O.; Komiya, H.; Rees, D. C. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 5730−5734.

(26) Chekalin, S. V.; Matveetz, Ya. A.; Shkuropatov, A.Ya.; Shuvalov, V. A.; Yartzev, A. P. *FEBS Lett.* **1987**, *216*, 245.

(27) Breton, J.; Martin, J. L.; Fleming, G. R.; Lambry, J. C. *Biochemistry* **1988**, *27*, 8276.

(28) Nagarajan, V.; Parson, W. W.; Davis, D.; Schenck, C. C. *Biochemistry* **1993**, *32*, 12324.

(29) Woodbury, N. W.; Peloquin, J. M.; Alden, R. G.; Lin, X.; Lin, S.; Taguchi, A. K. W.; Williams, J. C.; Allen, J. P. *Biochemistry* **1994**, *33*, 8101.

(30) Kirmaier, C.; Holten, D. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 3552.

(31) Scherer, P. O. J.; Fischer, S. F.; Lancaster, C. R. D.; Fritzsch, G.; Arlt, T.; Dressler, K.; Zinth, W. *Chem. Phys. Lett.* **1994**, *223*, 110.