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Biochimica et Biophysica Acta 1188 (1994) 278–286

BBA Biochimica et Biophysica Acta

Energy trapping in the purple sulfur bacteria *Chromatium vinosum* and *Chromatium tepidum*

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Received 5 May 1994

Abstract

Energy trapping in chromatophores of the purple sulfur bacteria *Chromatium vinosum* and *Chromatium tepidum* has been examined by means of picosecond transient absorption spectroscopy. In *C. vinosum*, time constants for excitation quenching in the core antenna by open and closed reaction centres were 50 and 230 ps, respectively. In *C. tepidum*, excitation quenching by closed reaction centres occurred with a single time constant of 310 ps. With open reaction centres, the kinetics of decay of excitations in the core antenna were more complicated. The decay was multi-exponential, with a major contribution from a 140 ps component and minor contributions from 40 ps and 530 ps components. By comparison with the kinetics of oxidation of the primary donor, the 140 ps component was identified as being due to excitation trapping by open reaction centres. This trapping time is slow as compared with other purple bacteria, and is most likely a consequence of the red-shift of the core antenna with respect to the reaction centre. The origin of the 40 ps component is not entirely clear, but it probably represents energy transfer to the reaction centre as well. The 530 ps component may reflect a fraction of bacteriochlorophyll *a* that transfers its energy to the reaction centre very inefficiently, or not at all. In *C. vinosum* as well as in *C. tepidum*, no kinetic components were found that could be associated with internal core antenna relaxation, which implies that equilibration within the core antenna occurred within our time resolution of 10–15 ps.

Keywords: Purple bacterium; Energy transfer; Antenna; Reaction center; Energy trapping; (*C. vinosum*); (*C. tepidum*)

1. Introduction

In the past decade energy migration in photosynthetic bacteria has been studied extensively by means of picosecond time-resolved spectroscopic techniques. This applies in particular to the purple bacteria, which have a relatively simple photosynthetic apparatus and spectrally well-resolved absorption bands (for a recent review, see Ref. [1]). With a few exceptions [2,3], all of this research has been focused on the purple non-sulfur bacteria, primarily the bacteriochlorophyll (BChl) *a* containing species *Rhodobacter sphaeroides* and *Rhodospirillum rubrum*.

In purple bacteria the so-called core antenna complex, LH1, is associated with the reaction centre [4]. It is generally assumed that energy transfer to the reaction centre from other antenna complexes, like B800–850 in *Rb. sphaeroides* occurs via the core antenna [5]. No crystal structure of the core antenna is as yet available, and as a consequence detailed understanding of migration mechanisms in the core antenna is lacking. Nevertheless, theoretical models were developed that seem to be able to account for some of the dynamic features observed in energy transfer [6,7].

There now appears to be general agreement that the lifetime of excitations in the antenna of purple bacteria in the presence of open, i.e., photochemically active, reaction centres is 50 to 60 ps [1]. Recent studies indicate that this time constant is determined by the rate of energy transfer from the antenna BChls to the reaction centre [2,8–10], rather than by the rate of charge separation [11]. Upon closure of the reaction centres, i.e., oxidation of the primary electron donor, the lifetime increases by a factor of three to five [1] but

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SSDI 0005-2728(94)00134-0

there are also reports of bi-exponential decays with closed reaction centres [12,13].

Relatively few studies have been reported on the purple sulfur bacteria [2,3]. In this paper we present a picosecond transient absorption study of energy trapping in two species of Chromatiaceae, *Chromatium vinosum* and *C. tepidum*, the latter of which has only recently been discovered [14]. Both species contain BChl *a* as light-harvesting and photochemically active pigment, and their reaction centre have been isolated [15–17]. The reaction centre of *C. vinosum* possesses a tightly bound tetraheme cytochrome [18], similar to that in the BChl *b* containing purple bacterium *Rhodopseudomonas viridis*. Remarkably, two kinds of reaction centre have been isolated from *C. tepidum*, one with a bound cytochrome and one without [17]. The one containing the cytochrome has recently been obtained in a crystalline form [19]. The reaction centres are associated with the core antenna complexes, B890, absorbing at 890 nm, in *C. vinosum* [20] and B920, absorbing at 918 nm, in *C. tepidum* [21,22]. Both species synthesize peripheral antennae as well: *C. vinosum* contains varying amounts of B800–850 and B800–820 complexes, depending on growth conditions [20], whereas *C. tepidum* has a B800–850 complex [21,22].

There is one distinct property of *C. tepidum* that merits attention. The core antenna has an absorption maximum at 918 nm, while the absorption of the primary donor peaks at 900 nm, which is at considerably higher energy. *C. tepidum* shares this property with some other photosynthetic organisms. In heliobacteria, for instance, the antenna pigment absorbing at the longest wavelength, BChl *g* 808, is red-shifted by 10 nm with respect to the primary donor P-798 [23]. *Rps. viridis* shows an even more extreme red-shift of 30 nm; its primary donor absorbs at 985 nm while the antenna absorption peaks at 1015 nm [24]. The uphill transfer from the antenna to the reaction centre would not seem to provide an optimal condition for photochemical trapping of excitations. The question thus arises how this relates to the assumption that photosynthetic systems have been optimised in the course of evolution [25].

The energy gap in *C. tepidum* is almost as large as the one found in *Rps. viridis*. Fluorescence polarisation measurements have shown that the core antenna in *C. tepidum* is spectrally inhomogeneous [26], in contrast to *Rps. viridis* which has a homogeneous antenna [27–29]. This indicates that with respect to its antenna organisation, *C. tepidum* is more related to other BChl *a* containing purple bacteria, including *C. vinosum*, which have an isoenergetic antenna-reaction centre system. This makes energy trapping in *C. tepidum* a very interesting subject of study. Our measurements will show that the red-shift of the antenna has a profound effect on the trapping efficiency.

2. Materials and methods

Chromatium vinosum was cultured in Hutner malate medium [30] at 30°C in rectangular vessels of 5×12 cm. They were illuminated from both sides at a light intensity of 3500 lux from fluorescent tubes and incandescent lamps, and grown to an absorbance of about 0.5 per cm at 850 nm. The cultures were bubbled with nitrogen. Cells of *Chromatium tepidum* were grown in closed 1 l bottles in the medium of Ref. [14] at 50 °C. The cells were concentrated by centrifugation and incubated with 2 mg DNAse per 100 ml cell suspension during 15 min at room temperature. Then the cells were sonicated for 20 min at 0 °C, and subsequently centrifuged for 10 min at $12\,000 \times g$ to remove unbroken cells and large cell fragments. Chromatophores were collected by ultracentrifugation. The sediment was resuspended in 10 mM Tris buffer at pH 8.0 and stored in liquid nitrogen (*C. tepidum*) or −20° C (*C. vinosum*) until used.

Absorbance difference measurements on a millisecond time scale were performed with the apparatus described elsewhere [26]. The picosecond laser system employed for our time-resolved measurements has been described in Ref. [31]. The most important features relevant to this paper are briefly summarised.

One or two dye lasers, synchronously pumped by the frequency doubled output of an actively mode-locked Nd:YAG laser (Spectron Laser Systems) with a repetition rate of 76.6 MHz and an output power of 15 W at the fundamental wavelength were used to perform one-colour and two-colour transient absorption measurements. Using the dye LDS 925, the dye laser tunability ranged from 880 nm to 960 nm. In the two-colour experiments, one of the dye lasers was operated with rhodamine 6G to obtain excitation pulses at 590 nm. The dye laser output power varied between 15 mW and 40 mW, depending on wavelength. The laser power of the excitation beam was attenuated to about 1 mW, while that of the probe beam was further reduced by more than a factor of 10. The autocorrelation of the dye laser operated with LDS 925 had a width of about 10 ps. The width of the cross correlation in the two-colour mode was 15–25 ps due to jitter between the two dye lasers. The angle between the polarisations of pump and probe beams was set at the magic angle of 54.7° by placing a Glan-Thomson polariser in one of the beams.

In order to avoid accumulation of closed reaction centres and antenna triplet states, the samples were contained in a circular sample cell with a diameter of 10 cm and an optical path length of 1.4 mm, rotating at 3000 rpm. At this rotation speed a given sample volume was exposed to approximately 150 laser pulses on moving in and out of the focus. For each series of measurements the samples were prepared by suspend-

ing the chromatophores in a 30% glycerol/10 mM Tris buffer at pH 8.0 to an absorbance of about 0.4 at the maximum of the Q_y band of the core antenna and purged with nitrogen gas to remove oxygen. Glycerol was added to increase the viscosity of the medium, thus preventing sedimentation in the rotating sample cell. Energy densities were of the order of 10^{11} photons/cm^2 per pulse which means that with the laser tuned near the absorption maxima, about 0.01% of the BChl *a* molecules in the sample were excited by a single pulse.

For measurements with closed reaction centres, the chromatophores were suspended in Tris-glycerol buffer without any further addition. Under these conditions, the continuous pulse train kept the primary donor in the oxidized state, which has a lifetime of several hundreds of milliseconds. For measurements with open reaction centres, 30 mM sodium ascorbate and 30 μM phenazine methosulfate were added to the buffer. All measurements were performed at room temperature.

3. Results

3.1. Trapping dynamics in C. vinosum

The absorption spectrum of chromatophores of *C. vinosum* was similar to that published earlier for intact cells [32]. Bands and shoulders near 800, 820 and 850 nm showed the presence of the two types of peripheral complexes, B800–820 and B800–850 [20], whereas the core antenna complex showed a Q_y band at 890 nm. In Fig. 1 the flash-induced absorbance difference spectrum due to oxidation of the primary electron donor P is presented. The maximum bleaching is located at 885 nm, and a band shift around 800 nm is also observed. The spectrum is similar to that originally published by

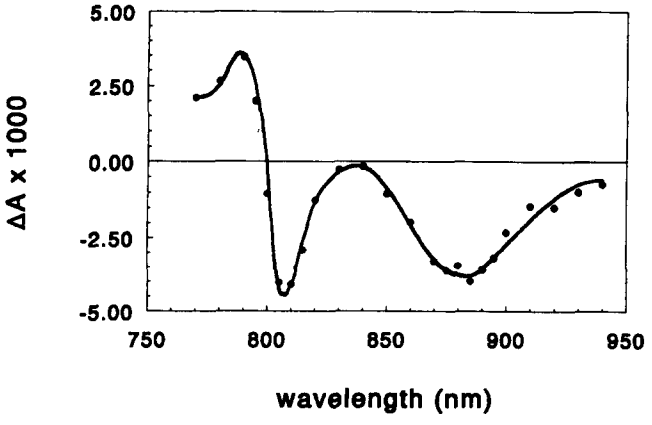

Fig. 1. Absorbance difference spectrum due to photooxidation of the primary electron donor in chromatophores of *C. vinosum*. The spectrum was taken at 2 ms after a saturating laser flash. A negative absorbance difference denotes a bleaching. The absorbance of the sample was 0.5 at 890 nm.

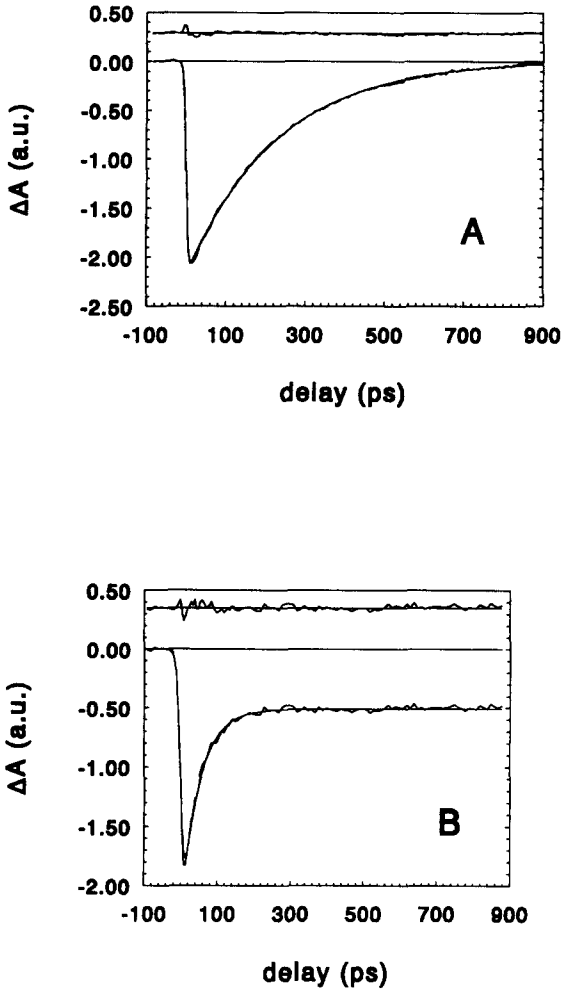

Fig. 2. (A) Kinetics of absorbance changes measured in chromatophores of *C. vinosum* with closed reaction centres in a one-colour experiment at 891 nm. The smooth line represents a monoexponential fit with a time constant of 230 ps. (B) Absorbance changes at 891 nm measured in chromatophores with open reaction centres in the presence of 30 mM ascorbate and 30 μM phenazine methosulfate. The smooth line represents a monoexponential fit with a time constant of 50 ps and a long-lived end level. The residuals of the fits are shown in the upper part of the figures (a.u., arbitrary units).

Duysens et al. [33] upon illumination of a cell suspension, and the band of the primary donor is clearly red shifted with respect to that of isolated reaction centres [15].

Fig. 2A shows a kinetic trace taken at 891 nm in chromatophores of *C. vinosum* with closed reaction centres obtained in a picosecond one-colour experiment. An instantaneous bleaching of BChl *a* absorption was observed that decayed monoexponentially with a time constant of 230 ps. We assign this time constant to quenching of excited BChl *a* by closed reaction centres. The fact that the decay of excited BChl *a* was monoexponential indicates that equilibration within the core antenna was completed within the time resolution of our laser system.

Fig. 2B shows a one-colour kinetic trace taken at 891 nm with open reaction centres. It displays a bleaching that decayed monoexponentially with a time constant of 50 ps to a long-lived end level. We attribute the time constant of 50 ps to excitation trapping by open reaction centres, and the long-lived end level to the formation of the oxidized primary donor. The rates found for excitation quenching by closed and open reaction centres are very similar to those found for BChl *a* containing purple non-sulfur bacteria, like *Rb. sphaeroides* and *R. rubrum* [1,3,12].

We also performed two-colour experiments on chromatophores of *C. vinosum* with open reaction centres upon excitation at 590 nm. The probe wavelengths ranged from 885 to 930 nm. Fig. 3 shows a typical kinetic trace at 897 nm. The kinetics at this and other wavelengths could be fitted with a rise time of 12 ps, a major decay time of 50 ps, a minor one of 230 ps and a long-lived end level. Under these conditions the Q_x bands of all BChl *a* spectral types are non-selectively excited. As the bulk of the BChl *a* present in the antenna is located in the B800–820 and B800–850 peripheral complexes, most of the excitations will originate there. Therefore, we may assume that the 12 ps rise time reflects energy transfer from the peripheral antenna complexes to the core complex. This rise time was not observed upon direct excitation of the core antenna, as in the experiments shown in Fig. 2.

The absorbance difference spectra of the four kinetic components are plotted in Fig. 4. The spectra of the 50 and 230 ps components are very similar to each other and are attributed to the decay of excited BChl *a* in the core antenna. Both show a maximum bleaching at 902 nm and approach an isosbestic point at 885 nm. Unfortunately, due to limitations in our laser system,

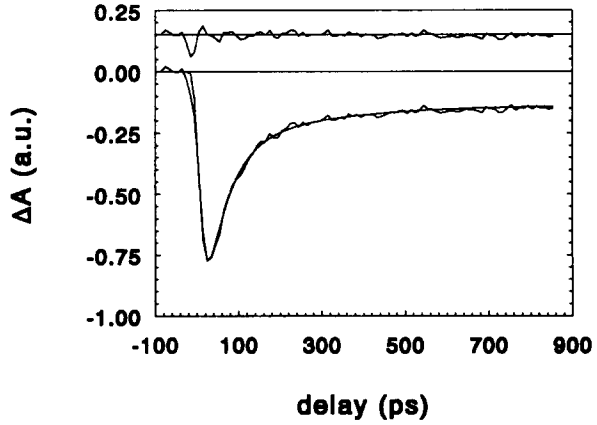

Fig. 3. Kinetic trace taken at 897 nm in a two-colour experiment upon excitation at 590 nm of chromatophores of *C. vinosum* with open reaction centres. The smooth line represents a three-component exponential fit with a rise time of 12 ps and decay times of 50 ps and 230 ps, and a long-lived end level, in an amplitude ratio of −1.11:1:0.22:0.14. The residual of the fit is shown in the upper part of the figure.

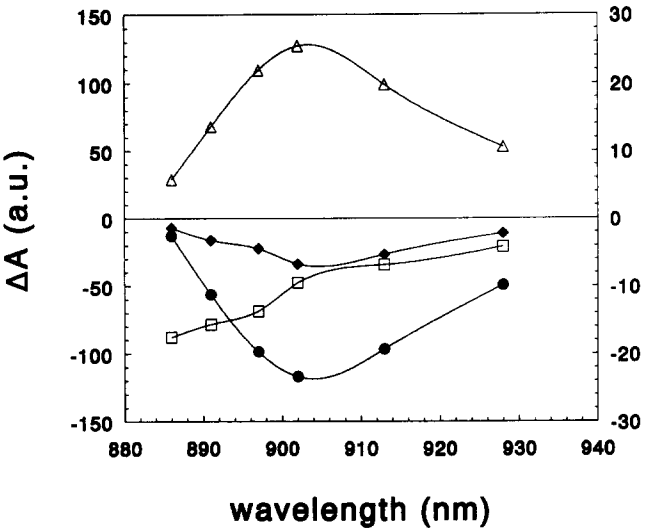

Fig. 4. Absorbance difference spectra of the four kinetic components derived from two-colour experiments on chromatophores of *C. vinosum* with open reaction centres. Triangles denote the 12 ps component, circles the 50 ps component, diamonds the 230 ps component (all on the left-hand scale) and the squares the long-lived end level (right-hand scale). Note that the spectrum of the 12 ps component represents the formation of the absorbance difference signal of excited BChl, and has therefore the opposite sign of the spectra of the 50 ps and 230 ps decay components. The same applies to the corresponding spectra of Figs. 6 and 9.

we were not able to measure kinetics at wavelengths shorter than 885 nm. In accordance with the results obtained in the one-colour experiments, we assign the 50 ps decay component to excitation trapping by open reaction centres, and the 230 ps component, which amounts to about 20% of the total signal, to excitation quenching by a small fraction of closed traps. The absorbance difference spectrum of the long-lived component showed a maximum bleaching at 885 nm and may be ascribed to the formation of the oxidized primary donor. Experiments at different excitation energies (not shown) demonstrated that the relative contribution of the 230 ps component increased with increasing excitation energy, whereas the amplitude ratio of the 50 ps component and of the long-lived end level remained constant, in agreement with the above assignments.

3.2. Trapping dynamics in C. tepidum

The absorption spectrum of chromatophores of *C. tepidum* was similar to those published earlier [21,22]. The Q_y bands of the peripheral B800–850 complex peaked at 795 nm and 855 nm, and the Q_y band of the B920 core complex was located at 918 nm. The absorbance difference spectrum of the photo-oxidation of the primary electron donor has been published by Garcia et al. [21] and more recently by Kleinherenbrink [26]; it showed a maximum bleaching at 900 nm.

Two-colour picosecond measurements with excitation at 590 nm and probe wavelengths ranging from 880 to 950 nm were performed on chromatophores of *C. tepidum* with closed reaction centres. A typical kinetic trace, with probe wavelength at 929 nm, is shown in Fig. 5. Similar to the situation in *C. vinosum*, the signal had a rise time of about 15 ps which can be ascribed to energy transfer from the peripheral antenna to the core antenna. The decay was monophasic with a time constant of 310 ps and a long-lived end level amounting to maximally 4% of the total amplitude. With the time constants 15 ps and 310 ps and a long-lived end level, the kinetics at the other wavelengths could also be adequately fitted. The resulting absorbance difference spectra are presented in Fig. 6. The 310 ps component showed a maximum bleaching at 930 nm, an isosbestic point at 907 nm and an increase in absorbance at shorter wavelengths. Such a shape for the absorbance difference spectrum of excited BChl *a* in the core antenna has been observed earlier in antenna systems of other purple bacteria [2,34]. The absorbance increase at the short-wavelength side can be ascribed to excited-state absorption of BChl 920. At the extreme red side of the absorbance difference spectrum, the apparent bleaching is mainly due to stimulated emission. We conclude that the 310 ps component reflects quenching of excited BChl *a* in the B920 complex by closed reaction centres. As in *C. vinosum*, the occurrence of a single decay component indicates that equilibration within the core antenna took place within our time resolution. The spectrum of the 15 ps component had a similar shape but opposite sign, in line with the assumption that it reflects arrival of excitations from the peripheral antenna on the core antenna.

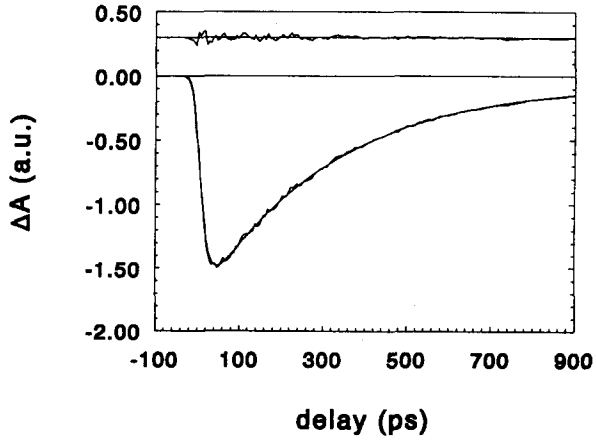

Fig. 5. Kinetic trace taken at 929 nm in a two-colour experiment upon excitation at 590 nm of chromatophores of *C. tepidum* with closed reaction centres. The smooth line represents a two-component exponential fit with a rise time of 15 ps and a decay time of 310 ps. The residual of the fit is shown in the upper part of the figure.

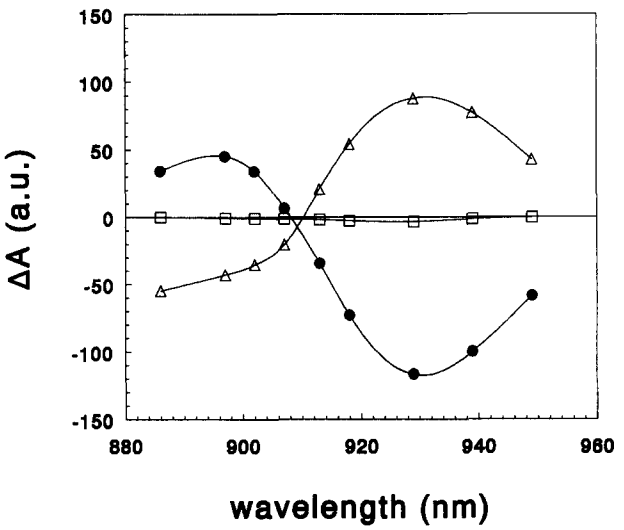

Fig. 6. Absorbance difference spectra of the three kinetic components derived from two-colour experiments on chromatophores of *C. tepidum* with closed reaction centres. Triangles denote the 15 ps component, circles the 310 ps component and squares the long-lived end level.

With open reaction centres the kinetics were much more complex. We performed one-colour experiments with wavelengths ranging from 897 to 950 nm. Fig. 7 shows the kinetic traces at 902 nm and 918 nm. Three time constants of 40 ± 5 ps, 140 ± 20 ps and 530 ± 150 ps and a long-lived end level were required to fit the kinetics at all wavelengths.

We also performed two-colour experiments with excitation at 590 nm under conditions of open reaction centres. Fig. 8 shows the two-colour kinetics at four representative wavelengths. The kinetics were fitted with the above-mentioned time constants of 40 ps, 140 ps and 530 ps and a long-lived end level, and a rise time of 15 ps. The absorbance difference spectra of all five kinetic components are plotted in Fig. 9. The absorbance difference spectra of the three decay components and the long-lived end level agreed with those obtained from the one-colour measurements (not shown). As in the other two-colour experiments, the 15 ps component may be attributed to energy transfer from the peripheral antenna to the core antenna. The long-lived end level had a negative sign at all wavelengths and a maximum at 900 nm, and is assigned to the formation of the oxidized primary donor. The 40, 140 and 530 ps components showed a bleaching at the red side and an absorbance increase at the blue side of the B920 absorption band, indicating that they represent excited BChl 920. As most of the decay was formed by the 140 ps component, we attribute this component to trapping by open reaction centres. The spectrum of the 40 ps component, together with the monoexponential decays measured in chromatophores with closed reaction centres, excludes that it is due to a

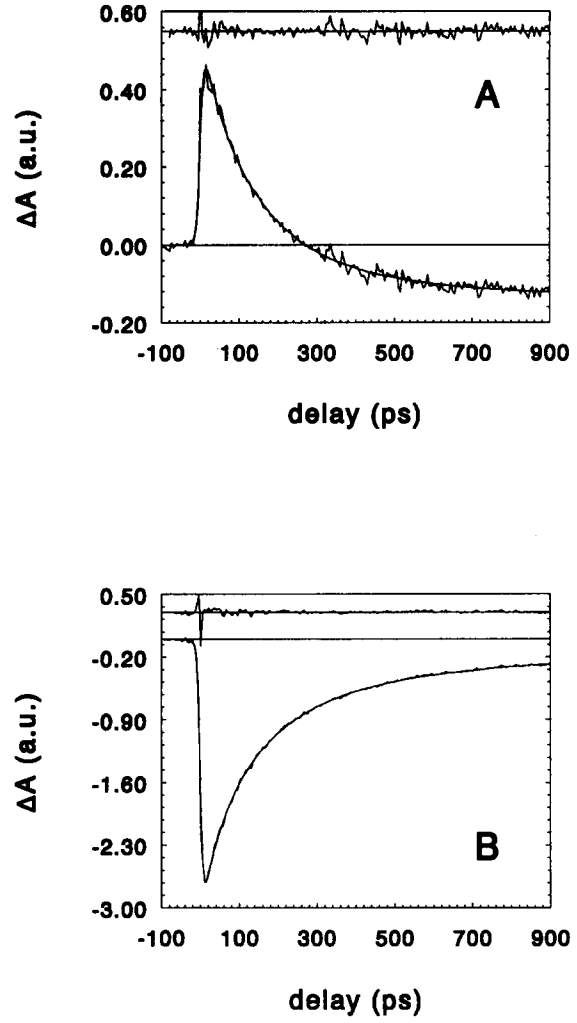

Fig. 7. Kinetic traces taken in one-colour experiments on chromatophores of *C. tepidum* with open reaction centres at 902 nm (A) and 918 nm (B). The smooth lines represents three-component exponential fits with decay times of 40 ps, 140 ps and 530 ps, and a long-lived end level, with amplitude ratios of 0.16:1:0.24:−0.29 (A) and 0.36:1:0.46:0.08 (B), respectively. The residuals of the fits are shown in the upper part of the figures.

spectral equilibration process within the core antenna. This indicates that the 40 ps component is most likely due to trapping by open reaction centres as well. The 530 ps component is slightly blue-shifted with respect to the 140 ps component. It may be caused by BChl *a* that either transfers its energy to the reaction centre very inefficiently, or not at all, as this time constant approaches the one found in isolated core antenna complexes of *R. rubrum* [35] and in a mutant of *Rb. sphaeroides* lacking reaction centres [36].

Of particular interest were the one-colour kinetics at 907 nm, shown in Fig. 10. Almost no instantaneous absorbance change was observed, indicating that this is near the isosbestic wavelength for absorbance changes due to excitation of BChl 920 (see also Fig. 9). A long-lived bleaching was now observed which developed with a time constant of 140 ps, and which must primarily be attributed to oxidation of the primary electron donor. Thus, this experiment gives strong evidence that the 140 ps component, dominant in all our one-colour and two-colour measurements, is due to energy trapping by open reaction centres. We also observe that there is a small negative amplitude of the 530 ps component, which implies that there is no 'true' isosbestic point at this wavelength, but rather that the three (small) antenna components cancel each other at zero delay. No direct evidence could be obtained in this way for the origin of the 40 ps component. Its contribution to the kinetics at 907 nm was negligible (< 10%), but this may be explained by a slightly blue-shifted difference spectrum for this component, in such a way that the contributions by the antenna and the primary donor roughly compensate each other at 907 nm. It is worthwhile to note that this kinetic trace is very similar to its two-colour counterpart shown in Fig. 8B, apart from the fast feature around zero delay which has a lifetime of 15 ps. The appearance of this fast feature in the two-colour experiment at 907 nm is very likely due to excitation and subsequent de-excitation of BChl 850, which is distinguishable at this wavelength because the absorbance changes due to excited BChl 920 are very small. This interpretation was confirmed by two-colour kinetic traces measured at wavelengths shorter than 885 nm (not shown), where the contribution by excited BChl 850 to the initial absorbance changes also exceeded that by excited BChl 920.

4. Discussion

Our measurements have shown that excitation lifetimes in the core antenna of *C. vinosum* with open and closed reactions centres were 50 ps and 230 ps, respectively. These values are very similar to the ones found in other purple bacteria like *R. rubrum, Rb. sphaeroides, Rb. capsulatus* and *C. minutissimum*, using both transient absorption [1,2] and time-resolved fluorescence [1,3,12] techniques. The monoexponentiality of the decays also indicates that at room temperature equilibration of excitations within the core antenna occurred in a time less than our resolution of 10 ps. This differs from the observations made by Sundström et al. [37] with chromatophores of *R. rubrum*, but is in line with many other reports [1,3,10,11]. Excitation transfer from the peripheral antenna to the core antenna takes place in about 12 ps, similar to the values found in other purple bacteria [1,3,11].

The situation in *C. tepidum* appears to be more complicated. Excitation quenching in the core antenna by closed reaction centres occurred with a single time constant of 310 ps. Again, the monoexponentiality of

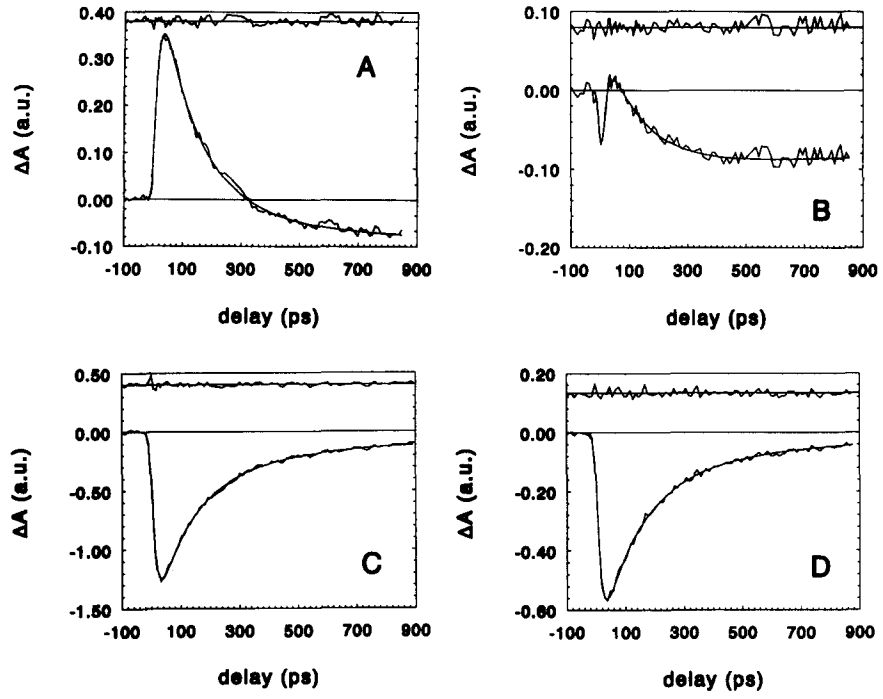

Fig. 8. Kinetic traces obtained in two-colour experiments on chromatophores of *C. tepidum* with open reaction centres. The excitation wavelength was 590 nm. The probe wavelengths were (A) 896 nm, (B) 907 nm, (C) 928 nm and (D) 949 nm. Four time constants of 15 ps, 40 ps, 140 ps, and 530 ps and a long-lived end level are used to fit the data. Residuals of the fits are shown in the upper part of the figures.

the measured kinetics showed that equilibration within the core antenna of *C. tepidum* was complete within our time resolution. In this respect, *C. tepidum* behaves very much like other purple bacteria. This is certainly not the case when energy trapping by open reaction centres is considered. A multi-exponentional quenching was observed, and most of the excitations were quenched with a time constant of 140 ps. Our measurements clearly indicate that this time constant reflects energy transfer to the reaction centre. This process is slow as compared with other purple bacteria, including *C. vinosum*, for which trapping times of 50–60 ps are common [1]. Presumably, the trapping time has been slowed down by the red-shift of the antenna. As discussed earlier, the 40 ps component may also be due to energy transfer to the reaction centre, but direct evidence is lacking in this case. The excitation densities that we used for the experiments were sufficiently low

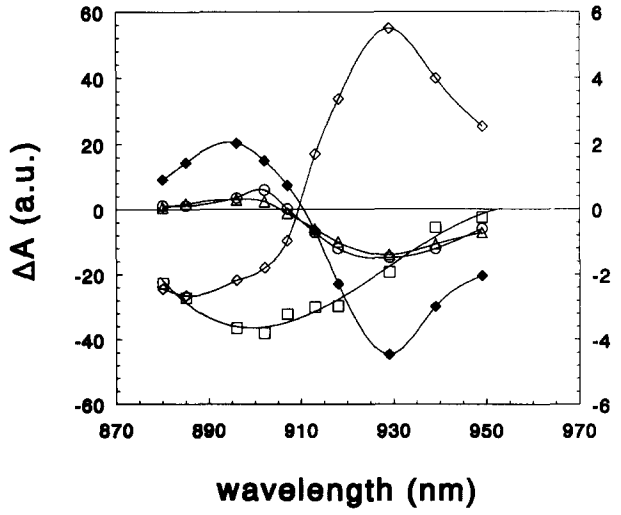

Fig. 9. Absorbance difference spectra of the five kinetic components derived from two-colour experiments on chromatophores of *C. tepidum* with open reaction centres. Open diamonds denote the 15 ps component, circles the 40 ps component, filled diamonds the 140 ps component, triangles the 530 component (all on the left-hand scale) and the squares the long-lived end level (right-hand scale).

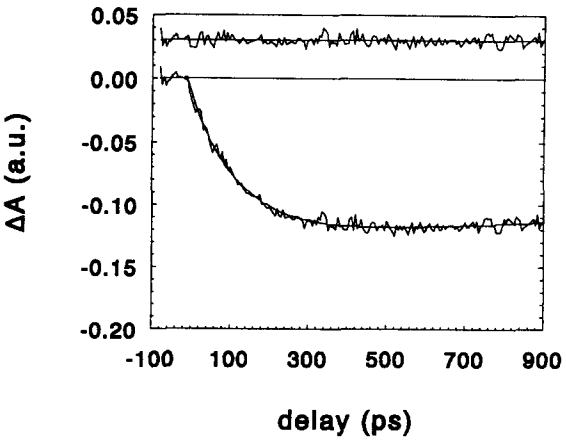

Fig. 10. Kinetic trace taken at 907 nm in a one-colour experiment on chromatophores of *C. tepidum* with open reaction centres. The smooth line represents a fit with time constants of 40 ps, 140 ps and 530 ps and a long-lived end level with amplitude ratios of 0.02:1:−0.27:−0.80. The residual of the fit is shown in the upper part of the figure.

to exclude the possibility that it is caused by singlet-singlet or singlet-triplet annihilation. The 530 ps component is most likely caused by a fraction of loosely bound, inefficiently or non-transferring BChl *a*. Multi-exponential and bi-exponential decays with open reaction centres have been occasionally reported for *R. rubrum* [37] and *Rb. capsulatus* [11]. Recently, in mutants of *Rb. sphaeroides* with decreased charge separation rates and elevated radical-pair redox energies, bi-exponential decays have been observed as well [10]. There are also several reports on Photosystem I and Photosystem II preparations showing bi-exponential trapping dynamics [38–42]. In most of these cases, the bi-exponential quenching was explained by assuming a back-reaction of the primary radical pair, and subsequent energy transfer from the reaction centre to the antenna. The slow phase would then be caused by a stabilization of the oxidized primary donor by electron transfer to the secondary acceptor. We believe, however, that this is not the case in *C. tepidum*, because upon the addition of sodium dithionite to the sample, which reduces the quinones and inhibits secondary electron transport, no drastic change in kinetics was observed (results not shown).

It is of interest to make a comparison between *C. tepidum* and *Rps. viridis*, which shows a comparable red-shift of the antenna with respect to the reaction centre. Trapping in *Rps. viridis* was reported to take place in 50–60 ps [13,27,28], essentially the same value as in purple bacteria with an iso-energetic antenna-reaction centre system, and is thus more efficient than in *C. tepidum*. Closed reaction centres are also relatively efficient quenchers of excitations in *Rps. viridis* [27,43], which is evidently not the case in *C. tepidum*. Furthermore, it was concluded that the antenna of *Rps. viridis* is spectrally homogeneous [27–29], in contrast to those of other purple bacteria including *C. tepidum* [26]. These observations suggest a different organisation of the antenna in *Rps. viridis* as compared with other purple bacteria, perhaps involving a shorter distance between the primary donor and the nearest antenna BChls [27].

The information obtained so far on the core antenna of purple sulfur bacteria suggests structural similarities with the core antenna of BChl *a* containing purple non-sulfur bacteria, but also points to some differences. A B820 subunit similar to those obtained from core antennae of *R. rubrum*, *Rb. sphaeroides* and *Rb. capsulatus* [44] has been isolated from the marine purple sulfur bacterium *C. purpuratum* [45], indicating that in Chromatiaceae the core antenna consists of an array of BChl *a* dimers as well. Low-temperature picosecond fluorescence measurements on chromatophores of *C. minutissimum* [3] and fluorescence polarisation measurements on chromatophores of *C. tepidum* [26] have shown that their core antennae are spectrally heterogeneous, as in *Rb. sphaeroides* and *R. rubrum* [1,7]. However, a conservative double circular dichroism band observed in the core antenna band of *Rb. sphaeroides* [46,47] has neither been found in *C. vinosum* [20] nor in *C. tepidum* [22], which implies that pigment-pigment or pigment-protein interactions in the core antennae of Chromatiaceae are different from those in purple non-sulfur bacteria.

One aspect of our kinetic measurements that is of specific interest is the amplitude of the long-lived component due to oxidation of the primary donor. This amplitude is small as compared with that of the antenna bleaching, even at low, non-saturating excitation energy. In *C. vinosum*, the maximum amplitude of the 50 ps component is 8-times larger than the amplitude of the long-lived end level (Fig. 4). The contribution by stimulated emission to the total antenna signal is at most 50%. This implies that per photon, the absorbance difference induced by excitation of antenna BChl *a* molecules, corrected for stimulated emission, is at least 4 times larger than the absorbance difference induced by the oxidation of the primary donor. In *C. tepidum* (Fig. 9) the combined amplitude of the 40 ps and 140 ps components, after correction for stimulated emission, is at least 8-times larger than that of the long-lived end level. A similar phenomenon can be observed in the absorbance changes reported by Abdourakhmanov et al. [2] in core-reaction centre complexes of *C. minutissimum*. A bleaching of antenna BChl *a* was found (not corrected for stimulated emission) that was 15-times larger than that associated with oxidation of the primary donor. Danielius et al. [48] concluded from picosecond absorbance difference measurements on chromatophores of *R. rubrum* that per absorbed photon an anomalously large maximum bleaching appeared at the red side of the absorption band that corresponded to ground state bleaching of four monomeric BChls. As a possible explanation of these results, Novoderezhkin et al. [6] have suggested a model which involves excitonic interactions between BChl molecules that are arranged in a circularly symmetric oligomeric structure. They showed that due to the collective nature of the initial excitation, absorption of one photon by the antenna can lead to an absorbance difference signal at the long-wavelength side of the absorption band with a magnitude which corresponds to the bleaching of several monomeric BChl molecules, and to an absorbance increase at the short-wavelength side. Such a model may also, at least partly, account for our observations.

Acknowledgements

The authors wish to thank Dr. P.I. van Noort and R.J.W. Louwe for useful advice concerning the pi-

cosecond laser system, E.M. Franken for providing the absorbance difference spectrum of Fig. 1, C. Francke for measuring the absorption spectrum of *C. tepidum*, and A.H.M. de Wit for carefully culturing the bacteria. They also wish to acknowledge the gift of cells of *C. tepidum* from Dr. A. Verméglio and Dr. M.T. Madigan. The investigations were supported by the Foundation for Biophysics and the Life Science Foundation (SLW), both subsidised by the Netherlands Organisation for Scientific Research (NWO), and were also supported by the European Union (contract no. SCI*-CT92-0796).

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