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Antenna and reaction-center processes upon picosecond-flash excitation of membranes of the green photosynthetic bacterium *Chloroflexus aurantiacus*

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The formation and decay of antenna-excited states and the primary charge separation in membranes of the green photosynthetic bacterium *Chloroflexus aurantiacus* were studied by means of picosecond absorbance difference spectroscopy. After chemical oxidation of the primary electron donor, a 35 ps excitation pulse at 532 nm produced singlet- and triplet-excited states of carotenoid and of bacteriochlorophyll *a*. Excitation of bacteriochlorophyll *a* caused a bleaching of its Q_y absorption band and induced a blue shift of several neighboring bacteriochlorophyll molecules. The singlet-excited state decayed biphasically with lifetimes of about 200 ps and 1.2 ns. A decrease in the lifetime at increasing flash intensity was attributed to singlet–singlet annihilation. In the presence of active reaction centers also the primary-charge separation and secondary electron transfer were observed. The charge separation consisted of the transfer of an electron from the primary donor, P-865, to the primary-acceptor complex of bacteriopheophytin *a* and bacteriochlorophyll *a*. Electron transfer to a secondary acceptor occurred with a time constant of 400 ± 50 ps, which is about 30% longer than had been observed with isolated reaction centers (Kirmaier, C., Holten, D., Mancino, L.J. and Blankenship, R.E. (1984) Biochim. Biophys. Acta 765, 138–146). When this secondary acceptor was prereduced chemically, the lifetime of the primary radical pair increased to 10 ns or more.

Introduction

Chloroflexus aurantiacus is a thermophilic facultatively photo-autotrophic bacterium that has been classified as a green photosynthetic bacterium on the basis of its pigment composition and structure [1]. Like the green sulfur bacteria its contains chlorosomes, and its major pigment is BChl *c*. However, recent studies have shown that the antenna organization of the cytoplasmic membrane, which contains BChl *a*, bears more resemblance to that of purple bacteria than to that of green sulfur bacteria (for recent reviews, see Refs. 2 and 3). The membrane contains a light-harvesting complex, B808–866 [4,5], and a protein complex that contains the reaction center [6]. Studies with isolated reaction centers have shown that the primary electron acceptor is a complex involving bacteriopheophytin *a* (BPh *a*) and bacteriochlorophyll *a* (BChl *a*) [7], whereas the secondary elec-

Abbreviations: P-865, primary electron donor bacteriochlorophyll; BPh, bacteriopheophytin; BChl, bacteriochlorophyll; I, primary acceptor; Q, quinone acceptor; Car, carotenoid; BChl 808 and BChl 866, bacteriochlorophyll molecules having an absorption maximum at 808 and 866 nm, respectively; B808–866 complex, light-harvesting complex containing BChl 808, BChl 866 and carotenoid; PMS, *N*-methylphenazonium methosulfate.

tron acceptor is a quinone (menaquinone) [8], as in purple bacteria.

In previous publications [9–11] we have shown that sensitive absorption difference spectroscopy in the picosecond and nanosecond region permits a study of the excited states of the antenna pigments and of the primary charge separation and secondary electron transport in photosynthetic membranes. The present communication reports a study by means of picosecond absorption difference spectroscopy of membranes of *C. aurantiacus*. Because of a relatively high reaction center to antenna BChl ratio these membranes were found to be quite suitable for a study of the antenna-excited states as well as of electron transport in the reaction center. The results provide information on the lifetimes of the excited BChl *a* and on the rate of energy transfer from short-wave to long-wave absorbing BChl *a*. Data are also reported on primary and secondary electron transport which afford a comparison between the properties of reaction centers when contained in the photosynthetic membrane with those of isolated reaction centers obtained by detergent solubilization.

Materials and Methods

C. aurantiacus strain J-10-fl, was grown phototrophically as described by Pierson and Castenholz [1]. Cells were harvested by centrifugation and resuspended in 10 mM Tris-HCl buffer (pH 8.0). Purified cytoplasmic membranes were isolated by the method described by Feick et al. [12], with minor modifications. To obtain membranes devoid of any residual chlorosome fragments, the final purification step on a discontinuous NaI gradient was repeated once.

Picosecond absorbance-difference measurements were performed by means of the apparatus described in Ref. 11. The 35 ps 532 nm excitation pulse was obtained from a frequency-doubled, mode-locked Nd/YAG laser (maximum energy density, about 2.5 mJ/cm^2). The 1064 nm radiation that remained after frequency doubling was focused in a water cell to generate a white continuum. The 35 ps probe pulse was obtained from this continuum by means of a monochromator placed before the sample. All measurements were performed at room temperature.

Results and Interpretation

Oxidized reaction centers

The measurements in this section were performed on cytoplasmic membranes with the primary donor P-865 oxidized by ferricyanide and continuous background illumination. Thus, no charge separation was observed and only processes taking place in the antenna system of the B808–866 complex of *C. aurantiacus* could be monitored.

Fig. 1 shows the absorbance difference spectra in the region 550–900 nm of the membrane fragments with coincident excitation and probe pulses (0 ps, solid circles) and at 2 ns (open circles) after the 35 ps excitation pulse. The 0 ps spectrum shows a prominent bleaching around 880 nm. We ascribe this bleaching to the disappearance of bacteriochlorophyll (BChl) ground states due to singlet excitation of these pigment molecules. Similar bleachings of the Q_y band of antenna BChl have been observed in chromatophores of purple bacteria [11,13], and in membrane fragments of *Prosthecochloris aestuarii* [9] and *Heliobacterium chlorum* [10]. In *C. aurantiacus* the excitations are mainly localized on the BChl 866 molecules; the shallow trough around 805 nm in the 0 ps spectrum may be due to the presence of some excitation on BChl 808. The absorbance increase between about 750 and 850 nm is also related to the formation of singlet excitations on BChl. Its structure and the position of the wavelength of maximal bleaching (880 nm) with respect to the ab-

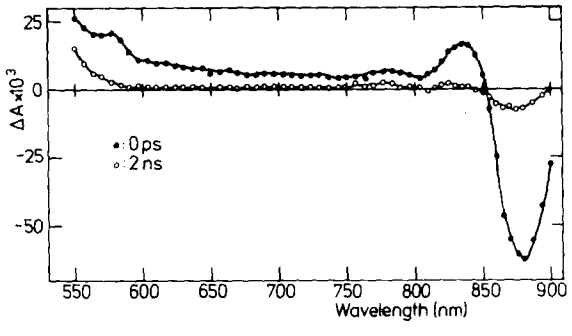

Fig. 1. Absorbance-difference spectra of membrane fragments of *C. aurantiacus* at 0 ps (●) and 2 ns (○) after the excitation pulse in the presence of 2 mM $K_3Fe(CN)_6$. Continuous background illumination was given at 528 nm. The flash-energy density was 0.9 mJ/cm^2. The absorbance of the sample was 0.45 at 532 nm in a 2 mm cell.

sorption maximum at 866 nm will be discussed below.

The absorbance changes in the region above 750 nm remaining at 2 ns (open circles) are ascribed to some triplet and residual singlet excitations on BChl 866 (see below). The absorbance below 590 nm in the 2 ns spectrum is tentatively ascribed to the formation of carotenoid triplet states (Car^T). The main carotenoid in membranes of *C. aurantiacus* is γ-carotene [14,5]. Formation of carotenoid triplets has also been observed following excitation with a 15 ns pulse at 532 nm (Vasmel, H., unpublished data). At 0 ps, the absorbance increase below 650 nm is probably mainly due to singlet-excited carotenoid. Similar absorbance changes were also observed in membranes of *P. aestuarii* and of *Rhodospirillum rubrum* [9,11].

Fig. 2A shows the kinetics of absorbance changes at 880 nm. They reflect the formation and the decay of singlet-excited BChl 866 (BChl*866). The rise-time of the bleaching is mainly determined by the convolution of the temporal profiles of the exciting and probing pulses. From 200 ps after the flash onwards the data can be fitted by a bi-exponential decay with lifetimes of 200 ps and 1.2 ns, and a constant component; the relative amplitude of the 200 ps phase is more than 80% of the total. The fit is shown by the solid line. The constant phase is ascribed to the formation of triplet states of BChl ($BChl^T$). The first 200 ps of the decay are distorted due to convolution with the shape of the pulses and to the occurrence of singlet–singlet annihilation, as has also been observed for *Rhodospirillum rubrum* chromatophores [11].

In Fig. 2B the kinetics at 550 nm are depicted. The initial rapid reversal of the absorbance increase is determined mainly by the temporal profiles of the excitation and probe pulses and is probably due to the disappearance of singlet-excited carotenoid (Car*) which is directly formed by the excitation at 532 nm. From about 250 ps onwards a constant component is observed which reflects the presence of carotenoid triplets. A 200 ps decay component is not observed which indicates that the contribution of BChl* at 550 nm is very small. The 0 ps spectrum of Fig. 1 in the region 550–650 nm could thus be largely due to

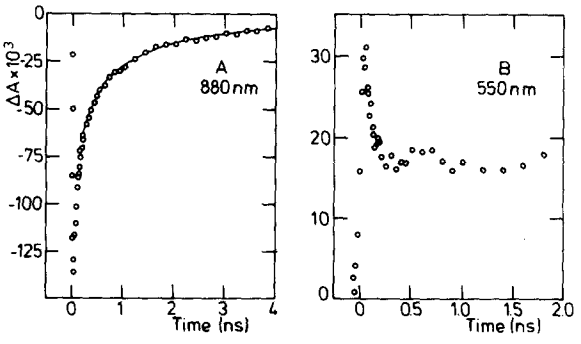

Fig. 2. Kinetics of absorbance changes of membranes of *C. aurantiacus* at 880 nm (A) at a flash-energy density of 1.0 mJ/cm², and at 550 nm (B) at 1.1 mJ/cm². Further conditions as for Fig. 1. The solid curve in (A) shows the best fit for a bi-exponential decay with decay times (1/e) of 200 ps and 1.2 ns (see text).

the formation of Car*, and perhaps of Car^1 below 590 nm.

Fig. 3 shows the dependence of the absorbance decrease at 880 nm on the flash-energy density. We ascribe the apparent saturation of the bleaching to singlet–singlet annihilation. This process shortens the average lifetime of the excitations, as has been observed in the fluorescence decay of the antenna of purple bacteria after a short intense laser flash [15], and reduces the fluorescence yield

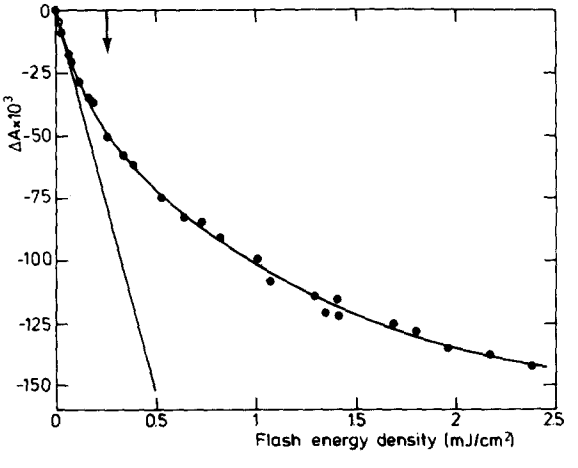

Fig. 3. Flash-energy dependence of the bleaching at 880 nm at 0 ps. Further conditions as for Fig. 1. The arrow indicates the energy density at which on the average one excitation per domain is generated (see text). The straight line is extrapolated from the initial linear part of the curve. See text for details.

[16]. When the lifetime of the excitations becomes of the order of the duration of the pulses or shorter, also the amplitude of the absorbance changes is diminished [11]. From the theory of singlet–singlet annihilation [17] it follows that, under the condition of small domains and efficient energy transfer between the pigments, the fluorescence yield will be decreased by 37% when on the average one excitation per domain is generated, where a domain is defined as the complex of molecules among which the excitations can be transferred. Since the intensity of fluorescence and the amplitude of the absorbance change are proportional, the situation of one excitation per domain occurs when the measured amplitude of the absorbance changes is decreased by 37% as compared to the extrapolated amplitude calculated from the initial linear region of the saturation curve (straight line in Fig. 3), i.e., at a flash-energy density of 0.25 mJ/cm^2, indicated by the arrow. From the absorbance of the sample at 532 nm, from a transfer efficiency from carotenoid to BChl 866 of 40% [5] and from the extinction coefficient of BChl 866 at 866 nm of 137 mM^{-1} · cm^{-1} [5] it then follows that one excitation per domain corresponds to one excited state per 20–25 BChl 866 molecules. This suggests that the domains in *C. aurantiacus* may be as small as one photosynthetic unit [5]. A similar result was obtained from fluorescence measurements (Vos, M., unpublished results).

Open reaction centers

In the presence of PMS and ascorbate both the antenna-excited states and the charge separation in the reaction centers were observed. Fig. 4 shows the absorbance difference spectra in the region 550–900 nm at 150 ps (triangles) and at 2 ns (circles) after the pulse. Above 600 nm the 2 ns spectrum mainly represents the formation of the state P-865$^+$Q$^-$. In the near-infrared region the spectrum is similar to that obtained with isolated reaction centers [6,8]. The oxidation of P-865 causes the bleaching of its Q_y and Q_x absorption bands around 865 and 605 nm, respectively, while the maximum and minimum at 800 and 815 nm are probably due to a blue shift of a BChl *a* band around 813 nm. The absorbance increase at 760 nm can be ascribed to bacteriopheophytin *a* (BPh *a*) and arises from both the oxidation of P-865 and the reduction of Q [7]. The shoulder at 880 nm in the negative band around 865 nm is probably caused by some remaining excited antenna BChl *a* (cf. Fig. 1); the absorbance increase between 550 and 595 nm indicates the formation of Car^T. The 150 ps spectrum shows, in addition to absorbance changes related to oxidation of P-865 and formation of Car^T, a large bleaching around 880 nm due to BChl*866 and, in the region 600–830 nm, changes that are related to the reduction of the primary electron acceptor I. In order to separate these absorbance changes from those due to P-865, we subtracted the 2 ns spectrum from that measured at 150 ps. The resulting difference spectrum contained an appreciable contribution by BChl*. The amplitude of this contribution was calculated from the amplitudes of the difference spectra at 880 nm, the difference of which was assumed to be due to BChl* only. The difference spectrum thus corrected for absorbance changes of antenna BChl is shown in the inset of Fig. 4. It should depict the absorbance difference between I$^-$Q and I Q$^-$ which can be mainly ascribed to the absorbance difference between I$^-$ and I in this region. Inspection of the figure shows that reduction of I causes a bleaching around 765

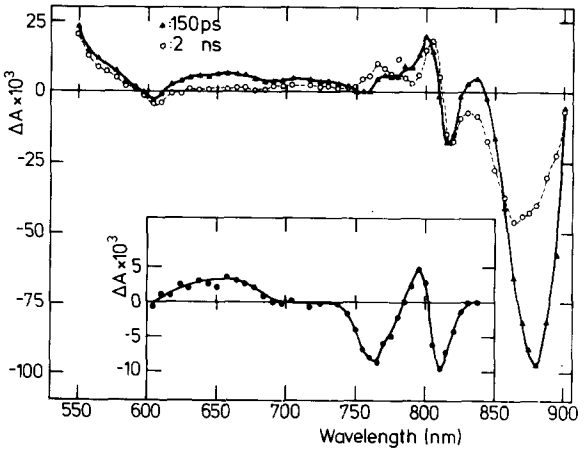

Fig. 4. Absorbance-difference spectra of membranes of *C. aurantiacus* at 150 ps (▲) and 2 ns (○) after the flash in the presence of 10 mM ascorbate and 20 μM PMS. Inset: difference between the 2 ns and 150 ps spectra corrected for absorbance changes in the antenna (see text). The flash-energy density was 1.1 mJ/cm^2. The absorbance of the sample at 532 nm was 0.45 in a 2 mm cell.

and 810 nm, and an increase around 795 nm. Similar changes have been observed by Kirmaier et al. in isolated reaction centers of *C. aurantiacus* [7]. These authors concluded that I is a complex involving BPh *a* and BChl *a*, and that reduction of I induced bleachings of BPh *a* at 765 nm and of BChl *a* at 810 nm. The absorbance increase around 650 nm observed by us is probably due to the absorption of the anionic species.

Fig 5 shows the absorbance difference spectrum in the region 550–900 nm at 2 ns after the flash in the presence of dithionite to reduce Q chemically. The bleaching around 875 nm can be ascribed to both $P\text{-}865^+$ and excited BChl 866. The broad increase in absorption between 610 and 700 nm and the bleaching around 760 nm indicate the reduction of BPh *a*. The asymmetry of the band shift around 810 nm (compare with the 2 ns spectrum of Fig. 4) shows the presence of a bleaching around 810 nm, which is also seen in the spectrum in the inset of Fig. 4. These results demonstrate that upon chemical reduction of Q the lifetime of I^- is increased. A possible reason for the smaller amplitude of the absorbance changes in Fig. 5 as compared to Fig. 4 is discussed below.

Fig. 6 displays the kinetics of the absorbance changes at various wavelengths in the presence of either PMS and ascorbate (open circles) or dithionite (solid circles). In each case, the rise-time of the initial absorbance changes is determined by

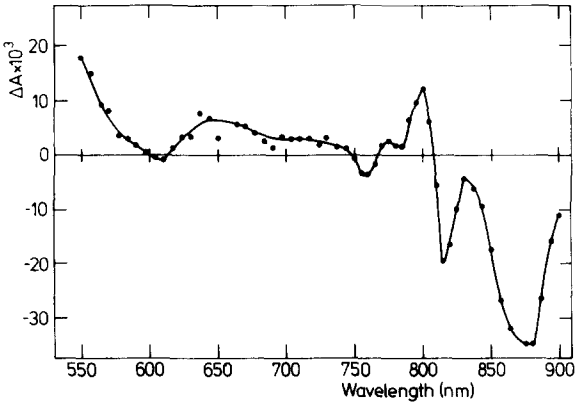

Fig. 5. Absorbance-difference spectrum of membranes of *C. aurantiacus* at 2 ns after the flash in the presence of dithionite, at a flash-energy density of 0.9 mJ/cm^2. The absorbance of the sample at 532 nm was 0.45 in a 2 mm cell.

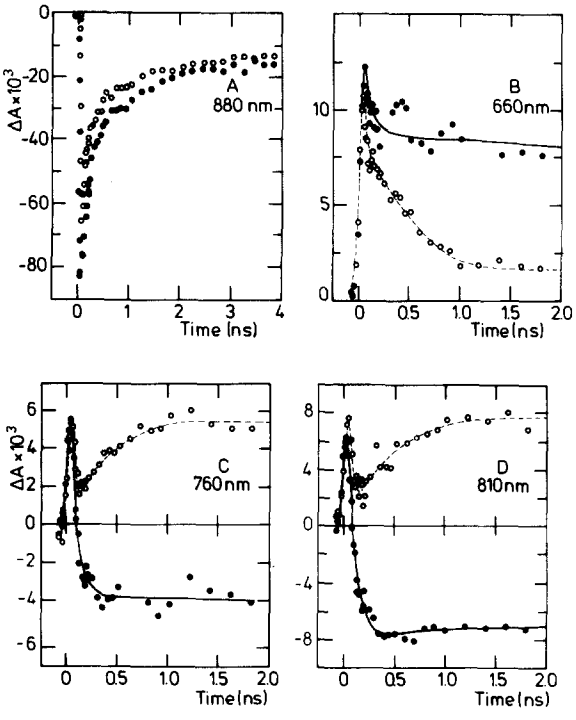

Fig. 6. Kinetics of absorbance changes of membranes of *C. aurantiacus* in the presence of 10 mM ascorbate and 20 μM PMS (○), or of dithionite (●) at 880 nm (A); 660 nm (B); 760 nm (C); and 810 nm (D). The excitation-energy density was 1.0 mJ/cm^2.

the convolution with the temporal profile of the pulses. The decay of the bleaching at 880 nm (A) up to 2 ns is due to the recovery of the antenna BChl 866 ground-state absorption. The residual bleaching is mainly caused by the oxidation of P-865. From the kinetics with dithionite it can be observed that the lifetime of $P\text{-}865^+$ when the reaction center is in the state $P\text{-}865^+I^-Q^-$ is probably more than 10 ns.

At 660 nm (B) the first 200 ps of the decay mainly represents the disappearance of antenna excited singlet states (cf. Fig. 1). The residual increase, which is due to reduction of I (cf. Fig. 4, inset and Fig. 5) decays with a time-constant of about 400–450 ps in the presence of PMS and ascorbate, and reflects the reoxidation of I^-, probably by Q. A 310 ps lifetime of I^- was observed in isolated reaction centers of *C. aurantiacus* [7]. No significant decay of I^- up to 2 ns is observed after chemical reduction of Q (solid circles). At 760 nm

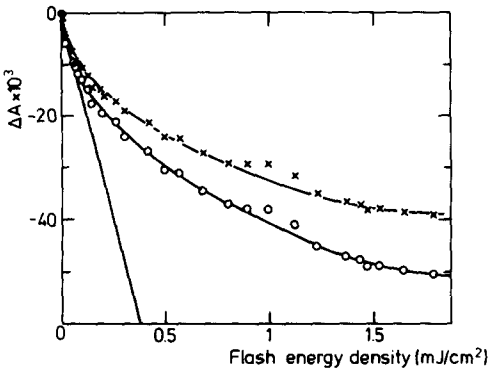

Fig. 7. Flash-energy density dependence of the bleaching at 865 nm at 2 ns after the flash in the presence of 10 mM ascorbate and 20 μM PMS (○), and curve calculated for the saturation of P-865$^+$ formation (×, see text). The straight line corresponds to a quantum yield of charge separation of 100%.

(C) and 810 nm (D) the decay of the initial increase is again due to the disappearance of antenna BChl*a. At both wavelengths an increase in absorption with a rise-time of about 350 ps is observed in the presence of PMS and ascorbate which may be ascribed to the electron transfer from I^- to Q. This increase is lacking after chemical reduction of Q and the absorbance change now rapidly reaches a constant negative level, reflecting the state P-865$^+I^-Q^-$.

These results all indicate that the electron transport from I^- to Q takes 350–450 ps, and that the lifetime of I^- is increased to at least several nanoseconds upon chemical reduction of Q. As judged from our data, a charge recombination between P-865$^+$ and I^- under these conditions, as observed for purple bacteria [18], could take place with a time constant of 10 ns or more.

Fig. 7 shows the flash-energy density dependence of the bleaching at 865 nm at 2 ns after the flash, in the presence of PMS and ascorbate (circles). The absorbance decrease is largely due to oxidation of P-865 (cf. Fig. 4, circles), but a contribution from excited antenna BChl 866 is also present, as judged from the 2 ns spectrum of Fig. 1. The magnitude of this contribution at 865 nm can be estimated from the saturation curve of BChl*866 formation (Fig. 3). After subtraction, the bleaching due to P-865 oxidation is obtained (Fig. 7, crosses). Our data indicate a maximal amplitude of $4 \cdot 10^{-2}$ at saturation, which corresponds to one reaction center per approx. 22 BChl 866 molecules, applying the known extinction coefficients at 865 nm for P-865 and BChl 866 [2,5]. Including the BChl 808 molecules, a BChl a-to-reaction center ratio of about 33 is obtained, in agreement with the value of 30–35 obtained by Vasmel et al. [5].

The quantum yield of charge separation upon excitation of antenna BChl, as calculated from the estimated initial slope of the bleaching of P-865 and from an efficiency of 40% for energy transfer from carotenoid to BChl, is at least 80% or more. The drawn line corresponds to a quantum yield of 100%.

Discussion

Excitation of membrane fragments of *C. aurantiacus* with a 35 ps flash at 532 nm causes absorbance changes due to formation and decay of excited states in the antenna and, when open reaction centers are present, also those due to the primary charge separation and subsequent electron transport. In the antenna system direct excitation of carotenoid (Car) gives rise to the formation of singlet excited Car (Car*), and BChl a (BChl*a). In the presence of oxidized reaction centers, the decay of BChl*866 occurs in two phases with time constants of about 200 ps and 1.2 ns. Possibly, the 1.2 ns component, which has a relative amplitude of less than 10%, reflects the decay of excitations in B808–866 complexes that, as a result of the isolation procedure, were detached from reaction centers. The same fluorescence lifetime of about 1.1 ns has been measured in the isolated B800–850 light-harvesting complex of *Rps. sphaeroides* [19], The 200 ps phase is similar to that observed in the antenna system of *R. rubrum* when the reaction centers were oxidized [20]. The small constant component observed after the decay of BChl*866 probably arises from the presence of triplet states. As in *R. rubrum* [20], one would expect the 200 ps phase to be replaced by a faster decay when monitoring the decay of BChl*a in the presence of open reaction centers. At the rather high-energy density at which the kinetics of Fig. 6A were measured, however, most or all of the reaction centers already became closed

during the flash, and so the effect of trapping on the lifetime of BChl*866 was not observed.

Within 150 ps after direct carotenoid excitation, Car^T states are formed, and no increase in the amount is observed in at least the following 2 ns. Since at 150 ps after the flash BChl*866 has only partly decayed, the formation of Car^T from Car* probably proceeds without excited states of BChl *a* as intermediates. In fact, formation of Car^T via BChl**a* and $BChl^T a$ in purple bacteria is usually observed to occur with a time constant of about 20 ns [21]. The $BChl^T$ detected in *C. aurantiacus* could thus be a precursor of later to be formed triplet states of Car. A rapid formation of Car^T after direct excitation of Car has also been observed in *R. rubrum* [11]. It has been proposed that in *R. rubrum* as well as in *Rps. sphaeroides* [22] singlet fission of Car* can give rise to the formation of Car^T states without excited states of BChl *a* as intermediates. On the basis of our results such a mechanism seems likely for *C. aurantiacus* as well, and could account at least partly for the fate of the 60% of the excitation energy on carotenoid that is not transferred to BChl *a* [5].

The absorbance-difference spectrum of BChl*866 formation shows a maximal bleaching at 880 nm (Fig. 1). This maximum is situated at about 14 nm longer wavelength than the maximum in the absorption spectrum. In addition there is an absorbance increase on the blue side of the bleaching. From measurements of fluorescence polarization at low temperature it has been concluded that the BChl 866 absorption band is due to a spectrally inhomogeneous population of BChl molecules [5]. It seems unlikely, however, that a Boltzmann equilibrium between excitations on these BChl *a* molecules could induce the red shift of about 14 nm in our room-temperature difference spectrum. It is more probable that the apparent red shift results from the absorbance increase observed on the blue side. A similar effect was encountered in the antenna complex of *R. rubrum* [11]. As in *R. rubrum*, the shape of the absorbance difference spectrum can be accounted for by the assumption that it is composed of a bleaching with the shape of the absorption band, due to excitation of BChl, and a blue shift of at least five surrounding molecules caused by the loss of exciton interaction with the excited BChl. Fig. 8 (solid circles) depicts the absorbance-difference spectrum measured at an intensity where on the average one excitation per reaction center is created. The amplitude was corrected for the effect of singlet-singlet annihilation (see Fig. 3). The two other curves show the proposed decomposition into a bleaching of six BChl 866 molecules per photosynthetic unit (open circles) and the formation of an absorption band around 858 nm of five molecules (squares). It was assumed that the bleaching has the shape and the combined amplitudes of the Q_y absorption bands of the BChl 866 molecules. The shape of the new band around 858 nm was calculated by subtracting the bleaching from the measured spectrum. We thus propose that the excitation of one BChl 866 molecule induces the bleaching of its Q_y absorption band and a blue shift of at least five surrounding BChl *a* molecules to about 858 nm.

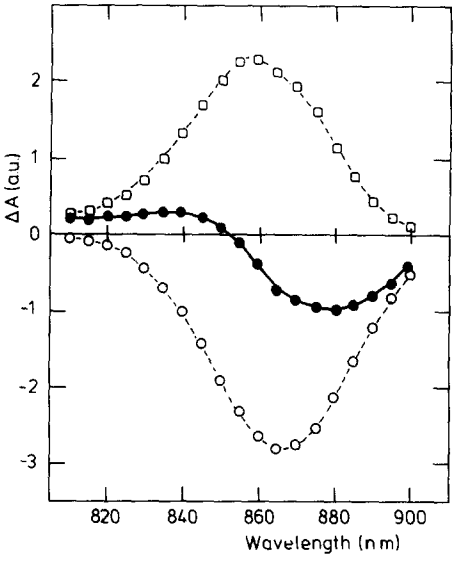

Fig. 8. Measured absorbance-difference spectrum of membranes of *C. aurantiacus* at 0 ps in the presence of $K_3Fe(CN)_6$ (●), and its decomposition into a bleaching (○) and an absorbance increase (□) (see text for details).

The minimum near 805 nm (Fig. 1) may be due to the presence of some excitations on BChl 808. The amplitude of the bleaching is estimated to be $6 \cdot 10^{-3}$. From the flash-energy density at which the spectrum of Fig. 1 was recorded, and assuming

that one-third of the excitations transferred from Car to BChl *a* are directly transferred to BChl 808, a maximal bleaching of $3 \cdot 10^{-2}$ would be expected if no energy transfer from BChl 808 to BChl 866 occurred. The difference between the calculated and observed absorbance decrease is caused by the short lifetime of BChl*808. A simulation of this effect by convolution of a mono-exponential decay of BChl*808 with 35 ps gaussian excitation and probe pulses indicates a maximal lifetime of BChl*808 of 6 ps, in good agreement with the minimal rate of energy transfer from BChl 808 to BChl 866 of $1.6 \cdot 10^{11}$ s^{-1} calculated from the low-temperature emission spectrum [5].

As to the primary-charge separation, our results on membrane fragments are in general agreement with those obtained by Kirmaier et al. [7] on isolated reaction centers of *C. aurantiacus*. The primary electron acceptor, I, is a complex involving both BPh *a* and BChl *a*. Whether the bleaching of the BChl *a* band at 810 nm observed upon reduction of I is due to the creation of some electron density on BChl *a*, or to a redistribution of oscillator strength of BChl *a* as a result of the disruption of the excitonic interaction between BChl *a* and the reduced BPh *a* is still unclear. The re-oxidation of I^- by the secondary acceptor, menaquinone, appears to proceed with a time constant of 350–450 ps, which is somewhat longer than then the 310 ps reported for the isolated reaction centers [7]. This difference, if real, could perhaps be due to changes induced during the removal of the reaction center from the membrane. Alternatively, energetic factors could play a role here. The position of the equilibrium BChl*866 $\leftrightarrow$ P-865$^+$I$^-$ is determined, amongst other factors, by the energy difference between the two states, and it governs the rate at which I^- is observed to become oxidized. If the establishment of the equilibrium is fast compared to the rate of I^- reoxidation, the increase of the time constant for the reoxidation of I^- by a factor of about four-thirds as compared to that in isolated reaction centers should be reflected in a correspondingly lower steady-state concentration of P-865$^+$I$^-$. Thus it follows that the equilibrium constant [P-865$^+$I$^-$]/[BChl*866] $\approx$ 3. This, combined with the assumption that all of the approx. 22 BChl 866 molecules that are present per reaction center take part in the equilibrium, would correspond to an energy difference between BChl*866 and P-865$^+$I$^-$ of about 100 meV, similar to the values of 50 –150 meV that have been reported for purple bacteria [23].

After chemical reduction of Q, the lifetime of I^- is increased, and no decay of I^- is observed up to 2 ns. Similar to what has been observed in purple bacteria [18], a recombination between P-865$^+$ and I^- might occur under these conditions. In *C. aurantiacus* the lifetime of the primary radical pair is probably 10 ns or longer. In the state P-865$^+$I$^-$Q$^-$ the amounts of P-865$^+$ and the amounts of BChl*866, present at 2 ns after the pulse (Fig. 5), seem to be smaller and larger, respectively, than in the state P-865$^+$I Q$^-$, present at 2 ns after the flash (Fig. 4). These observations support the above-discussed equilibrium.

Concluding we can say that the application of sensitive picosecond absorbance-difference spectroscopy to membranes of *C. aurantiacus* has allowed for the first time a comparison of results obtained on the primary photochemistry of isolated reaction centers to those of a more intact photosystem, and furthermore has the advantage of providing information on the antenna-excited states as well.

Acknowledgements

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