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Excited states and primary charge separation in the pigment system of the green photosynthetic bacterium *Prosthecochloris aestuarii* as studied by picosecond absorbance difference spectroscopy

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Picosecond absorbance difference spectra at a number of delay times after a 35 ps excitation flash and kinetics of absorbance changes were measured of the membrane vesicle preparation Complex I from the photosynthetic green sulfur bacterium *Prosthecochloris aestuarii*. After chemical oxidation of the primary donor the excitation pulse produced singlet and triplet excited states of carotenoid and bacteriochlorophyll *a*. With active reaction centers present also the flash-induced primary charge separation and subsequent electron transfer were observed. The singlet excited state of the carotenoid, formed by direct excitation at 532 nm, is characterized by an absorbance band peaking at 590 nm. Its average lifetime was calculated to be about 1 ps. Excited singlet states of bacteriochlorophyll *a* were characterized by a bleaching of their ground state Q_y absorption bands. Singlet excited states, localized on the so-called core complex, were produced by energy transfer from excited carotenoid. Their lifetime was about 70 ps. A decay component of about 280 ps was ascribed to singlet excited bacteriochlorophyll *a* in the bacteriochlorophyll *a* protein. These singlet excitations were partly converted to the triplet state. With active reaction centers, oxidation of the primary donor, P-840, characterized by the bleaching of its Q_y and Q_x absorption bands, was observed. This oxidation was accompanied by a bleaching between 650 and 680 nm and an absorbance increase between 680 and 750 nm. These changes, presumably due to reduction of bacteriopheophytin *c* (Van Bochove, A.C., Swarthoff, T., Kingma, H., Hof, R.M., Van Grondelle, R., Duysens, L.N.M. and Amesz, J. (1984) Biochim. Biophys. Acta 764, 343–346), were attributed to the reduction of the primary electron acceptor. Electron transfer to a secondary acceptor occurred with a time-constant of 550 ± 50 ps. Since no absorbance changes due to reduction of this acceptor were observed in the red or infrared region, we tentatively assume that this acceptor is an iron-sulfur center.

Introduction

Until fairly recently little was known about the primary and associated electron-transport reactions of green sulfur bacteria. One reason for this lack of knowledge was undoubtedly the large antenna size and correspondingly low reaction-center content of these organisms [1,2]. Moreover, isolated reaction centers, like those obtained from purple bacteria and recently also from Chloroflexaceae, have not been isolated from green sulfur bacteria (see Refs. 1–3 for recent reviews).

Abbreviations: BChl, bacteriochlorophyll; BPh, bacteriopheophytin; Car, carotenoid; P-840, primary electron donor bacteriochlorophyll; PMS, *N*-methylphenazonium methosulfate.

The photosystem of the green sulfur bacterium *Prosthecochloris aestuarii* consists of a large antenna system of BChl *c* molecules, the chlorosome, and a base plate that contains the soluble BChl *a* protein [1,2,4,15].

Excitation energy captured by the chlorosome is thought to be funnelled through the base plate to the core complex. This core complex contains BChl *a* and carotenoid, and is closely associated with the reaction center [5,6].

An important advance toward the study of electron transport in the reaction center of green sulfur bacteria has been the development of methods to obtain preparations with a strongly enhanced reaction center content. These preparations contain little or no BChl *c* and have a BChl a^-/reaction center ratio that ranges from about 100 to about 20. They include the membrane vesicle preparation 'Complex I' with about 80 BChl *a* molecules per reaction center and various pigment-protein complexes derived from the cytoplasmic membrane [5–9].

Optical and ESR measurements with these preparations have provided evidence that the electron-acceptor chain in green sulfur bacteria resembles more that of Photosystem I of green plants than that of purple bacteria. Photoaccumulation experiments indicated that iron-sulfur centers function as secondary electron acceptors [10,11], and also suggested the involvement of monomeric BChl *a* with a Q_y band at 814 nm in the electron-transport chain [12]. More recently, nanosecond flash spectroscopy of a preparation in which secondary electron transport was inhibited suggested that BPh *c* or another pigment absorbing at 670 nm acts as primary electron acceptor [13]. Evidence was given for a back reaction between the reduced primary acceptor I^- and the oxidized primary electron donor P-840$^+$ yielding the triplet state of P-840. The half-time of this reaction was 25 ns.

This paper reports the results of a flash spectroscopic study of Complex I in the picosecond region. Evidence will be presented that a 35 ps, 532 nm laser flash, in addition to excited singlet and triplet states of BChl *a* and carotenoid, generates the primary charge pair P-840$^+I^-$. Our results confirm the suggestion that I is BPh *c*, and show that the electron is transferred in about 550 ps from I^- to a secondary electron acceptor. No evidence was found for a significant absorption in the red or infrared region by the secondary acceptor, suggesting that this acceptor is an iron-sulfur center, rather than BChl *a*.

Materials and Methods

P. aestuarii, strain 2 K, was grown anaerobically in a mixed culture as described by Holt et al. [14]. The membrane vesicle preparation Complex I was prepared according to Ref. 7, and the BChl *a* protein according to Ref. 15. Samples were suspended in 10 mM phosphate/10 mM ascorbate buffer (pH = 7.4).

The picosecond absorbance measurements were performed by means of the apparatus briefly described in Ref. 16. The light source was a mode-locked Nd-YAG laser generating pulses at 1064 nm with a duration of 35 ps. Part of the radiation was frequency-doubled to 532 nm, at which wavelength the samples were excited. The maximum excitation energy used was about 3 mJ/cm^2. The remaining infrared radiation was focussed into a 15 cm cell filled with water in order to generate a broad wavelength continuum. The measuring pulse was obtained from this continuum by means of a small monochromator placed before the sample. Its wavelength was varied between 550 and 900 nm. Due to variations in the focussing of the laser beam on the monochromator, the error in the wavelength reading was about 2 nm. Part of the pulse was deflected by a beam splitter onto a photodiode as a reference. The remaining measuring pulse light was transmitted through the sample and detected by a second photodiode. A time delay of maximally 5 ns between the exciting and probe pulses was obtained by means of an optical delay line. Stray exciting light and fluorescence were prevented from reaching the photodiode by diaphragms, a Schott KV 550 and, when needed, suitable interference filters. Absorbance changes were measured by comparing the ratio of measuring and reference pulse intensities with the ratio obtained with a measuring pulse preceding the excitation pulse. By this method remaining effects of stray light and fluorescence, if any, were largely eliminated. Both the excitation and measuring pulses were vertically polarized. The repetition rate

of the pulses was 0.5 Hz. By averaging over about 25 laser flashes a resolution of about $(1-2)\cdot 10^{-3}$ units of absorbance was obtained. All experiments were performed at room temperature.

Results and Interpretation

The absorbance spectrum of the membrane vesicle preparation Complex I of *P. aestuarii* is shown in Fig. 1. The near-infrared absorbance band (maximum at 810 nm) is due to antenna BChl *a*, for the largest part contained in the BChl *a* protein and partly in the so-called core complex [5]. The corresponding Q_X bands are located around 600 nm. Membrane-bound BPh *c* is partly responsible for the absorbance band at 670 nm, but artefacts of the preparation, including solubilized BChl *c*, may also absorb at this wavelength. The band at 740 nm is due to residual BChl *c* of the chlorosome [2,7,9].

Illumination of Complex I at 532 nm excites mainly carotenoids (rhodopin and (OH)-chlorobactene). A laser flash at this wavelength produced absorbance changes that could be ascribed both to the formation of excited states of antenna pigments and to charge separation in the reaction center. In order to distinguish between these phenomena, we measured the absorbance difference spectra and the kinetics of the absorbance changes both under reducing and oxidizing conditions. In the first case the preparation showed full reaction center activity, whereas in the second case the primary electron donor P-840 was in the oxidized state ($P\text{-}840^+$) before the flash, so that the reaction centers were closed and no light-induced charge separation was observed.

Oxidized reaction centers

Fig. 2 shows the flash-induced absorbance difference spectrum obtained during the flash in the presence of 20 mM ferricyanide and with a weak background light to keep P-840 in the oxidized state throughout the experiment. The bleaching between 800 and 850 nm, with a maximum near 820 nm, can be ascribed to disappearance of antenna BChl *a* ground states due to excitation of the antenna molecules. A similar bleaching with a lifetime of about 60 ps, ascribed to singlet-excited BChl *a* (BChl *a**) has been observed in the antenna of purple bacteria [17].

The kinetics of the bleaching at 840 nm are shown in Fig. 3B. The light-on response is probably determined by the temporal profile of the exciting and the probing pulses. Simulation of the decay kinetics by convolution of a monoexponential decay with 35 ps Gaussian excitation and

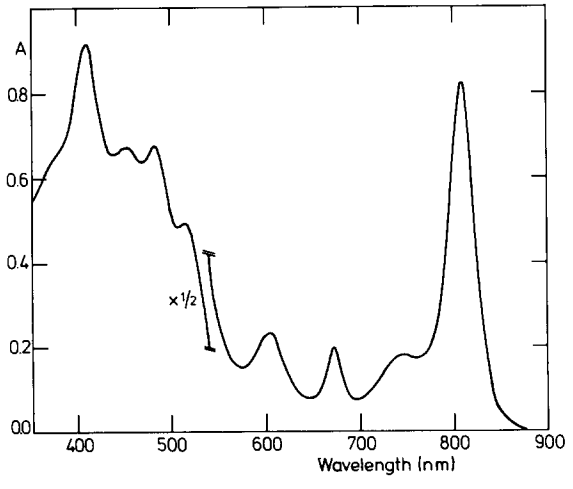

Fig. 1. Absorption spectrum of Complex I of *P. aestuarii* suspended in 10 mM phosphate/10 mM ascorbate buffer (pH = 7.4).

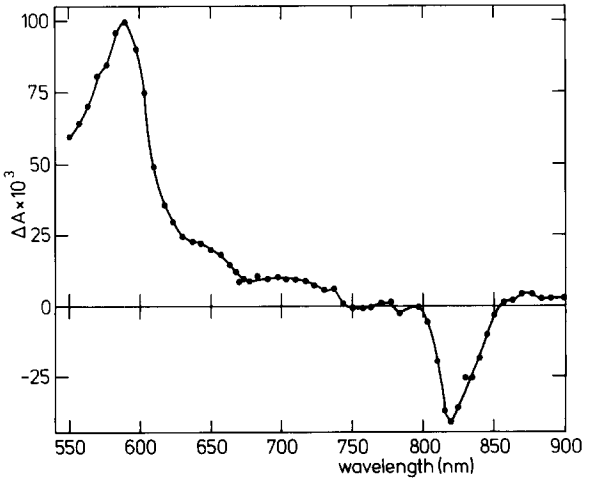

Fig. 2. Absorbance difference spectrum of Complex I with coincident excitation and measuring pulses after addition of 20 mM $K_3Fe(CN)_6$ and under continuous (weak) background illumination at 528 nm. The absorbance at 532 nm was 1.5 in a 2 mm cell.

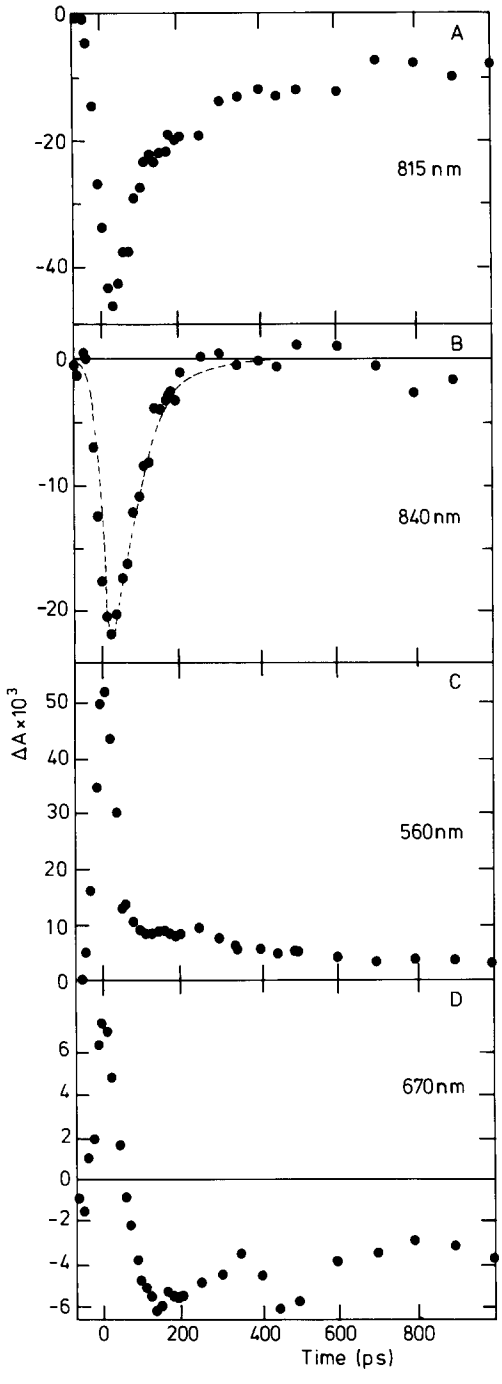

Fig. 3. Kinetics of absorbance changes at various wavelengths. Conditions as for Fig. 2; $t = 0$ corresponds to simultaneous 35 ps Gaussian excitation and probe pulses. The broken line in Fig. 3B corresponds to a simulation obtained by convolution of a 70 ps decay (see text).

probe pulses indicated a lifetime τ $(1/e)$ of BChl a^* of 70 ± 10 ps, as shown by the broken line in Fig. 3B. A similar decay component of about 70 ps was observed at 815 nm (Fig. 3A), but at this wavelength at least two other components could be discerned; one with a lifetime of approx. 250 ± 50 ps and a much slower one that remained approximately constant at the time-scale of our experiment. The first of these may be due to a longer-lived excited singlet state of BChl *a* (see below); the second one may be attributed to a triplet state (BChl a^T), produced from BChl a^* by intersystem crossing. The absence of long-lived components at 840 nm indicates that no flash-induced oxidation of P-840 occurred, and that thus practically all P-840 was in the oxidized state before the flash.

Another prominent feature of the difference spectrum of Fig. 2 is a strong increase in absorbance at 550–650 nm with a maximum at 590 nm and a weaker tail extending to 750 nm. The kinetics of the absorbance changes at 560 nm are shown in Fig. 3C. The absorbance change consists of several components. The time dependence of the initial increase and rapid decrease is largely determined by the temporal profile of the excitation and probe pulses, and thus reflects the formation and the decay of a species with a lifetime short compared to the duration of the pulses (i.e., a few picoseconds). This short-lived component accounts for the larger part of the absorbance increase. Similar absorbance changes have recently been shown to occur in the antenna of chromatophores of *Rhodospirillum rubrum*. Spectral evidence indicated that these changes were caused by transient formation and decay of the singlet excited state of the carotenoid spirilloxanthin [16]. Analogously, the short-lived component in Complex I may be caused by singlet excited carotenoid (rhodopin or (OH)-chlorobactene) formed by direct excitation of these carotenoids during the pulse.

The absorbance changes due to the formation of Car* (at 590 nm) and BChl a^* (at 815 nm) during the excitation flash are plotted in Fig. 4 (filled circles and crosses, respectively) as a function of the energy of the excitation pulse. Apparently, the formation of both excited states proceeds approximately linearly with energy density,

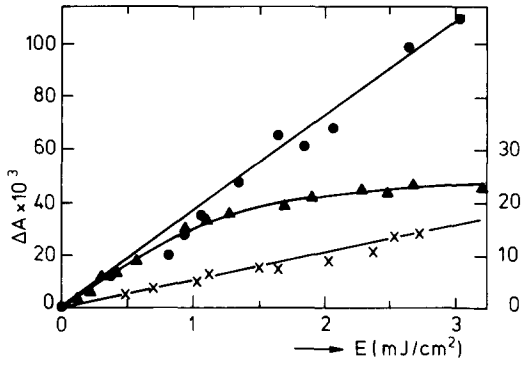

Fig. 4. Absorbance changes vs. the incident excitation-energy density. ●, absorbance increase at 590 nm at $t = 0$ (left hand scale); ×, bleaching at 815 nm at $t = 0$ (left hand scale), both under oxidizing conditions as for Fig. 2. ▲, bleaching at 840 nm after 500 ps (right hand scale), under reducing conditions as in Fig. 7. The sign of the absorbance changes at 815 and 840 nm was reversed.

and is far from saturated at the maximal flash energy used.

Absorbance difference spectra of Complex I measured at 130 ps and 4 ns after the excitation pulse are given in Fig. 5. At 130 ps after the pulse the singlet carotenoid (Car*) has largely or completely disappeared, but a considerable amount of BChl *a** is still present, as indicated by the bleaching in the near-infrared region. Part of the absorbance increase in the region 550–600 nm, which is also seen in the 4 ns spectrum, may be ascribed to carotenoid triplets (Car^T) [13]. Formation of

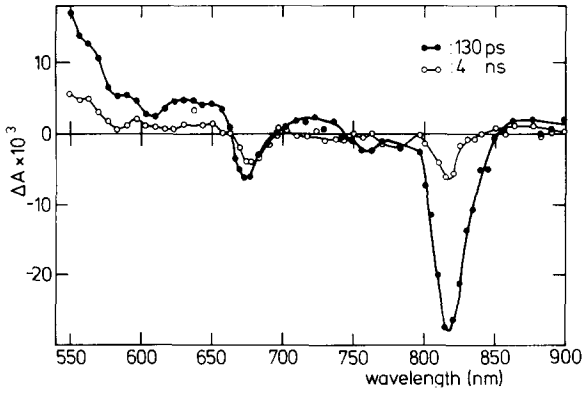

Fig. 5. Absorbance difference spectra of Complex I under oxidizing conditions at 130 ps (●) and 4 ns (○) after the excitation flash. Conditions as for Fig. 2.

these triplets then accounts for the small constant component in the kinetics at 560 nm (Fig. 3C). The small residual bleaching at 817 nm observed in the 4 ns spectrum may be likewise ascribed to triplets of BChl *a* (Bchl a^T), as mentioned above.

The spectra measured after 130 ps and 4 ns also show a fairly strong bleaching at 675 nm. Most of the absorption at this wavelength is due to 'native' BPh *c*, which showed effective energy transfer to BChl *a* of the core complex [5,18], but part of the absorption and most of the relatively strong fluorescence observed near 675 nm have been attributed to a detached form of BPh *c*, which may be an artefact of the preparation, and to degradation products [18,19]. Since these products presumably do not transfer their energy to other pigments, their excited singlet (and perhaps triplet) states may contribute significantly to the difference spectrum measured at 4 ns. At shorter times the contribution of 'native' BPh *c** may be more important. Part of the absorbance increase measured in the region 550–600 and 700–750 nm could likewise be due to these excited pigments.

The BChl a protein

Approximately three-fourth of the BChl *a* of Complex I is contained in the BChl *a* protein [5,18]. It is therefore of interest to compare the absorbance changes of Complex I with those of the isolated BChl *a* protein.

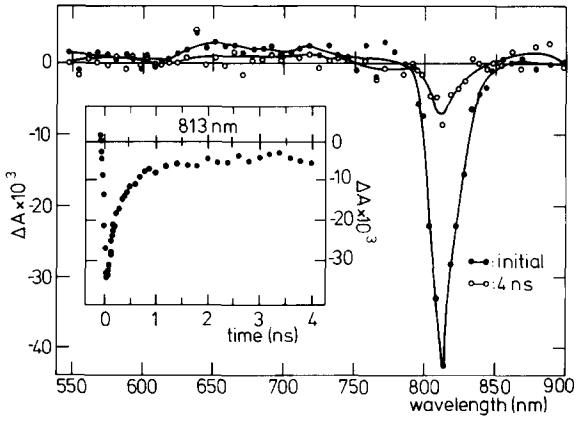

Fig. 6. Absorbance difference spectra of the isolated BChl *a* protein with coincident excitation and probe pulses (●), and after 4 ns (○). The insert shows the kinetics of absorbance changes at 813 nm. The absorbance of the sample was 0.92 at 810 nm in a 2 mm cell.

The difference spectra of the isolated BChl *a* protein recorded during the excitation flash and after 4 ns are shown in Fig. 6. Both spectra exhibit a bleaching with a maximum at 813 nm, coinciding with that of the absorption spectrum. The kinetics of the absorbance changes at 813 nm are given in Fig. 6 (inset). The bleaching decays with a time constant of 280 ± 20 ps to a level constant on a nanosecond time-scale. We attribute the fast component to the decay of BChl *a**, and the constant component to BChl a^T. Formation of a triplet of BChl *a* in the BChl *a* protein has recently also been shown by absorbance-detected magnetic resonance at 1.2 K [20]. The triplet is probably produced by intersystem crossing from the excited singlet state Comparison of the difference spectra observed for Complex I and the BChl *a* protein indicates that for Complex I at least an appreciable fraction of the excited singlet states is not located on the BChl *a* protein (and thus probably resides on the core complex). We will return to this point in the discussion.

Open reaction centers

Fig. 7 shows the absorbance difference spectrum of Complex I with coincident exciting and probe pulses under reducing conditions. These and other measurements reported in this section were performed in the presence of 5 μM PMS and 20 mM ascorbate to obtain complete rereduction of

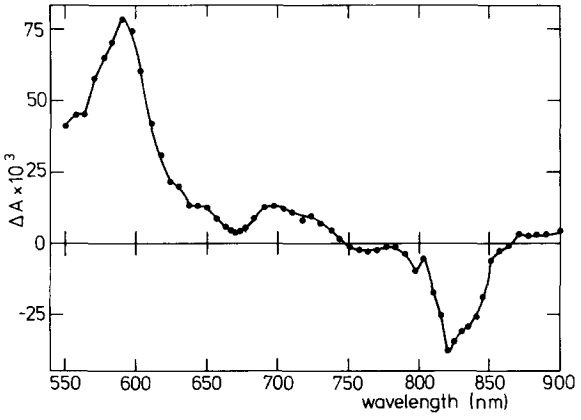

Fig. 7. Absorbance difference spectrum of Complex I with coincident excitation and measuring pulses in the presence of 5 μM PMS/20 mM ascorbate. The absorbance at 532 nm was 1.5 in a 2 mm cell.

P-840$^+$ between flashes. The absorbance increase in the visible region with a maximum near 590 nm can again for the larger part be attributed to Car*. The kinetics at 560 nm (Fig. 8B) were similar to those obtained in the presence of ferricyanide (see Fig. 3C). Above 750 nm a comparison with the spectrum of Fig. 2, with closed reaction centers, reveals a shoulder near 840 nm and an additional weak maximum and minimum near 800 nm. This indicates that in addition to BChl *a**, P-840$^+$ was now formed during the flash. In the region 650–750 nm, the trough at 670 nm as well as the absorbance increase around 700 nm were more pronounced than in the presence of ferricyanide. As will be discussed below, these features are probably due to reduction of the primary electron acceptor.

Absorbance difference spectra measured at 130 ps, 1 ns and 4 ns after the excitation pulse are given in Fig. 9. The absorption changes in the region above 750 nm measured after 4 ns and after 1 ns (not shown) include negative bands around 835 nm and near 790 nm, which are lacking in the corresponding spectra with closed reaction centers (Fig. 5). In this region the spectrum resembles closely that measured by Swarthoff and Amesz [9] at a much longer time, which was attributed to the oxidation of P-840 together with the formation of a reduced secondary acceptor (X). The shape of the 4 ns spectrum in the region 650–700 nm may be explained by bandshifts of pigments absorbing near 670 nm that accompany the oxidation of P-840 [9], and by the long-lived excited states that were also observed with closed reaction centers; the bleaching at 615 nm may be ascribed to the disappearance of the Q_x band of P-840. The same features can be seen in the spectrum measured after 1 ns (Fig. 9).

The kinetics of the absorbance changes at 815 and 840 nm are shown in Fig. 8A and 8C, respectively. At both wavelengths a bleaching is observed which decays with a lifetime of 70 ± 10 ps (obtained by convolution as described above) to a level constant on the time scale of the experiment. We again interpret the 70 ps phase which corresponds closely to that observed for samples with oxidized reaction centers to the decay of BChl *a**. As is apparent from Fig. 8C, no change in absorbance at 840 nm occurs after the decay of the

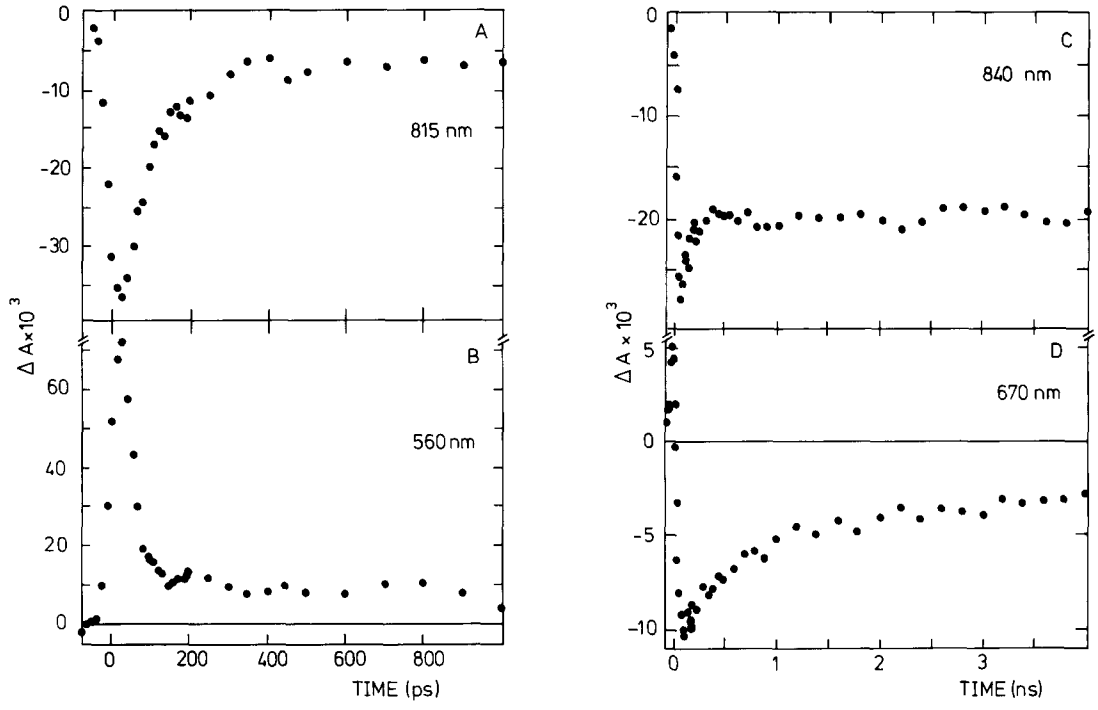

Fig. 8. Kinetics of absorbance changes at various wavelengths (note the different time scales). Reducing conditions as for Fig. 7.

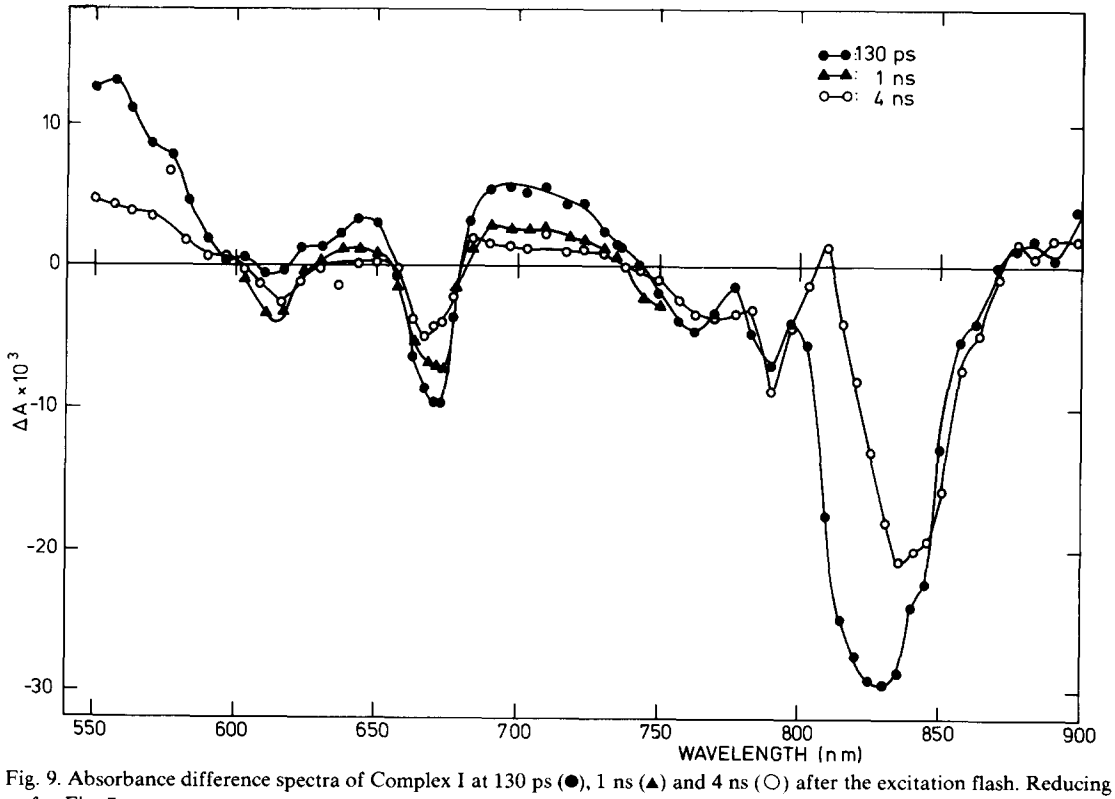

Fig. 9. Absorbance difference spectra of Complex I at 130 ps (●), 1 ns (▲) and 4 ns (○) after the excitation flash. Reducing conditions as for Fig. 7.

70 ps phase, indicating that P-840 oxidation occurs within the lifetime of BChl *a**. From the intensity dependence of P-840$^+$ formation (Fig. 4) it can be seen that the excitation pulses were of saturating intensity with respect to charge separation. The maximal absorbance change at 840 nm due to P-840$^+$ formation (about $20 \cdot 10^{-3}$) corresponded well with the maximal absorbance change detected under continuous illumination (about $18 \cdot 10^{-3}$) (not shown).

The spectrum measured after 130 ps (Fig. 9) shows contributions by P-840$^+$ and BChl *a** in the near-infrared region. Below 600 nm it resembles that obtained with closed reaction centers (Fig. 5). The spectrum shows a relatively large bleaching at 670 nm, and a large increase in absorption around 700 nm in comparison to the spectra measured at longer times after the pulse. The absorbance changes in the region 650–750 nm in the spectra of Figs. 7 and 9 are clearly distinct from those in the corresponding spectra with closed reaction centers (Figs. 2 and 5), the bleaching at 670 nm and especially the increase at 690 nm being more pronounced. Since these changes appear and disappear in times to be expected for an intermediary between P-840 and secondary electron acceptors, and do not occur in the presence of ferricyanide, we attribute these changes to reduction and re-oxidation of the primary electron acceptor. The kinetics of the absorbance changes at 670 nm are shown in Fig. 8D. The initial increase can again be attributed to Car*. The bleaching which is left after Car* has disappeared decayed with a half time of 550 ± 50 past to a constant level. Absorbance changes with a similar difference spectrum but with a lifetime of about 25 ns were observed by Van Bochove et al. [13] in a preparation in which secondary electron transport was inhibited, and were tentatively attributed to reduction of BPh *c*. The 550 ps decay time in Complex I then would reflect the transfer of the electron from the primary to a secondary acceptor. The residual bleaching at 670 nm can be partly ascribed to long-lived excited states, partly to the band shift that is associated with P-840$^+$, mentioned above. Thus, apart from absorbance changes due to excited states (Bchl *a** mainly), the 130 ps spectrum of Fig. 9 can be ascribed to formation of the radical pair P-840$^+$I$^-$, in which I is the primary electron acceptor. The 4 ns spectrum reflects the state P-840$^+$I X$^-$, in which X is a secondary acceptor.

Discussion

Application of picosecond time-resolved absorbance difference spectroscopy has permitted for the first time to observe the primary photosynthetic processes of a relatively large intact photosystem derived from a green photosynthetic bacterium. Excitation with a 35 ps flash at 532 nm of Complex I of *P. aestuarii* causes absorption changes due to formation of singlet and triplet excited states of BChl *a* and carotenoid, and in open reaction centers also those due to the primary charge separation and subsequent electron transfer.

At 532 nm most of the incident light is absorbed by carotenoids, which are thus directly excited by the flash. Car* probably has a lifetime of a few ps (see below). At 4 K, less than 10% of the carotenoid singlet excitations is transferred to the BChl *a* of the core complex, as is indicated by the low-temperature fluorescence excitation spectra (Kramer H.J.M. et al., unpublished data). Some BChl *a** is also formed by direct excitation in the BChl *a* protein and in the core complex, but from the excitation spectrum mentioned above it can be deduced that the amount will be relatively low.

The absorption of Complex I around 815 nm is made up of contributions from both the core complex and the BChl *a* protein, whereas that at 840 nm is due to the core complex alone [2]. Since for Complex I (Fig. 2) the maximum bleaching in the near-infrared region, due to BChl *a** formation, is at longer wavelength than for the isolated BChl *a* protein (Fig. 6), we conclude that an appreciable part of the singlet excitations in Complex I is, at least initially, localized on the core complex. The decay kinetics of the absorbance changes at 840 nm (Fig. 3B) show that the lifetime of BChl *a** in the core complex is 70 ± 10 ps. This decay time differs significantly from the 280 ps decay of singlet excitations in the isolated BChl *a* protein (Fig. 6, inset). The 250 ± 50 ps decay component in Complex I, observed at 815 nm (Fig. 3A), and the shift of the maximum bleaching from 820 nm during the flash (Fig. 2) to 817 nm at

130 ps (Fig. 5) suggest that some longer-lived singlet excitations remain on the BChl *a* protein. Part of this BChl *a** may be formed by direct excitation of BChl *a* at 532 nm, part by energy transfer from BChl *a** in the core complex.

Using a somewhat similar preparation from *Chlorobium limicola*, Borisov et al. [21] measured fluorescence lifetimes and yields by means of a phase fluorimeter. At high intensity of excitation they observed an increase in yield and a decrease in average lifetime from 2.4 to 1.6 ns. These results were interpreted in terms of two fluorescence components: a 'background' with a constant intensity and a lifetime of 2.7 ns and one with a variable intensity and lifetime (40 ± 20 to 400 ± 200 ps). The first number would apply to a system with open traps. Our results provide little evidence for a significant 'background' component in BChl *a** deexcitation, but it should be kept in mind that such a component may be more prominent in phase fluorimetric measurements. Our 70 ps decay phase, which is observed both in the absence and in the presence of ferricyanide does not appear to be affected by the rate of trapping by the reaction center. In this connection it should be remarked that even without ferricyanide at least an appreciable fraction of the reaction centers already becomes closed during the flash. Therefore, our experiments do not exclude the possibility of a considerably faster component that would reflect the rate of trapping by the reaction center. Govindjee et al. [22] measured fluorescence lifetimes of intact cells of green sulfur bacteria using a phase fluorimeter. The decay time increased from about 600 ps to 1 ns upon increasing the excitation intensity. However, since broad band detection was used in these experiments, the measured fluorescence presumably originated mainly from the chlorosomes [23].

The absence of a bleaching at 840 nm in the 4 ns absorbance difference spectrum of Fig. 5 suggests that BChl a^T formed in complex I is localized on the BChl *a* protein. This triplet is probably formed by intersystem crossing from the excited singlet state, similarly as for the isolated BChl *a* protein (Fig. 6).

As stated, the absorbance changes at 560 nm, remaining after the decay of Car* (Figs. 3C and 8B) might be attributed to carotenoid triplet states. Formation of these triplets could take place via the mechanism of singlet fission of Car*, as has been proposed for purple bacteria [24,25]. Recently, it has been shown that in *Rhodospirillum rubrum* antenna carotenoid triplets are formed within 100 ps after direct carotenoid excitation [16].

The lifetime of Car* is probably much shorter than the duration of the flash, and, unlike that of BChl *a**, cannot be measured directly. However, our data allow an indirect estimate from the amplitude of the absorbance changes in the carotenoid region during the flash. Since the carotenoids are directly excited at 532 nm, and no energy transfer is assumed to occur between them, the ground state transition moments of the excited molecules will be more or less parallel to the vertical polarization direction of the excitation pulse. In view of the long chain-like structure of the carotenoids, it is likely that the transition moment of Car*, corresponding to the absorption band around 590 nm in Fig. 2, is also vertically polarized. Since the measuring pulse is polarized parallel to the excitation pulse, it is necessary to correct for photodichroism when specific extinction coefficients are used which normally refer to randomized transition moments. If no depolarization occurs, the optical density measured parallel to the polarization direction is three times that measured perpendicular to this direction [26]. From this it follows that a correction for photodichroism is obtained by dividing the measured absorbance changes in the carotenoid region by a factor of 9/5. This correction should not be applied to the BChl *a* bands. Due to the transfer of excitation energy from carotenoid to BChl *a* and among BChl *a* molecules, the bleaching at 820 nm is probably only weakly polarized, as is also indicated by measurements of fluorescence polarization (Kramer, H.J.M. and Westerhuis, W.H.J., unpublished results). We assume that the extinction coefficient of Car* at 590 nm is 150 $mM^{-1} \cdot cm^{-1}$, i.e., roughly equal to that of the ground-state absorption at 460–475 nm [27]. Then, the amount of Car* observed can be compared with that excited during a flash, as the intensity of the flash and thus the number of absorbed quanta are known. Simulation of the absorbance changes at 590 nm by convolution of a monoexponential decay with 35 ps Gaussian excitation and probe

pulses indicated an average lifetime for Car* of approx. 1 ps. Comparison of the amplitude of the absorbance changes due to formation of BChl *a** at 820 nm with that of Car*, by applying a similar convolution procedure for BChl *a** (using a lifetime of 70 ps), yielded a lifetime of less than 3 ps for Car*. An efficiency of transfer from carotenoid to BChl *a** of less than 10%, like that at 4 K, was assumed in this calculation. A decay time of 1 ps is in reasonable agreement with the value calculated for the excited singlet state of spirilloxanthin in solution (Nuijs, A.M. et al., unpublished results), and agrees well with the upper limit of 1 ps of the lifetime of singlet excited β-carotene, which was estimated from the absence of any detectable Raman peak [28].

Comparison of the difference spectra for samples with closed and open reaction centers indicates that the absorbance changes due to excited states in the antenna are approximately the same in both preparations. Thus, when these are subtracted from the absorbance changes obtained with open reaction centers, only changes caused by the charge separation and electron transport should remain. The result of such a subtraction for the spectral region 600–750 nm is shown in Fig. 10. The spectrum obtained at 130 ps clearly resembles the $P\text{-}840^{+}I^{-}$ spectrum as measured by Van Bochove et al. [13]. On the other hand, apart from a shift of about 3 nm, the 4 ns spectrum is in good agreement with the spectrum recorded by Swarthoff and Amesz [9] upon continuous illumination, which spectrum was attributed to $P\text{-}840^{+}X^{-}$, where X is an iron-sulfur center [11]. It should be noted that our data do not provide evidence for the involvement of the BChl *a* monomer, the reduction of which was observed previously in continuous light at low ambient redox potential and high pH. This monomer was proposed to be an electron acceptor intermediate between I and an iron-sulfur center [12]. Reduction of this BChl *a* was accompanied by the bleaching of its Q_y band at 814 nm. As shown by Fig. 8A no such bleaching concomitant with the 550 ps decay of the absorbance changes of I^{-} is seen. Thus the function of BChl *a* 814 in the electron chain remains unclear. Reduction of the BChl *a* 814 could either be a side reaction taking place under the non-physiological conditions applied, or alternatively, its redox reactions might be too fast to be observed under our conditions.

Our data thus can be summarized by the fol-

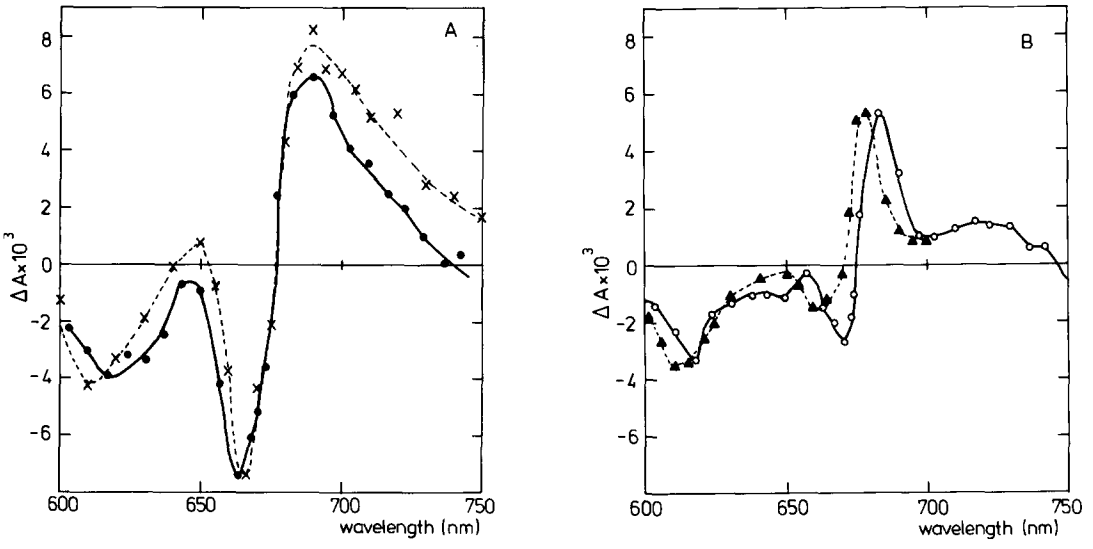

Fig. 10 (A) ●, Absorbance changes of Complex I, 130 ps after a flash obtained by subtracting the spectrum of an oxidized sample from that of a reduced one (see text). ×, Absorbance difference spectrum of the reaction center pigment-protein complex, 7 ns after a flash [13]. The two spectra are normalized at 665 nm. (B) ○, Absorbance changes of Complex I, 4 ns after a flash obtained by subtracting the spectrum of an oxidized sample from that of a reduced one (see text). ▲, Absorbance difference spectrum of Complex I obtained after 1 s of saturating continuous illumination [9]. The two spectra are normalized at 680 nm.

lowing scheme:

$$\text{P-840IX} \xrightarrow{h\nu} \text{P 840*IX} \xrightarrow{< 35\ \text{ps}} \text{P-840}^{+}\text{I}^{-}\text{X} \xrightarrow{550\ \text{ps}} \text{P-840}^{+}\text{IX}^{-}$$

in which I is probably BPh *c* and X is an iron-sulfur center.

It has been previously argued that the reaction center of green sulfur bacteria, in contrast to that of purple bacteria and Chloroflexaceae, resembles that of Photosystem I of green plant photosynthesis [12]. However, the present results indicate that its electron-acceptor chain combines features of both plant photosystems, especially if one takes into account that the porphyrin system of BPh *c* is chemically more closely related to that of pheophytin *a* than to that of BPh *a*: the primary electron acceptor is similar to that of Photosystem II, whereas the secondary acceptor system consists of iron-sulfur centers, as in Photosystem I. This may suggest a fairly complicated evolutionary relationship among the various groups of photosynthetic organisms.

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References

1 Amesz, J. and Knaff, D.B. (1985) in Environmental Microbiology of Anaerobes (Zehnder, A.J.B., ed.), John Wiley and Sons, New York, in the press
2 Olson, J.M. (1980) Biochim. Biophys. Acta 594, 33–51
3 Parson, W.W. and Ke, B. (1982) in Photosynthesis, Vol. I, Energy Conversion by Plants and Bacteria (Govindjee, ed.), pp. 331–386, Academic Press, New York
4 Matthews, B.W., Fenna, R.E., Bolognesi, M.C., Schmid, M.E. and Olson, J.M. (1979) J. Mol. Biol. 131, 259–285
5 Vasmel, H., Swarthoff, T., Kramer, H.J.M. and Amesz, J. (1983) Biochim. Biophys. Acta 725, 361–367
6 Hurt, E.C. and Hauska, G. (1984) FEBS Lett. 168, 149–154
7 Fowler, C.F., Nugent, N.A. and Fuller, R.C. (1971) Proc. Natl. Acad. Sci. USA 68, 2278–2282
8 Olson, J.M., Philipson, K.D. and Sauer, K. (1973) Biochim. Biophys. Acta 292, 206–217
9 Swarthoff, T. and Amesz, J. (1979) Biochim. Biophys. Acta 548, 427–432
10 Knaff, D.B. and Malkin, R. (1976) Biochim. Biophys. Acta 430, 244–252
11 Swarthoff, T., Gast, P., Hoff, A.J. and Amesz, J. (1981) FEBS Lett. 130, 93–98
12 Swarthoff, T., Gast, P., Van der Veek-Horsley, K.M., Hoff, A.J. and Amesz, J. (1981) FEBS Lett. 131, 331–334
13 Van Bochove, A.C., Swarthoff, T., Kingma, H., Hof, R.M., Van Grondelle, R., Duysens, L.N.M. and Amesz, J. (1984) Biochim. Biophys. Acta 764, 343–346
14 Holt, S.C., Conti, S.F. and Fuller, R.C. (1966) J. Bacteriol. 91, 311–323
15 Olson, J.M. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 161–178, Plenum Press, New York
16 Nuijs, A.M., Van Bochove, A.C., Joppe, H.L.P. and Duysens, L.N.M. (1984) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. I, pp. 65–68, Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht, the Netherlands
17 Borisov, A.Yu., Gadonas, R.A., Danielus, R.V., Kozlovskii, V.S., Piskarskas, A.S., Razzhivin, A.P. and Kharchenko, S.G. (1982) Dokl. Akad. Nauk SSSR 266, 482–485
18 Kramer, H.J.M., Kingma, H., Swarthoff, T. and Amesz, J. (1982) Biochim. Biophys. Acta 681, 359–364
19 Karapetyan, N.V., Swarthoff, T., Rijgersberg, C.P. and Amesz, J. (1980) Biochim. Biophys. Acta 593, 254–260
20 Vasmel, H., Den Blanken, H.J., Dijkman, J.A., Hoff, A.J. and Amesz, J. (1984) Biochim. Biophys. Acta 767, 200–208
21 Borisov, A.Yu., Fetisova, Z.G. and Godik, V.I. (1977) Biochim. Biophys. Acta 461, 500–509
22 Govindjee, Hammond, J.K. and Merkelo, H. (1972) Biophys. J. 12, 809–814
23 Swarthoff, T., Amesz, J., Kramer, H.J.M. and Rijgersberg, C.P. (1981) Isr. J. Chem. 21, 332–337
24 Rademaker, H., Hoff, A.J., Van Grondelle, R. and Duysens, L.N.M. (1980) Biochim. Biophys. Acta 592, 240–257
25 Kingma, H. (1983) Thesis, University of Leiden
26 Albrecht, A.C. (1961) J. Mol. Spectr. 6, 84–108
27 Foppen, F.H. (1971) Chrom. Rev. 14, 133–298
28 Dallinger, R.F., Woodruff, W.H. and Rodgers, M.A.J. (1980) Photochem. Photobiol. 33, 275–277