Biochimica et Biophysica Acta 851 (1986) 1–5
Elsevier

BBA 42086

Picosecond spectroscopy of isolated membranes of the photosynthetic green sulfur bacterium *Prosthecochloris aestuarii* upon selective excitation of the primary electron donor

V.A. Shuvalov *, J. Amesz ** and L.N.M. Duysens

Department of Biophysics, Huygens Laboratory of the State University, P.O. Box 9504, 2300 RA Leiden (The Netherlands)

(Received February 6th, 1986)

Key words: Photosynthesis; Charge separation; Bacteriochlorophyll *c*; Picosecond spectroscopy; (*P. aestuarii*)

Picosecond excitation at 850 nm of isolated membranes of the photosynthetic green sulfur bacterium *Prosthecochloris aestuarii*, when the primary electron donor P-840 was in the oxidized state, caused the formation of excited states of bacteriochlorophyll (BChl) *a* in the antenna, but not those of other pigment molecules, as had been observed upon excitation at shorter wavelengths. When, however, P-840 was initially in the reduced state, the flashes produced in addition absorption changes that could be attributed to reaction center components. These changes included bleachings of the absorption bands of P-840 and of BChl *c*. The latter bleaching, caused by BChl *c* reduction, decayed in 700 ± 70 ps, due to electron transfer to the next electron acceptor, whereas the bleaching of the bands of P-840 did not reverse in the time region studied (up to 3 ns). Analysis of the absorbance changes appeared to indicate a 1:1 stoichiometry for P-840 oxidation and BChl *c* reduction. The primary charge separation occurred in less than 10 ps.

Introduction

Experiments in various laboratories have demonstrated that the organization of the photosynthetic membrane and the primary and secondary electron transport reactions in green sulfur bacteria are in many respects different from those in the much more extensively studied purple photosynthetic bacteria and also from those in the green filamentous bacterium *Chloroflexus aurantiacus* [1–3].

Attempts to prepare isolated reaction center complexes from green sulfur bacteria have been unsuccessful to far [4], which suggests that part of the antenna pigments is bound to the same intrinsic protein that binds the components of the reaction center. For this reason, studies of primary and secondary electron transport in the reaction center of green sulfur bacteria have been performed either with isolated membranes or with pigment-protein complexes that contained the reaction center together with part of the antenna pigments.

The primary electron donor of green sulfur bacteria, P-840, is probably a BChl *a* dimer [1,2]. From measurements of the absorbance changes in the pico- and nanosecond region [3,5] it has been concluded that a pigment absorbing near 670 nm functions as an early electron acceptor. At first it was tentatively assumed that this pigment would be bacteriopheophytin *c*, but recent studies demonstrated the virtual absence of this pigment in purified photochemically active pigment-protein

* Permanent address: Institute of Soil Science and Photosynthesis, USSR Academy of Sciences, Pushchino, Moscow Region (U.S.S.R.).

** To whom correspondence should be addressed.

Abbreviations: BChl, bacteriochlorophyll; I, primary electron acceptor, P-840 or P, primary electron donor.

complexes derived from membranes of *Prosthecochloris aestuarii,* and it was concluded that the pigment involved in electron transport can only be BChl *c*, a lipophilic form of which was found to be present in fairly large amounts in these preparations [6].

The picosecond absorbance-difference measurements mentioned above have been performed with excitation flashes at 532 nm. A disadvantage of this method is that, in addition to absorbance changes that can be ascribed to electron transport, these flashes produce relatively large amounts of excited carotenoid, BChl *c* and BChl *a* in the antenna.

In the present communication we report the results of picosecond experiments in which membranes of *P. aestuarii* were illuminated with 850-nm pulses. This wavelength was chosen in order to excite the primary electron donor P-840 selectively, together with the long-wave absorbing BChl *a* molecules in the so-called core complex [4] that may be assumed to transfer their energy directly to the reaction center. Our results confirm the notion that the primary electron acceptor in *P. aestuarii* in BChl *c*. Analysis of the absorbance changes indicates a 1 : 1 stoichiometry for P-840 oxidation and BChl *c* reduction. The primary charge separation was found to occur in less than about 10 ps.

Material and Methods

Prosthecochloris aestuarii, strain 2K, was grown anaerobically in a mixed culture as described by Holt et al. [7]. A membrane vesicle preparation ('Complex I') was prepared according to Ref. 8. The final preparation was dissolved in 10 mM sodium phosphate/10 mM sodium ascorbate buffer (pH = 7.4) and had an absorbance of 1.2 or 0.6 (as indicated in the figure legends) in a 2-mm cuvette.

The dual-beam spectrometer with 33-ps measuring and excitation pulses and equipped with an optical multichannel analyzer to register absorption difference spectra has been described in Ref. 9. Excitation at 850 nm was obtained by means of a parametric light generator pumped by 532-nm frequency-doubled Nd-YAG laser pulses. The intensity was about 0.2 mJ, the repetition rate 0.5 Hz. The excitation pulses were polarized; the probe pulses were not. All experiments were done at room temperature.

Results and Discussion

Fig. 1 shows absorption difference spectra of isolated membranes ('Complex I') obtained upon excitation at 850 nm with 33-ps flashes. The reaction centers were kept inoperative with P-840 in the oxidized state by continuous background illumination in the presnece of ferricyanide. The solid curves show the spectra measured at 0 ps (with coincident excitation and probe pulses); the dashed curves were measured at 350 ps after the

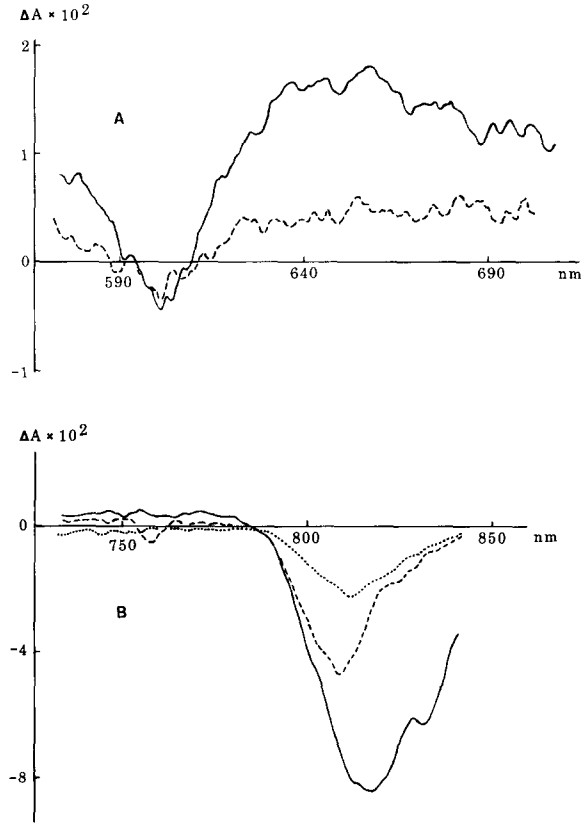

Fig. 1. Absorption difference spectra of isolated membranes ('Complex I') of *Prosthecochloris aestuarii* ($A_{830} = 0.6$) measured at 0 ps (———), 350 ps (- - - - - -) and 2 ns (· · · · · ·) after the 33-ps excitation flash at 850 nm. P-840 was kept in the oxidized state by continuous background illumination in the presence of 20 mM ferricyanide.

flash. In the near-infrared region the spectrum measured after 2 ns is also shown (dotted line). In the visible region the spectra are characterized by a bleaching at 600 nm and an absorbance increase in the 570–690 nm region that can be ascribed to excitation of antenna BChl *a*. No absorbance decrease is observed near 670 nm. This shows that excited BCh *c* was not produced under these conditions, in contrast to what was observed upon excitation at 532 nm [3]. The formation of excited BChl *a* is also reflected by a bleaching in the region 800–840 nm. The maximum bleaching is observed near 820 nm at 0 ps, and shifts to 810 nm after 350 ps. A similar, but smaller blue shift was observed by Nuijs et al. [3] upon excitation at 532 nm. It can be seen (Fig. 1B) that around 830 nm about 80% of the bleaching had disappeared after 350 ps.

Difference spectra obtained in the presence of open reaction centers are shown in Fig. 2. The spectra measured at 2 or 3 ns (dashed curves) represent the formation of P-840$^+$ in agreeement with earlier measurements [3] and show a bleaching of the absorption bands of P-840 at 610 nm and at 830–840 nm, together with red shifts of BChl *a* and BChl *c* near 800 and 675 nm, respectively. The amplitude of the absorbance changes indicated that the flashes were about 70% saturating.

The spectrum measured at 350 ps shows an additional bleaching at 800–825 nm that can be ascribed to excited antenna BChl *a*. In the visible region the spectrum shows the characteristics that have been attributed to the formation of P^+I^- (where I is the primary electron acceptor) with negative bands at 615 and 670–675 nm, a positive band near 650 nm, and a broad absorbance increase above 680 nm [3,5]. In contrast to absorption difference spectra measured upon excitation at 532 nm under similar conditions [3] the spectrum is not distorted by excited BChl *c*; a small correction for the presence of excited BChl *a* may be obtained by subtracting the spectrum measured with closed reaction centers (Fig. 1); such a correction would shift the spectrum in the region 640–710 nm downward by about $0.8 \cdot 10^{-2}$ absorbance units, but would not change its shape. The shape of the difference spectrum in the region 640–710 nm is clearly different from the spectrum

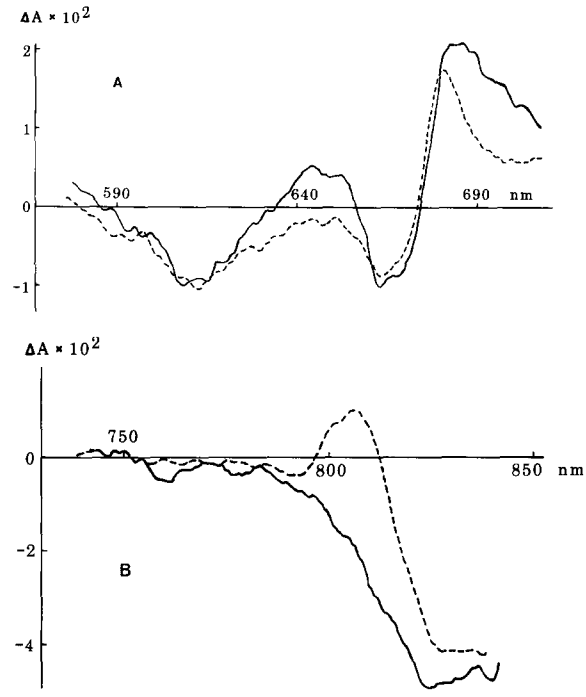

Fig. 2. Absorption difference spectra of membranes of *P. aestuarii* measured in the presence of ascorbate (10 mM) and *N*-methylphenazoniummethosulfate (10 μM) upon excitation at 850 nm. (A) Spectra in the visible region measured at 350 ps (———) and at 3 ns (- - - - -). $A_{830} = 1.2$. (B) Spectra in the near-infrared region measured at 350 ps (———) and at 2 s (- - - - -). $A_{830} = 0.6$.

measured for BChl *c* reduction in vitro [10]. This suggests that it contains a contribution both from the reduction of BChl *c* and from a red shift of some other BChl *c* molecule(s), presumably caused by the oxidation of P-840.

In order to obtain a correction for the effects due to P-840 oxidation and to gain more information about the spectral features of the primary electron acceptor, we plotted the difference between the spectrum measured at 3 ns [P^+–P] and those measured at earlier times (Fig. 3). The lowest spectrum of Fig. 3 shows the difference between the spectra measured at 250 ps and 3 ns. It shows a broad absorbance increase in the region of 600–700 nm together with a bleaching centered at 673 nm. This spectrum still contains a contribution by excited BChl *a* (see above), but otherwise it is similar to that obtained upon reduction of

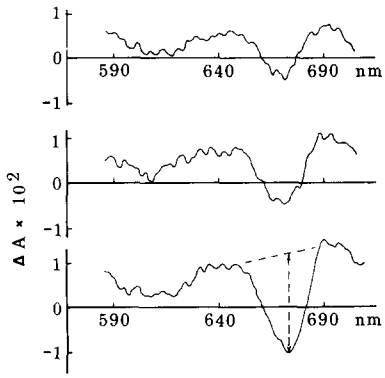

Fig. 3. Difference spectra obtained by subtraction of the difference spectrum obtained at 3 ns from the spectra measured at 1 ns (upper curve), 670 ps (middle curve) and 250 ps (bottom curve) after excitation at 850 nm. Conditions as for Fig. 2A.

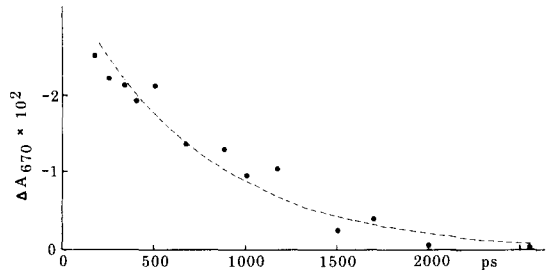

Fig. 4. The kinetics of ΔA_{673} measured as shown by the dashed vertical line in Fig. 3, bottom. The dashed curve shows exponential kinetics with a lifetime of 700 ps.

BChl *c* in vitro [10]. Corresponding difference spectra obtained at 670 ps (middle spectrum) and at 1 ns (upper spectrum) show the same features but with smaller amplitudes. Thus we conclude, as suggested earlier (see Refs. 3, 5 and 6), that the primary electron acceptor in *P. aestuarii* is probably BChl *c*.

To measure the kinetics of the 670-nm bleaching the amplitude of the trough near 670 nm (shown by an arrow in Fig. 3, bottom) was plotted as a function of time (Fig. 4). The kinetics are approximated by an exponential curve with a lifetime of 700 ± 70 ps, which is close to that measured earlier [3] and has been interpreted as the electron-transfer time from $BChl^-$ *c* to the next electron acceptor. Extrapolation of the kinetics shown in Fig. 4 to zero time shows that for equal concentration of reaction centers ΔA_{670} approaches a value which is 45% of the absorbance decrease at 830 nm due to P-840 oxidation (as measured at 2 ns after the flash; see Fig. 2B). If the extinction difference coefficient at 830 nm for P-840 is taken to be approx. 120 $mM^{-1} \cdot cm^{-1}$ [6], that for ΔA_{670} would be about 50–55 $mM^{-1} \cdot cm^{-1}$, which is close to the value observed in vitro (55 $mM^{-1} \cdot cm^{-1}$ [10]). Thus our results appear to indicate that P-840 and BChl *c* react in a 1 : 1 stoichiometry.

From earlier experiments it was concluded that the primary electron transfer reaction in *P. aestuarii* occurs in less than 35 ps [3]. Experiments where the center of the probing pulse preceded that of the excitation pulse indicated that the charge separation occurred significantly faster. Comparison of the bleaching at 670 nm with that at 615 nm indicated a low initial value and an increase of the ratio $\Delta A_{670}/\Delta A_{615}$ during the early phases of the excitation flashes. Although the signal-to-noise ratio did not allow an accurate determination, the results of these measurements, by use of the method described earlier [9,11] indicated a time constant for the primary charge separation of less than about 10 ps. If one takes into account that this number includes the time needed for energy transfer to the reaction center, this means that the rate of primary electron transfer in *P. aestuarii* is probably of the same order of magnitude as in purple bacteria [9]. However, these experiments do not rule out the participation of an early electron acceptor preceding BChl *c* in electron transport of green sulfur bacteria.

Although the evidence is still controversial [12,13], there are indications that in purple bacteria [9] and in the green filamentous bacterium *Chloroflexus aurantiacus* [11] BChl *a* serves as a primary electron acceptor. The midpoint potentials of BChl *a* and BChl *c* in solution are −0.86 V and −1.03 V, respectively [10,14]. Taking into account the midpointpotentials of P-840 (0.25 V [1]) and of P-870 in purple bacteria (0.45V [1,15]), this suggests that the energies of charge separation are close to 1.3 eV, both in purple and in green sulfur bacteria. Thus the low redox potential of BChl *c* results in a small difference between the energies of P* (about 1.45 eV) and of P^+BChl^-c, which is a necessary condition for fast electron transfer

between these states [16]. On the other hand, the relatively slow electron transfer from $BChl^-$ *c* to iron-sulfur centers [17] is accompanied by a large energy drop (> 0.6 eV) which decreases the rate of the back electron transfer by several orders of magnitude.

Acknowledgements

We thank F.T.M. Zonneveld for skillful preparative work. This investigation was supported by the USSR Academy of Sciences, by the Netherlands Foundations for Biophysics and for Chemical Resarch (SON), financed by the Netherlands Organization for the Advancement of Pure Research (ZWO), and by a ZWO visitor's grant to V.A.S.

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