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Picosecond charge separation upon selective excitation of the primary electron donor in reaction centers of *Rhodopseudomonas viridis*

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Measurement of picosecond absorbance changes in the near-infrared and visible region in reaction centers of *Rhodopseudomonas viridis* upon selective excitation of the primary electron donor P-960 showed that the time constant for electron transfer to bacteriopheophytin *b* in the presence of reduced quinone acceptor is 12 ± 2 ps. At earlier times simultaneous bleachings at 970 and 850 nm were observed. The corresponding difference spectrum does not appear to be due to excited P-960 and is attributed to electron transfer from P-960 to bacteriochlorophyll serving as an intermediate electron acceptor.

Studies by means of flash spectroscopy on isolated reaction centers of purple bacteria and of the green photosynthetic bacterium *Chloroflexus aurantiacus* [1–10] have shown that upon excitation of the primary electron donor P a very rapid electron transfer occurs to bacteriopheophytin (BPh). However, there is conflicting evidence on the mechanism of this process, and in particular on the role of the 'monomeric' or 'accessory' bacteriochlorophyll. Analysis of the absorption difference spectra obtained during the early phases of exciting 33-ps laser pulses provided evidence that the reduction of BPh in reaction centers of *Rhodobacter (Rhodopseudomonas) sphaeroides* and *C. aurantiacus* is preceded by the transfer of an electron to the BChl molecule located between P and BPh [9,10]. On the other hand, experiments with 150- and 800-fs flashes gave no indications for the existence of an early charge-separated state P^+BChl^- [4,11]. A discussion of these and other apparently conflicting results is given in Refs. 9 and 10.

The above-mentioned experiments have been done with BChl *a* containing reaction centers. Studies on BPh reduction in the BChl *b* containing species *Rhodopseudomonas viridis* thus far have only been performed after completion of the charge separation and in the near-infrared region, where the absorbance changes that can be ascribed to BPh *b* are relatively small [7,8]. The present communication extends these measurements to the region of the Q_x band of BPh at 545 nm and to a time resolution of a few ps. The results indicate that the mechanism of charge separation in *Rps. viridis* is similar to that in the BChl-*a*-containing bacteria, and that a transient reduction of BChl *b*-850 occurs at early times after the excitation of the primary donor.

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Abbreviations: BChl, bacteriochlorophyll; BPh, bacteriopheophytin; B_1, photoreactive BChl; H_1, photoreactive BPh, primary electron acceptor; P (P-960), primary electron donor.

Reaction centers of *Rps. viridis* were isolated as described in Ref. 12, and dissolved in buffer containing 50 mM Tris (pH 8.0). The absorbance at 830 nm was 1.0 in a 2-mm cuvette. In order to avoid accumulation of P^+ and oxidized cytochrome *c* in a flash series, quinone acceptors were kept in the reduced state during the experiments by background illumination in the presence of 10 mM ascorbate and 10 μM *N*-methylphenazonium methosulfate. Absorbance difference spectra were measured with 33-ps measuring and excitation pulses using an apparatus equipped with an optical multichannel analyzer [9]. A non-polarized measuring pulse was used in order to avoid photoselection effects. A parametric light generator was used to obtain excitation flashes at 960 nm with a repetition rate of 1 Hz. In some experiments the center of the probing pulse preceded that of the excitation pulse as indicated by negative numbers in the text and figures. In this way a time resolution of up to 1.5 ps was obtained [9]. The experiments were done at room temperature.

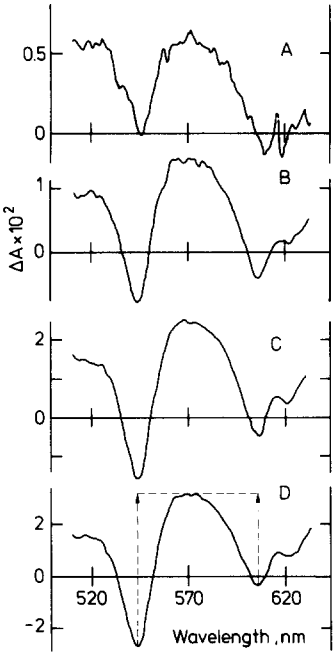

Fig. 1. Absorption difference spectra in the visible region of *Rps. viridis* reaction centers measured upon selective excitation at 960 nm at −30 ps (A), −17 ps (B), 0 ps (C) and +67 ps (D). Quinone acceptors were kept in the reduced state as described in the text.

Fig. 1 shows a family of absorption difference spectra of *Rps. viridis* reaction centers for the visible region measured at different times with respect to the excitation pulse at 960 nm. All spectra showed negative bands near 543 and 605 nm, that can be attributed to bleaching of the Q_x bands of BPh *b* and BChl *b*, respectively, but the relative amplitudes of the bands varied with time. The ratio of the amplitudes, measured as indicated by dashed arrows in Fig. 1D, increased from $\Delta A_{543}/\Delta A_{605} = 0.7$ at −30 ps to 1.65 at +67 ps. This indicates that the bleaching at 543 nm was delayed with respect to the bleaching of the absorption band of the primary electron donor P. Along with the bleaching at 605 nm a shoulder at 622 nm could be observed in the spectra measured at later times.

A similar set of absorption difference spectra for the infra red region is shown in Fig. 2. The spectrum (D) measured at 70 ps after the center of the excitation flash is similar to that reported by Kirmaier et al. [8], and is presumably due to a blue shift and a decrease of the dipole strengths of the absorption bands near 830 and 850 nm [8], together with a bleaching of the absorption band of P at 960–970 nm. Although the absorbance changes in the Q_y region of BPh are small as compared to those in BChl *a* and BPh *a* containing reaction centers [7,8], the difference spectrum in the visible region (Fig. 1D) strongly supports the assumption that the spectrum is caused by the formation of the state $P^+H_1^-$, where H_1 denotes the photoactive BPh *b*. It is not clear if the shoulder at 622 nm in the Q_x region of BChl *b* reflects exciton splitting of the dimer band of P (cf. Ref. 13) or a change in absorption of 'accessory' BChl.

The absorption difference spectrum measured at −38 ps (Fig. 2A) was different from that measured at 70 ps. The maximum bleaching in the short wave region was now at 850 nm. Its amplitude was about 40% of that of the bleaching of P at 970 nm. In addition, a weak shoulder can be seen near 835 nm, which gradually became more predominant and evolved into the main minimum at −10 ps (Fig. 2B and C). This indicates that, as earlier observed for reaction centers of *Rb. sphaeroides* [9] and *C. aurantiacus* [10], the difference spectra measured during the early phases

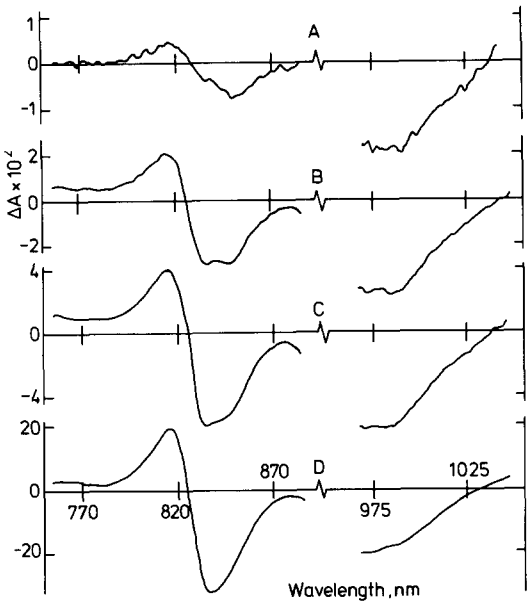

Fig. 2. Absorbance difference spectra in the infrared region at −38 ps (A), −30 ps (B), −10 ps (C) and +70 ps (D). Conditions as for Fig. 1.

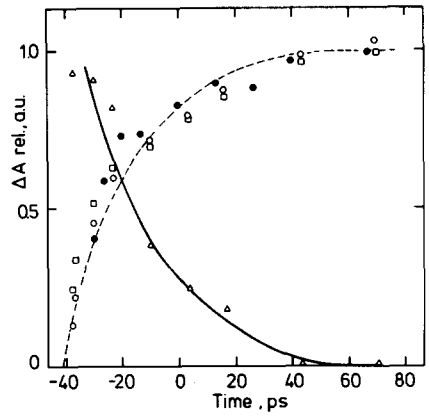

Fig. 3. Normalized kinetics of the ratios $\Delta A_{543}/\Delta A_{605}$ (●), $\Delta A_{815}/\Delta A_{975}$ (□), $\Delta A_{835}/\Delta A_{975}$ (○) and $\Delta A_{1032}/\Delta A_{975}$ (△) (see text). The amplitudes of the absorbance changes at 543 and 605 nm were measured as indicated by the vertical dashed arrows in Fig. 1D. The broken line shows the calculated kinetics for a time constant of 12 ps. a.u., arbitrary units.

of the excitation pulse cannot be ascribed solely $P^+H_1^-$: some earlier state of the reaction center must also be involved.

In the long-wave region, the spectrum measured at 70 ps has an isobestic point at 1032 nm. The spectra measured at earlier times have an additional bleaching at this wavelength, which may be attributed to stimulated emission from excited P, observed earlier for *Rb. sphaeroides* [3,4,9,11] and *C. aurantiacus* reaction centers [10].

Fig. 3 reflects the kinetics of the absorbance changes at various wavelengths obtained by plotting the ratio of the amplitudes to those of the bleachings of the Q_x and Q_y bands of P at 605 and 975 nm, respectively. It is assumed (see Refs. 9 and 10) that the kinetics of the bleaching of P at these wavelengths are proportional to the number of reaction centers which had absorbed a photon. The absorbance changes at 543 and 835 nm were found to have roughly the same kinetics, which upon deconvolution [9,10] could be fitted reasonably well by a single exponential with a rise time of 12 ± 2 ps. The kinetics at 1032 nm suggest that the stimulated emission decayed with a similar time constant. The kinetics at 815 nm were somewhat faster initially than those at the other wavelengths, perhaps by interference by the component that causes the bleaching at 850 nm. Since the bleaching of the BPh band at 543 nm presumably reflects the reduction of H_1, these results indicate that the absorbance changes at 835 nm may be attributed to arise mainly from the formation of $P^+H_1^-$ too.

These results indicate that the difference spectra measured at early times during the excitation flash (Fig. 2A–C) are composite spectra containing a contribution by $P^+H_1^-$ and an additional bleaching at 850 nm. At −38 ps the amplitude at 850 nm is about 40% of that at 975 nm. This ratio is in reasonable agreement with the ratio of the exctinction coefficients of the corresponding bands in the absorption spectrum [14], which indicates that a significant fraction of the absorption band at 850 nm is initially bleached upon excitation.

It seems unlikely that the bleaching at 850 nm can be attributed to formation of P*, the singlet excited state of P, since the difference spectrum of the formation of the triplet state of P, which may be assumed to be similar to that of that of P* formation [10], does not show a significant bleaching at this wavelength [15,16]. Therefore, the simplest explanation of our data, in analogy to that proposed for *Rb. sphaeroides* [9] and *C. aurantiacus* [10], is the formation of a transient state $P^+B_1^-$, where B_1 denotes the BChl *b* molecule

between P and H_1, in agreement with the pigment arrangement in the reaction center derived from optical measurements [17] and X-ray analysis [18]. This assumption implies that the 850-nm band, like the 810-nm band in *Rb. sphaeroides* [17] mainly belongs to the B_1 molecule [19]. Its relatively small extinction coefficient can be explained by dipole strength redistribution due to the excitonic interaction with P and other components of the reaction center.

In summary we conclude that the primary charge separation upon selective excitation of P in *Rps. viridis* reaction centers appears to occur between P and B_1. The time resolution in our experiments was insufficient to determine the time constant of this reaction, which appears to be less than 1.5 ps. Formation of $P^+H_1^-$ occurs in 12 ± 2 ps. The latter reaction is slower than in *Rb. sphaeroides* (4 ps [9]), but it cannot be excluded that this is caused by the presence of reduced quinone in the *Rps. viridis* reaction centers.

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References

1 Parson, W.W. and Ke, B. (1982) in Photosynthesis, Vol. I, Energy Conversion by Plants and Bacteria (Govindjee, ed.), pp. 331–385, Academic Press, New York
2 Shuvalov, V.A. and Klevanik, A.V. (1983) FEBS Lett. 160, 51–55
3 Parson, W.W., Woodbury, N.W.T., Becker, M., Kirmaier, C. and Holten, D. (1985) in Antennas and Reaction Centers of Photosynthetic Bacteria. Structure, Interaction and Dynamics (Michel-Beyerle, M.E., ed.), pp. 278–285, Springer-Verlag, Berlin
4 Martin, J.-L., Breton, J., Hoff, A.J., Migus, A. and Antonetti, A. (1986) Proc. Natl. Acad. Sci. USA 83, 957–961
5 Freiberg, A.M., Godik, V.I., Kharchenko, S.G., Timpmann, K.E., Borisov, A. Yu. and Rebane, K.K. (1985) FEBS Lett. 189, 341–344
6 Kirmaier, C., Holten, D., Feick, R. and Blankenship, R.E. (1983) FEBS Lett. 158, 73–78
7 Holten, D., Windsor, M.W., Parson, W.W. and Thornber, J.P. (1978) Biochim. Biophys. Acta 501, 112–126
8 Kirmaier, C., Holten, D. and Parson, W.W. (1983) Biochim. Biophys. Acta 725, 190–202
9 Shuvalov, V.A. and Duysens, L.N.M. (1986) Proc. Natl. Acad. Sci. USA 83, 1690–1694
10 Shuvalov, V.A., Vasmel, H., Amesz, J. and Duysens, L.N.M. (1986) Biochim. Biophys. Acta 851, in the press
11 Woodbury, N.W., Becker, M., Middendorf, D. and Parson, W.W. (1985) Biochemistry 24, 7516–7521
12 Den Blanken, H.J. and Hoff, A.J. (1982) Biochim. Biophys. Acta 681, 365–374
13 Breton, J. (1985) Biochim. Biophys. Acta 810, 235–245
14 Verméglio, A. and Paillotin, G. (1982) Biochim. Biophys. Acta 681, 32–40
15 Shuvalov, V.A. and Parson, W.W. (1981) Biochim. Biophys. Acta 638, 50–59
16 Hoff, A.J., Lous, E.J., Moehl, K.W. and Dijkman, J.A. (1985) Chem. Phys. Lett. 114, 39–43
17 Shuvalov, V.A., Shkuropakov, A.Ya., Kulakova, S.M., Ismailov, M.A. and Shkuropakova, V.A. (1986) Biochim. Biophys. Acta 849, 337–346
18 Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1984) J. Mol. Biol. 180, 385–398
19 Knapp, E.W., Fischer, S.F., Zinth, W., Sander, M., Kaiser, W., Deisenhofer, J. and Michel, H. (1985) Proc. Natl. Acad. Sci. USA 82, 8463–8467