Antennas and Reaction Centers of Photosynthetic Bacteria

Structure, Interactions, and Dynamics

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Contents

Part II Reaction Centers: Structure and Interactions

Femtosecond Studies of the Reaction Center of *Rhodopseudomonas viridis*: The Very First Dynamics of the Electron-Transfer Processes

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The X-ray structure analysis of crystals made up of reaction centers (RC) of Rhodopseudomonas viridis provides us with the information on the location and orientation of the various pigments in the protein matrix /1,2/. After many years of speculation one is now in the position to predict unequivocally the path of the electron in the RC following the absorption of a photon by the special pair in the RC. This note is concerned with the time-dependence of the very first events.

We present here results from ultrafast time-resolved experiments. The RC were excited by a first ultrashort light pulse which triggered the photochemical reactions. A second, properly delayed probing pulse monitored absorbance changes induced by the various transient intermediates. The measurements with highest time resolution (1×10^{-13} s) were made with exciting and probing pulses of 150 fs (1.5×10^{-13} s) duration at a wavelength of 620 nm. These pulses were generated in a cw dye-laser system operating in the colliding pulse mode (CPM) /3/. The exposure of the sample to light was held on a low level by the following two techniques: First, an electro-optical modulator operating at 100 KHz cuts short pulse trains of five individual pulses from the 100 MHz repetition rate emission of the CPM laser. Second rotation of the sample cell with 25 Hz ensured that each short pulse train illuminated a new portion of RC /4/. The light intensity was kept so low that in the excited-sample volume less than 10^{-2} of the reaction centers were excited. The changes of absorption were monitored by delayed probe pulses at the same wavelength. To supplement our femtosecond data, excite and probe measurements using single picosecond pulses from a Nd-glass laser system were made at different probe frequencies. Tuning of the probe pulse was achieved by frequency converters based on the stimulated Raman process. Excitation wavelength was 620 nm. In the picosecond experiments less than 15% of the RCs were excited.

The reaction centers of Rhodopseudomonas viridis studied here were prepared according to the procedure given in /1/. We used reaction center preparations where 20 mM ascorbate was added in order to reduce the quinones. In that way it was guaranteed that oxidized special pairs did not accumulate. The absorption spectra of the RC were studied prior to and after each experimental run. An absorbance change indicating a decomposition of

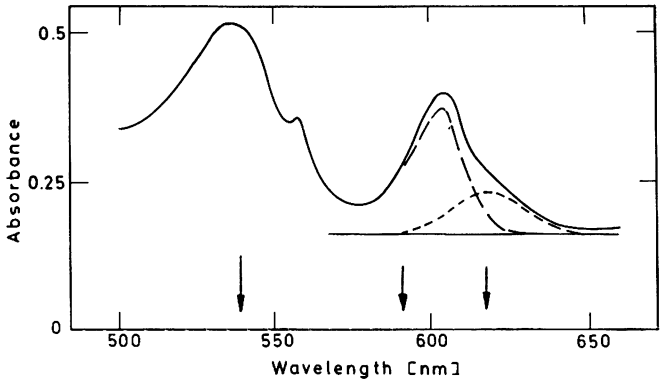

Fig. 1 Absorbance spectrum of a suspension of reaction center preparation of Rhodopseudomonas viridis. The broken lines represent a decomposition of the band around 605 nm into the contributions from the Q_x transition of the accessory $BChl_A$ ($\lambda_{max} \sim$ 605 nm) and of the special pair ($\lambda_{max} \sim$ 618 nm). (Sample: RC without ascorbate).

the RC during the picosecond and femtosecond experiments was not observed.

In Fig.1 part of the absorption spectrum of the reaction center in solution is depicted. The band at 610 nm corresponds to the Q_x transition of the two accessory bacteriochlorophyll b molecules ($BChl_A$) and the broad shoulder around 620 nm belongs to the Q_x transition of the special pair (P), the bacteriochlorophyll dimer. The position of the Q_x transition of P is well established from bleaching experiments /5/. Photooxidation of P reduces the absorption band at 960 nm, the Q_y transition of P, and simultaneously the absorption around 618 nm. The broad absorption at 530 nm in Fig.1 is made up of absorption bands of the two bacteriopheophytin b molecules (BPh) of the reaction center and of the four cytochrome units attached to the RC.

With light pulses of 620 nm we excited predominantly the special pair; approximately 20% of the incident radiation was absorbed by the neighboring $BChl_A$. In Fig.2 the absorption changes, initiated by the femtosecond excitation pulse and monitored by the delayed probe pulse, are presented as a function of delay time between the two pulses. Four successive processes are readily seen in the figure: During the passage of the excitation pulse of 150 fs the absorption of the sample decreases strongly. At the end of the excitation the absorption increases very rapidly with a time constant shorter than the pulse duration. The enhanced absorption decreases for approximately 1 ps and recovers with a time constant of 5 ps.

The experimental data suggest that four transient species are seen during the first 10 ps after excitation of the special pair: A first state having reduced absorbance (at 620 nm) lives shorter than the pulse duration; a second intermediate of enhanced absorbance lasts for 1 ps, a third one lives 5 ps, and a fourth one is stable during our subsequent observation time of 100 ps.

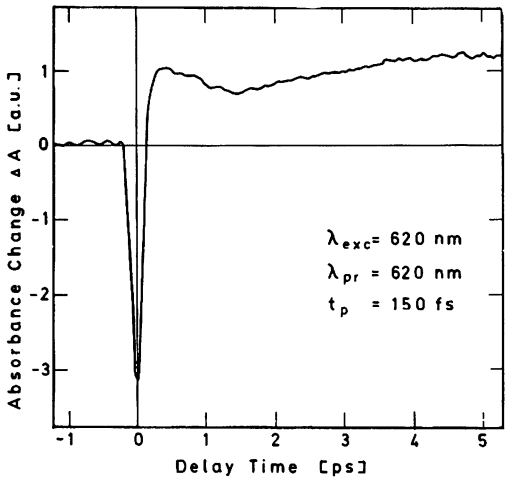

Fig. 2 Absorbance change induced by 150 fs pulses at λ = 620 nm measured as a function of time delay at the same wavelength. The transient absorbance changes indicate the existence of four intermediate states formed after optical excitation.

The assignment of the different absorption processes of Fig.2 to molecular states is supported by several sources of information. (i) As pointed out above, the x-ray work tells us the location of the different pigments within the protein, and thus gives strong indication on the course of the electron after excitation of the special pair. (ii) Our picosecond data (discussed below) at the frequency position of the BChl and BPh absorption bands help us to interpret our data for times exceeding 1 ps. (iii) Information on the absorption properties of the oxidized special pair P^+ may be deduced from the known spectra of the state P^+Q^-. Assuming that the negative charge at the quinone does not influence the visible spectrum, we deduce that P^+ has a broad absorption extending from a peak around 1.3 µm throughout the visible. Additional spectroscopic data /6/ give information on the absorbance changes induced by reducing the pigments BChl and BPh. In Fig.3 the absorption changes are depicted which occur when bacteriochlorophyll b and bacteriopheophytine b are reduced chemically to form $BChl^-$ and BPh^-, respectively. The curves in Fig.3 (redrawn from /6/) were taken in solutions of dimethylformamide ($BChlb^-$) and CH_2Cl_2 (BPh^-). While small shifts of the band are likely when going into the protein surrounding, the broad features of the spectra should basically remain unchanged, allowing the following discussion.

According to our present tentative picture the time-sequence of events is as follows: (i) During the excitation the special pair is promoted to the excited state; the reduced number of special pairs in the ground state leads to a decrease in absorption at the very beginning. The observed absorbance decrease is enhanced by the so-called coherence artifact, which exists only during the time duration of the exciting pulse. (ii) Immediately following the excitation, i.e. even during the excitation pulse of 150 fs, a second, strongly absorbing species is formed. To our

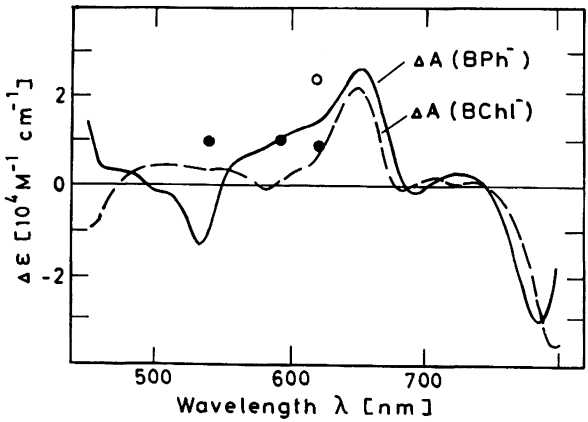

Fig. 3 Absorbance change induced by reduction of BChlb in dimethylformamide (solid curve) and of BPhb in CH_2Cl_2 (broken curve, after /6/). Absorption changes deduced from our time-resolved data are shown for the state P^{+-} ($\Delta A(P^{+-})$, open circle) and for the oxidized special pair ($\Delta A(P^+)$, full circle).

interpretation, fast charge separation in the excited special pair forms the P^{+-} state within 150 fs. (iii) The following decrease in absorption within 1 ps suggests to us the rapid passage of the electron at the neighboring $BChl_A$ forming a transient $P^+BChl_A^-$ state. The electron continues its path to the pheophytine within 5 ps; we see a new state P^+BPh^-. In our samples, where the quinones are chemically reduced, back-reactions return the RC to its initial state within less than 10^{-4} s.

Some comments should be added concerning the absolute values of the absorbance changes induced by the various intermediates. At late delay times one observes state P^+BPh^-. Its absorbance change ΔA is induced, in a first-order approximation, by oxidation of the special pair (giving $\Delta A(P^+)$) and reduction of a bacteriopheophytine ($\Delta A(BPh^-)$): $\Delta A(P^+BPh^-) \simeq \Delta A(P^+) + \Delta A(BPh^-)$. In a similar way we obtain $\Delta A(P^+BChl_A^-) \simeq \Delta A(P^{+-}) + \Delta A(BChl_A^-)$. Knowing $\Delta A(BChl^-)$ and $\Delta A(BPh^-)$ from Fig.3 we can deduce from Fig.2 the values of $\Delta A(P^+)$ (full circles in Fig.3) and $\Delta A(P^{+-})$ (open circle in Fig.3).

Additional information supporting the presented interpretation and yielding the absorbance changes $\Delta A(P^+)$ at other wavelengths can be deduced from the picosecond measurements: In Fig.4 the change of absorption at 592 nm is presented after picosecond excitation at 623 nm. As pointed out in Fig.1, at 592 nm the Q_x transition of $BChl_A$ absorbs stronger than the special pair. After excitation of the special pair at 623 nm the absorption at 592 nm rises during the pulse duration of 4 ps and remains constant for longer times. A more careful analysis of the data shows, however, that the build-up of the absorption is slowed down. The broken curve in the figure represents the measured integral over the autocorrelation curve. The difference between the instantaneous response and the experimental data are presented on an enlarged

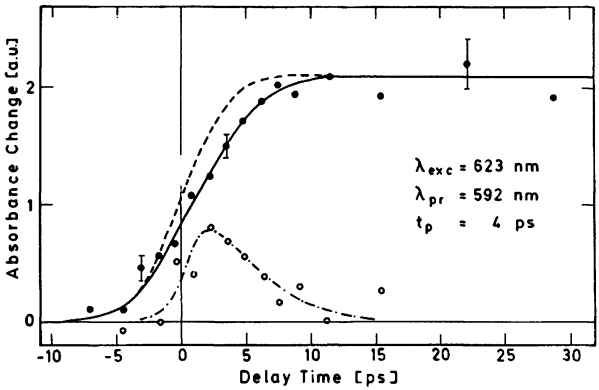

Fig. 4 Absorbance change induced by pulses at 620 nm with 4 ps duration measured at λ = 592 nm (full circles). The broken curve shows the integrated cross-correlation curve. Differences between the solid and the broken curve are drawn in an enlarged scale (x(-3)) as the open circles.

scale by the dash-dotted curve. One may explain the data of Fig.4 as follows: The excitation of the special pair at 623 nm leads to an enhanced absorption at 592 nm due to the rapid generation of P^{+-} and the slower formation of the final state $P^{+}BPh^{-}$ within 5 ps. The delay in the build-up is an indication of a short-lived intermediate state, which absorbs weaker than the final state $P^{+}BPH^{-}$. We believe that this intermediate state is $P^{+}BChl_{A}^{-}$. Indeed, according to the data of Fig.3, the absorbance change $\Delta A(BChl^{-})$ is considerably smaller than $\Delta A(BPh)$ at 592 nm.

The situation at the probing frequency at 540 nm is quite different. In Fig.5 the change of absorption after picosecond excitation at 620 nm is depicted. We find a rapidly rising (within

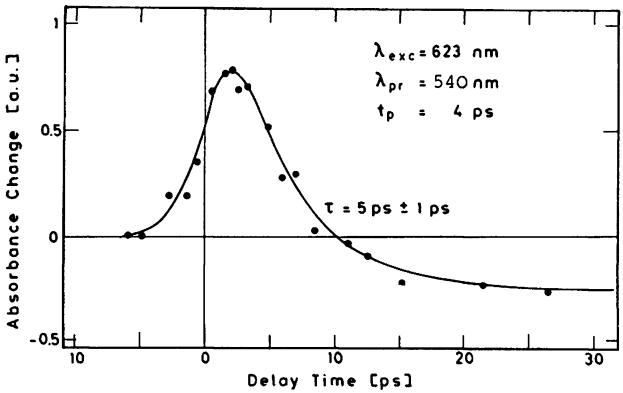

Fig. 5 Absorbance change induced by pulses at λ = 620 nm with 4 ps duration probed at λ=540 nm. At this wavelength the bacteriopheophytines give a negative absorbance change ΔA. The positive ΔA at earlier times is due to the oxidized special pair P^{+}.

the time resolution of 1 ps) enhanced absorption, followed by a negative absorption change. The short positive absorption change is believed to be due to a strong absorption of the P^{+-} and $P^{+}BChl^{-}$ states at 540 nm. These short-lived states are not resolved with pulses of a few picosecond duration. The negative absorption change for the transition BPh → BPh^{-} (see Fig.3) changes the sign of ΔA in Fig.5. In fact, the negative values of ΔA build up with a time-constant of 5 ps, as expected for the formation of the radical pairs $P^{+}BPh^{-}$. The final absorbance change is due to $\Delta A(P^{+})$ and $\Delta A(BPh^{-})$. From a numerical fit of the measured absorbance changes we estimate $\Delta A(P^{+})$ to be 80% of ΔA(BPh). The value $\Delta A(P^{+})$ determined by using $\Delta A(BPh^{-})$ is shown as full circle in Fig.3. It is interesting to note that the absorbance change induced by oxidation of the special pair $\Delta A(P^{+})$ is nearly constant in the observed spectral range.

A comment should be made concerning the intramolecular energy relaxation between the Q_x state and the lower lying Q_y state of the special pair. There exists strong evidence in the literature that in large polyatomic molecules intramolecular energy relaxation in the electronic excited state proceeds very fast, within 10^{-13} seconds /7/. It is very likely that the intramolecular energy relaxation in the special pair occurs during the first hundred femtoseconds prior to the formation of the P^{+-} state.

In summary we wish to say that experiments with the improved time resolution of 100 fs give new information not visible in previous picosecond experiments /8/. The proposed time sequence of charge separation and electron transfer

$$P \xrightarrow{h\nu} P^{*} \xrightarrow{<0.1\ ps} P^{+-} \xrightarrow{0.1\ ps} P^{+}BChl_{A}^{-} \xrightarrow{5\ ps} P^{+}BPh^{-}$$

is consistent with the present knowledge of the reaction center and with our femtosecond and picosecond experiments.

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