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Reaction Centers of Photosynthetic Bacteria

Feldafing-II-Meeting

With 165 Figures

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Part II

Native Reaction Centers: Electron Transfer Dynamics

Similarities of the Primary Change Separation Process in the Photosynthesis of *Rhodobacter sphaeroides* and *Rodopseudomanas viridis*

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Photosynthetic conversion of light into chemical energy starts via a series of electron transfer reactions in pigment-protein complexes called reaction centers (RC's). The most direct access to the primary reaction dynamics offers time resolved optical spectroscopy. During the past few years, this technique has been continuously improved permitting advanced experiments with high temporal and amplitude resolution. In this paper, we show, that RC's from Rhodobacter(Rb.) sphaeroides and Rhodopseudomonas (Rps.) viridis exhibit common features in the absorption transients. This points to a substantial similarity of the elementary molecular processes. This fact is not self-evident, since different polypeptides and different types of bacteriochlorophylls (BChl) and bacteriopheophytins (BPh) are present in various reaction centers, e.g. BChl a and BPh a are essential pigments in the reaction centers of Rb. sphaeroides while BChl b and BPh b are active in the RC's of Rps. viridis. For both reaction centers x-ray structures are now available /1-3/. It was shown that the prosthetic groups and neighbouring amino acids are in a very similar arrangement: most importantly are two BChl molecules in close contact which act as the primary electron donor P. The other pigments are arranged in two branches, A and B. Starting from the primary donor, the special pair P, one finds a monomeric bacteriochlorophyll (B), a bacteriopheophytin (H), and a quinone (Q) on each branch. It was shown that the electron transfer occurs via the A-branch and that after about 3 - 4 ps a radical pair $P^+H_A^-$ is formed. Approximately 200 ps later the electron reaches the quinone Q_A building the intermediate $P^+Q_A^-$. The role of the monomeric bacteriochlorophyll B_A is still in debate /4,5/. Recent experiments on Rb. sphaeroides have proven the existence of a previously undetected

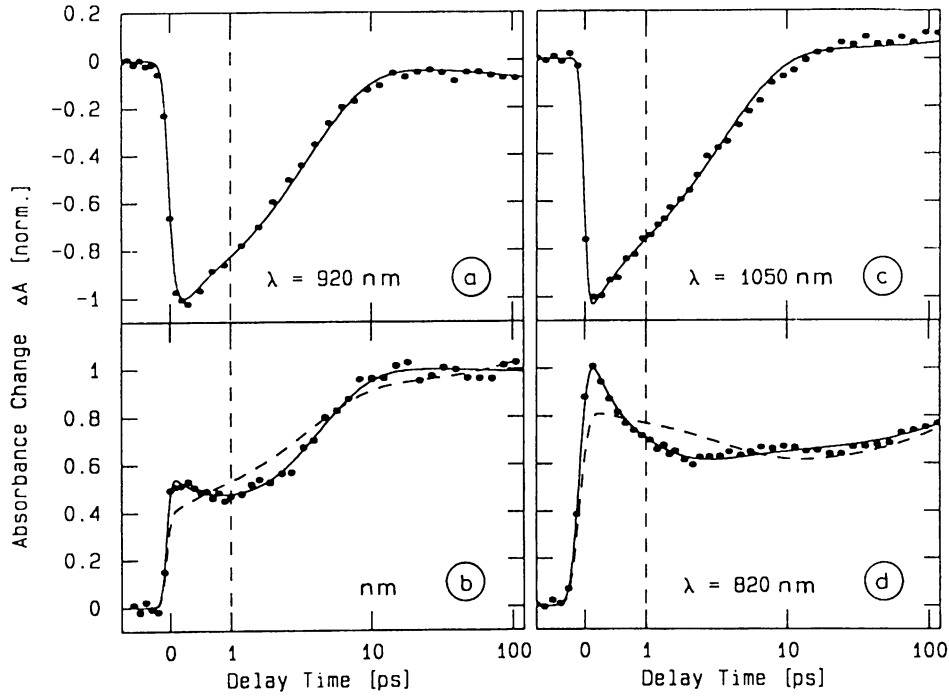

Fig. 1 Transient absorption data for reaction centers from Rb. sphaeroides (a,b) and Rps. viridis (c,d). The filled circles represent the experimental data, the solid lines correspond to model calculations with time constants given in the text. The broken lines are calculated without the fast (0.9 ps or 0.65 ps, respectively) kinetic. The excitation wavelengths are 860 nm and 955 nm for Rb. sphaeroides and Rps. viridis, respectively.

0.9 ps kinetic. A straightforward interpretation relates the corresponding transient to the radical pair $P^+B_A^-$, a real intermediate formed prior to $P^+H_A^-$ /6,7/.

The experiments presented here were performed using the excite and probe technique with weak subpicosecond pulses (pulse duration below 150 fs) generated by two different laser-amplifier systems with repetition rates of 10 Hz. The samples were excited in the lowest energy band of P (at 860 nm for Rb. sphaeroides and at 955 nm for Rps. viridis). Probing was performed by a 5 nm to 20 nm wide fraction of a femtosecond white light continuum. Exciting and probing pulses were parallel polarized. The reaction centers were prepared as described in Ref. /6/ and /8/. They were kept at room temperature under stirring.

Time-resolved absorption data for both types of bacteria are shown in Fig.1 for different probing wavelengths. The decay of the excited electronic state of the special pair is studied at λ_{pr} = 920 nm for Rb. sphaeroides (Fig.1a) and λ_{pr} = 1050 nm for Rps. viridis (Fig.1c). Both probing wavelengths are located on the long-wavelength side of the P absorption band (see Fig.2a and 2b), where the population of the first excited electronic state is readily detected via its stimulated emission. As shown in Fig.1a and 1c the rapidly appearing gain decays with a time constant around 3.5 ps. Quite different is the situation at wavelengths close to the absorption band of the monomeric bacteriochlorophylls. Here an additional fast kinetic component becomes evident (Fig.1b, 1d). In Rb. sphaeroides at 785 nm (Fig.1b) a very fast first absorption increase at time zero is followed by a brief relative absorption decrease before the absorption rises again with 3.5 ps. For Rps. viridis one finds the additional fast kinetic component quite clearly at 820 nm near the peak of the BChl absorption band (Fig.1d). Extensive studies at more than twenty different wavelengths gave the following numbers for the time constants for both RC's: the fastest process occurs with 0.9 ps +/- 0.4 ps in Rb. sphaeroides and with 0.65 ps +/- 0.3 ps in Rps. viridis. The other time constants are 3.5 ps +/- 0.4 ps, 220 ps +/- 50 ps and infinity in both RC's. The transient absorption measurements also supply amplitudes of the various kinetic components which allow to calculate difference spectra of the cross-sections of the intermediate states for specific sequential reaction models /7,9/.

The experiments clearly show, that the absorption curves can be described well by a multiexponential function with a minimum of four time constants. As a consequence, the reaction model comprises at least four intermediate states. However, the reaction scheme cannot be deduced uniquely from the transient absorption data. A certain reaction model can only be accepted if the deduced spectra of all intermediate states are not in contradiction with any other information. In the following, we discuss two linear models, which are distinguished by their different order of the early intermediates:

$$P \xrightarrow{h\nu} \underset{P^*}{I_1} \xrightarrow{3.5ps} \underset{P^+B_A^-}{I_2} \underset{0.65ps}{\xrightarrow{0.9ps}} \underset{P^+H_A^-}{I_3} \xrightarrow{220ps} \underset{P^+Q_A^-}{I_4} \xrightarrow{\infty} \ldots \qquad \text{(model A)}$$

$$P \xrightarrow{h\nu} I_1 \underset{0.65ps}{\xrightarrow{0.9ps}} I_2 \xrightarrow{3.5ps} I_3 \xrightarrow{220ps} I_4 \xrightarrow{\infty} \ldots \quad \text{(model B)}$$

$$P^* \qquad ? \qquad P^+H_A^- \qquad P^+Q_A^-$$

The difference cross-section spectra of intermediates I_1, I_3 and I_4 do not depend on the specific model A or B. The spectrum of intermediate I_1 is depicted in Fig. 2c (Rb. sphaeroides) and Fig. 2d (Rps. viridis). Intermediate I_1 exhibits a pronounced absorption decrease on the long-wavelength

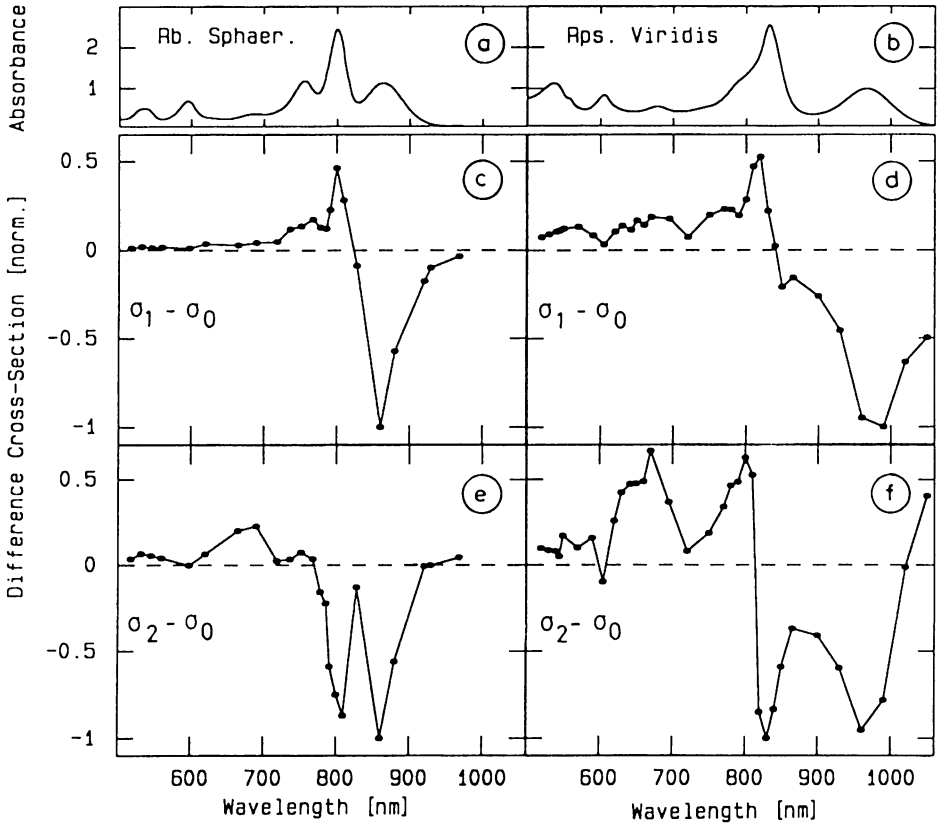

Fig.2 Spectral data for Rb. sphaeroides (a,c,e) and Rps. viridis (b,d,f). (a,b) give the absorption spectra, (c,d,e,f) show difference spectra calculated according to model A for the intermediates I_1 ($\sigma_1 - \sigma_0$) and I_2 ($\sigma_2 - \sigma_0$).

side of the P absorption band, which is due to optical gain from the electronically excited special pair P*. Excited state absorption is strong around 800 nm. The spectra of the intermediate I_2 differ significantly for the two reaction models. In model B the intermediate I_2 displays the same salient spectral features (not shown here) as I_1. However, its gain is reduced by 30%, a relative absorption decrease occurs in the Q_y band of the BChl and an increased absorption is found around 660 nm (in the anion band of the tetrapyrols). These observations indicate, that intermediate I_2 (in model B) contains an electronically excited special pair. The spectral difference between I_1 and I_2 may be explained by an excited state relaxation process or by a mixing of P* with a charge-transfer state $P^+B_A^-$. According to model B the bacteriopheophytin H_A would be the primary electron acceptor.

Quite different is the situation in model A where the 3.5 ps decay precedes the 0.9 ps (0.65 ps) process. Fig. 2e, 2f show the difference spectra of intermediate I_2 for model A. The salient features are: (i) disappearance of the absorption of the special pair P , (ii) absorption changes characteristic for P^+, (iii) strong absorption decrease in the Q_y band of the monomeric bacteriochlorophylls (at 800 nm for Rb. sphaeroides, at 820 nm for Rps. viridis), (iv) pronounced absorption increase around 660 nm in the BChl and BPh anion bands. (v) Furthermore transient dichroism experiments for Rb. sphaeroides indicate that the transition moment of the 660 nm band of I_2 is parallel to the direction expected for the BChl anion B^- /6/. It is remarkable that all five points support the assignment of I_2 being the radical pair $P^+B_A^-$. Thus the monomeric bacteriochlorophyll B_A should be the primary electron acceptor.

In conclusion: We have shown that the primary electron transfer in the bacterial reaction centers from Rb. sphaeroides and Rps. viridis proceeds according to a common reaction scheme, where a subpicosecond reaction is involved. We discussed two linear reaction models. So far the experiments cannot decide conclusively between the two. Model B leads to an additional excited electronic state (I_2) of which the functional relevance is unknown. On the other hand the structural arrangement of the chromophors in the RC's and all the spectral features (i) to (v) of intermediate I_2 in model A favour the radical pair $P^+B_A^-$ as a real transient in the electron transfer process. According to this model the electron transfer proceedes as follows: From the excited electronic state of the special pair P* an electron moves to the monomeric bacteriochlorophyll B_A within 3.5 ps for-

ming the radical pair state $P^+B_A^-$, which decays more rapidly with 0.9 ps (0.65 ps) to the radical pair state $P^+H_A^-$.

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