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# Femtosecond Reaction Dynamics

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## Contents

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# Is there a Bacteriochlorophyll Anion in the Primary Electron Transfer of Reaction Centers?

## Abstract

Femtosecond transient absorption spectroscopy is used to study the primary reaction dynamics of bacterial reaction centers from *Rhodopseudomonas viridis*. Experiments at a variety of probing wavelengths give strong indication, that the electron transfer proceeds via the monomeric bacteriochlorophyll molecule as a real electron carrier.

## 1. Introduction

Important questions on the organization of the reaction centers (RC's) have been answered by x-ray structural analysis (for a review see Deisenhofer 1989). From these studies one knows that the different chromophores are arranged in two symmetry related branches $A$ and $B$. Starting at the special pair $P$, a strongly coupled pair of bacteriochlorophyll (BChl) molecules, the two branches contain the monomeric bacteriochlorophyll (BChl) molecules $B_A$ and $B_B$, the bacteriopheophytins (BPhe) ($H_A$, $H_B$) and the quinones ($Q_A$, $Q_B$). Spectroscopy on reaction centers has revealed that the two pigment branches are spectroscopically non-equivalent and that electron transfer uses predominantly the $A$ branch. In functional reaction centers the photosynthetic action is initiated by optical excitation of the special pair, which acts as the primary electron donor. It is generally accepted that the electron transfer (ET) starts at the special pair $P$ and that the primary reaction is finished by the ET from the bacteriopheophytin $H_A$ to the quinone $Q_A$ which occurs with a time constant of 200 ps. However, there exist different opinions on the processes of the first part of the ET reaction which transfer the electron from the special pair $P$ to the BPhe $H_A$: Experimental observations have presented different kinetic processes which are related to the first ET steps. At room temperature, the decay of the electronically excited state $P^*$ of the special pair was found to proceed with approximately 3 ps (Breton 1986, Martin 1986, Holzapfel 1989, 1990). An additional subpicosecond component was found with timeconstants of 0.9 ps and 0.65 ps in *Rhodobacter* (*Rb.*) *sphaeroides* and *Rhodopseudomoras* (*Rps.*) *viridis* respectively. Recently, emission experiments with high dynamic range have shown bi(multi)-exponential features

in the decay of $P^*$ (Du 1992, Hamm 1993): There is a dominating 2.3 ps decay and a weak (relative amplitude around 20%) 7 ps contribution (Hamm 1993). Very weak slower emission processes occur in the ten to houndred picosecond regime. At cyrogenic temperatures the 3 ps and the subpicosecond process become faster and some oscillations appear in transient absorption traces. The basic interpretations of the experimental data follow two major lines:

(i) In the superexchange ET model the electron is transferred directly from the special pair $P$ to the bacteriopheophytin $H_A$ on the $A$ branch. The monomeric bacteriochlorophyll is only used as a virtual electron carrier (Breton 1986, 1988, Martin 1986, Fleming 1988). The additional time constants observed must be assigned in the superexchange model to an excited state vibrational relaxation or to a functional heterogeniety of the RC's.

(ii) In the stepwise ET model the monomeric bacteriochlorophyll $B_A$ is a real electron carrier and the electron undergoes two reaction steps, before it reaches the bacteriopheophytin. In this model the initial electron transfer to $B_A$ occurs in approximately 3 ps while a second transfer step to the bacteriopheophytin $H_A$ should be faster taking less than one picosecond (Holzapfel 1989, 1990). It causes the 0,65 ps (0,9 ps) time constant.

In this paper we present a discussion of the primary ET reaction. We will focus on the ET at room-temperature corresponding much better to physiological conditions than cryogenic temperatures. We will begin with a description of first order processes, which are well visible at least at one probing wavelength in transient absorption experiments. By this way, we believe to explain the photosynthetic action of the majority of RC's. In a second step, we extend the discussion to the more detailed measurements and will present working models for the description of the whole RC set.

## 2. Theoretical Aspects of Ultrafast Spectroscopy of Electron Transfer Reactions

In the standard nonadiabatic description of transient absorption spectroscopy one treats the molecular system as a set of electronic states where the vibrational levels of each state are in thermal equilibrium at some temperature $T$. As a consequence these states have well defined absorption properties. Transitions between the states are governed by reaction rates and the population of intermediate states may be calculated from a rate-equation system (Holzapfel et al. (1990) and Finkele et al. (1990)). For weak excitation the absorbance change is a sum of exponentials convolved with the instrumental response function. The number of exponentials is equal to the number of intermediate states populated during the reaction.

The situation becomes considerably more complicated when the selective, non-thermal population of specific vibrational levels has to be considered (see for instance Bixon et al. (1991) and references therein). Adiabatic processes have to be taken into account when the reaction i.e. the transition between different electronic states is faster than thermalization of the vibrational states within

each electronic state. An estimate for the relevant time scales is given by the vibrational relaxation times.

In photosynthesis large chromophore molecules with many vibrational modes are of relevance. Here relaxational processes of vibronic modes in the excited electronic state are known to be very fast; e.g. at room temperature and in the condensed phase, vibrational relaxation in the excited electronic state even of medium sized dye molecules was found to have dephasing times below 100 fs and vibrational energy relaxation times of a few 100 fs (Brito Cruz et al., 1988). As a consequence non-adiabatic theory is well suited to describe room temperature ET processes slower than 500 fs (Bixon et al., 1991).

## 3. Primary Reaction Dynamics in Rps. viridis

The experimental results presented in this paper are obtained on RC from *Rps. viridis*. The preparation of the reaction centers and details of the experimental system are described in Dressler et al. (1991). Characteristic experimental results are depicted in Fig. 1 and 2: The first state in the photosynthetic reaction sequence is the excited electronic level $P^*$ of the special pair $P$ populated directly by the optical excitation process. $P^*$ is readily studied by its stimulated emission on the long wavelength side of the special pair $Q_y$ absorption band. In Fig. 1 at the probing wavelength of 1050 nm, the absorption decrease around delay time zero is due to stimulated emission and depopulation of the ground state. Subsequent relaxation of the signal reflects the decay of $P^*$. Weak absorption changes seen at later delay times, $t_D > 50$ ps, can be related with the ET to the quinone $Q_A$. A more carefull inspection of the signal decay in the 0 ps to 50 ps regime leads to the following results: The best monoexponential fit of the data points

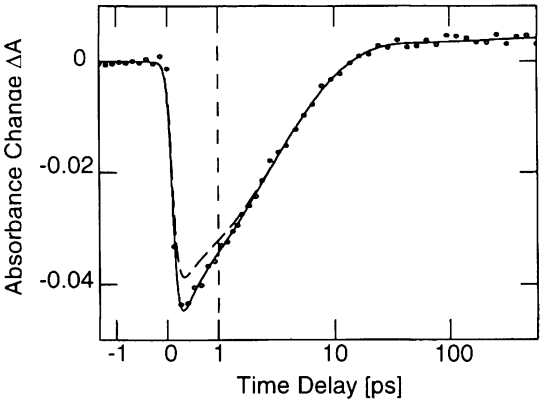


Fig. 1. Time resolved absorption data (circles) for RC from *Rps. viridis*. The curves are calculated model functions. For the solid curve five time constants, 0.65 ps, 2.3 ps, 7 ps, 200 ps, and infinity were used while the broken curve was calculated without the 0.65 ps component. A linear scale of the delay time is used for $t_D < 1$ ps; a logarithmic scale is applied at later delay times.

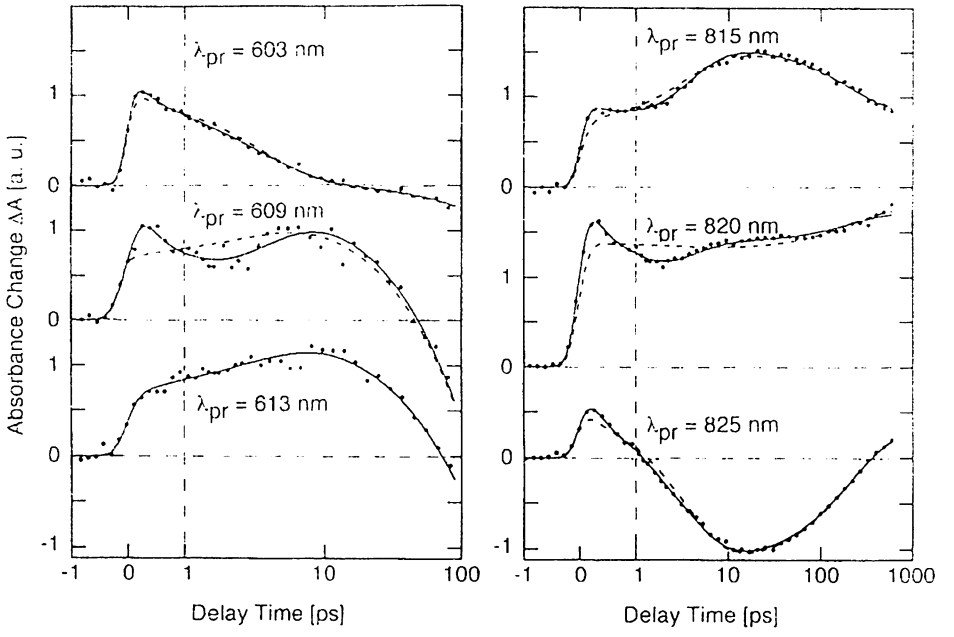


Fig. 2. Transient absorption data (points) for RC from *Rps. viridis* recorded in the $Q_x$ (left) and $Q_y$ (right) absorption band of the accessory bacteriochlorophyll. The solid curves are calculated for a four component (0.65 ps, 3.5 ps, 200 ps, infinity) the broken curve for a three component model (3.5 ps, 200 ps, infinity).

results in a time-constant of $\simeq 3.5$ ps. However there are deviations from the experimental data points at early delay times (which can be explained by a 0.65 ps component with negative amplitude, see below) and weaker ones around 10 ps (for details see last section). Additional information is obtained from probing wavelengths in the $Q_x$ absorption band of the BChl (Fig. 2, left) and in the vicinity of the BChl $Q_y$ absorption bands (Fig. 2, right). Here a fine tuning of the probing wavelength revealed rapidly changing spectral features with a clearly visible subpicosecond component. The distinct visibility of the fast component (time constant $\simeq 0.65$ ps) is restricted to very narrow spectral ranges. Nevertheless it amounts at some wavelengths to more than 30% of the peak absorption change. The fast kinetic component is also visible in the spectral range around 650 nm where the anions of BChl and BPhe absorb. At these wavelengths a final absorption transient occurs which is ($\tau \simeq 200$ ps) related with the ET from $H_A$ to the quinone $Q_A$.

The observation of three kinetic components with two time constants below 5 ps parallels the previous findings for *Rb. sphaeroides* (Holzapfel 1989, 1990). The experimental data strongly support the idea that the primary ET reactions of *Rb. sphaeroides* and *Rps. viridis* proceed via similar reaction models (Dressler 1990). While the qualitative agreement of the experimental results is striking it should be recalled that the subpicosecond kinetic component is somewhat faster

in RC of *Rps. viridis* than in RC of *Rb. sphaeroides*. In both RC's the fast kinetic component has the following properties: (i) At most wavelengths it appears with a rather small amplitude. (ii) Its amplitude is largest in spectral ranges where BChl ($Q_x$, $Q_y$) or BChl anions (660 nm) are known to have a strong absorption. While a qualitative inspection of the spectral dependence seems to relate the subpicosecond component with the monomeric BChl $B_A$ a sound interpretation has to take into account the mathematical description of the transient absorption experiment.

## 4. A First-Order Description of the Primary Electron Transfer

The structural arrangement of the RC supports the idea that the electron is transferred in several steps from the special pair $P$ via $B_A$, $H_A$ to $Q_A$. We now discuss this model in more detail (Model A of Fig. 3): The transient experimental data presented above do not give any contradiction against this reaction model. Far from it, the analysis of the transient data using reaction model A yields the spectra of the intermediates and would expect from in vitro measurements of the chromophores (Fajer 1973, 1976). This finding can be illustrated using the data of Fig. 1. In this experiment the transient absorption at 1050 nm in the gain region was investigated. Surprisingly there was some faster initial decay of the signal at early delay times which is not seen in the short wavelength side of the gain. Within a stepwise ET-model one would not expect to see a rapid initial decay of the gain. Therefore this decay must be due to another mechanism: Data analysis using reaction model A indicates that such a rapid initial decay of the signal occurs if the second intermediate $I_2$ has an increased absorption in this spectral range. This observation fits well to the interpretation of $I_2$ being the radical pair state $P^+B_A^-$ as spectra of the BChl *b* anion show a distinct absorption band around 1050 nm (Fajer 1973, 1976). A similar qualitative estimate holds at all wavelengths, where the in vitro spectra of BChl *b* and its anion differ strongly: in a spectral region where a bleaching of a BChl band upon anion formation should occur (605 nm and 825 nm) a positive amplitude of the 0.65 ps kimetic component is expected. On the other hand in spectral regions where the BChl anion has stronger absorption (around 650 nm and 1050 nm) a negative amplitude should occur. In Fig. 4b, the amplitude $\Delta A$ of the 0.65 ps kinetic component determined from a number of different transient experiments is plotted as a function of the probing wavelength. The experimentally found values of $\Delta A$ agree well with the predictions given by the in vitro spectra.

In model B based on the superexchange ET there is no intermediate which could be related to the subpicosecond kinetic component. In order to consider the subpicosecond kinetic component one has to introduce another intermediate or a modified reaction scheme: a first possibility would use two excited electronic states related by vibrational relaxation. However this assumption does not fit to the temperature dependence of the reaction rates (Lauterwasser 1992).

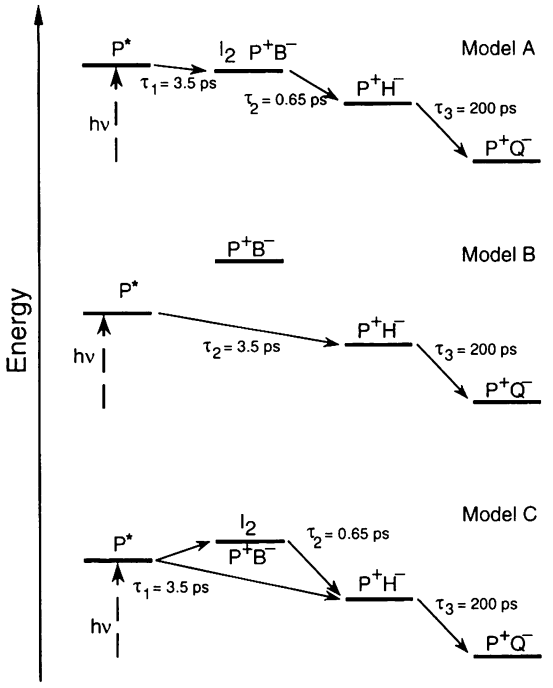


Fig. 3. Schematic representation of possible reaction models for the primary photosynthetic ET. The time constants shown in the figure represent the values for room temperature.

Another interpretation would involve a distribution of reaction rates (Kirmaier 1990, Du 1992). This possibility will be dicussed below in more detail.

Very promising is model C which interpolates between the stepwise and the superexchange ET in a consistent picture (Bixon 1989, 1991): Here the energy of state $P^+B_A^-$ is the relevant parameter. For an energy of state $P^+B_A^-$ far below and above the energy of $P^*$ the stepwise ET-mechanism and the (coherent)

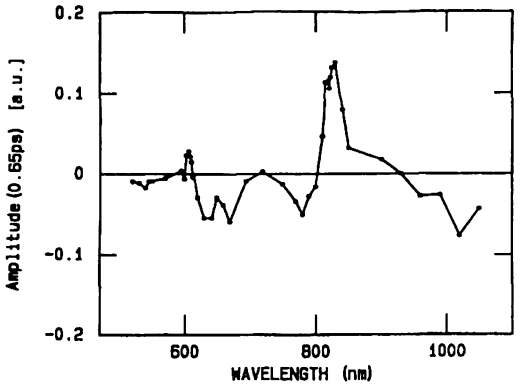


Fig. 4. Amplitude spectrum related to the 0.65 ps kinetic component. The bands fit well the in vitro spectral properties of BChl anions.

superexchange-mechanism applies respectively. For an energy of state $P^+B_A^-$ close to the energy of $P^*$ both reaction mechanisms may occur in parallel. In model C $I_2$ must have the spectral properties of a radical pair state $P^+B_A^-$. From this one obtains restrictions for the model parameters. We find a consistent spectra for an energy of state $P^+B_A^-$ which is about 200 $cm^{-1} \pm 150$ $cm^{-1}$ below that of $P^*$. As a consequence, a maximum yield of the direct superexchange transfer at room temperature is below 10%.

## 5. Higher-Order Effects in Primary Electron Transfer

Very careful inspection of the measurements at $\lambda = 1050$ nm (see Fig. 1a) suggests a weak systematic deviation of the data from a model function with a 3.5 ps decay time in the later part of the decay (for this reason the curve in Fig. 1 was calculated using a 2.3 ps and a 7 ps kinetic component). This observation is an indication for additional kinetic components in the decay of $P^*$. While it is difficult to observe this deviation from a monoexponential decay in transient absorption experiments, transient stimulated emission techniques having a larger signal to background ratio show this phenomenon convincingly (Hamm 1993, Du 1992). The results of such experiments are: In standard RC preparations of *Rb. sphaeroides* the time constant of 3.5 ps breaks up into a 2.3 ps process with large amplitude and a 7 ps process with a small amplitude of approximately 20% (Hamm et al. 1992, 1993). Similar results were obtained by Du et al. (1992). When we introduce the additional time constant in the fit of the transient absorption data, we obtain a small amplitude of the 7 ps kinetic component, which is, within experimental accuracy, at all wavelengths proportional to the amplitude of the strong 2.3 ps component.

The observation of an additional time constant requires an extension of the reaction models: Assuming a homogeneous sample with only one type of RC one has to assign the longer emission decay time to a new intermediate state (we call it $N$). The experimental observation of $N$ in emission indicates that it is coupled directly to $P^*$. There are several possibilities to introduce the new state in a reaction model. Most promising is the assignment of $N$ being the radical pair state $P^+B_B^-$. In this model the electron can transiently reach the monomeric BChl on the inactive $B$-branch. Another explanation is given by the assumption of a functional heterogeneity of the RC in the sample (Kirmaier 1990, Du 1992). In this case one would deal with two or more types of RC having different speeds of the primary ET reaction. This possibility explains well the bi-(multi-) exponential decay of the emission and the fixed amplitude ratio observed in the experiment for the amplitudes of the 2.3 ps and the 7 ps components. The origin of the 'heterogeneity' of the RC may be a distribution of the energy of an intermediate level or a distribution of the distance between two chromophores. Recently it was argued whether a microheterogeniety or a distribution of RC' properties may also account for the subpicosecond kinetic component. Taking this assumption for serious one obtains quite unusual spectral

properties of this subset of RC (In detail: Their special pair absorption remains unchanged but their emission is red-shifted; the BChl absorption decreases upon decay of $P^*$ but the electrochromic shift due to the formation of $P^+H_A^-$, which is observed for the other RC's, is missing; around 650 nm, the $P^*$ absorption must be weaker or the BPhe anion absorption must be higher than in the slower RC's; however in the 605 nm region the opposite behaviour occurs: the $P^*$ absorption must be higher or the BPhe anion absorption must be weaker than in the slower RC's). As this combination of properties is quite unlikely we may use this as an argument to support the notion, that the subpicosecond kinetic component is due to an intermediate state and not to a heterogeniety of the RC.

## 6. Conclusions

Time resolved spectroscopy on reaction centers of the purple bacterium *Rps. viridis* indicate that electron transfer for the major fraction of native RC occurs stepwise using the different chromophores of the *A*-branch as real intermediate electron carriers. The observation of the bi-(multi-)exponentiality seen in emission and in transient absorption spectroscopy may be taken as an indication for a transient population of the radical pair state $P^+B_B^-$ on the 'inactive' *B* chromophore branch or for a microheterogeniety of the reaction centers. The relative amplitudes observed in the experiments suggest, that only a minor fraction of the reaction centers follows the slower reaction mechanism.

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