The Photosynthetic Bacterial Reaction Center II

Structure, Spectroscopy, and Dynamics

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FEMTOSECOND SPECTROSCOPY OF THE PRIMARY ELECTRON TRANSFER IN PHOTOSYNTHETIC REACTION CENTERS

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INTRODUCTION

In the primary photosynthetic process of bacterial reaction centers (RC) light energy is stored by a rapid electron transfer (ET). The structural arrangement of the reaction centers with the six chromophores kept in two symmetry related branches A and B predetermines the ET path. The branches begin at two strongly interacting bacteriochlorophyll molecules forming the special pair P which acts as a primary electron donor. Subsequently each branch contains a monomeric bacteriochlorophyll (BChl) molecule B_A and B_B, a bacteriopheophytin (BPhe) (H_A, H_B) and a quinone (Q_A, Q_B) [1, 2]. Spectroscopy on reaction centers has revealed that the two pigment branches are spectroscopically non-equivalent and that electron transfer uses predominantly the A branch. It is generally accepted that the ET starts after excitation by light from 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 first part of the ET reaction: In the super-exchange ET model the electron is transferred directly from the special pair P to the bacterio-pheophytin H_A on the A branch. The monomeric bacteriochlorophyll is only used as a virtual

electron carrier [3-7]. 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. This model is supported by recent experimental results on RC from Rhodobacter (Rb.) sphaeroides which indicate that the electron transfer to B_A occurs in approximately 3.5 ps while the second transfer step to the bacteriopheophytin H_A should be faster taking less than one picosecond (0.9 ps) [8, 9].

In this paper we give additional information on the primary ET reaction obtained by transient absorption and emission experiments. In a detailed discussion we will use these results in order to obtain new insight into the nature of the primary electron transfer.

MATERIAL AND METHODS

RC from Rps. viridis and Rb. sphaeroides R26.1 were prepared as described in Ref. 8, 10. Room temperature experiments were performed in cuvettes with a pathlength of 1 mm under stirring. Measurements on low temperature RC were performed on quinone depleted RC from Rb. sphaeroides strain R26.1 desolved in glycerol [12]. The time resolved absorption experiments used the excite-and probe technique. Details of the experimental set-up are described elsewhere [8, 10]. The main features of the experiments are: Excitation beam: short pulses of a duration of about 200 fs at a repetition rate of 10 Hz, excitation wavelength 955 nm (Rps. viridis) and 875 nm (Rb. sphaeroides), less than 10 % of the RC are excited per laser pulse. Probing pulses: 5-10 nm wide portion of a femtosecond white-light-continuum selected by a dispersion compensated spectrometer in front of the sample, parallel polarisations of exciting and probing pulses, probe intensities at least 30 times smaller than excitation intensities. The width of the instrumental response function was between 250 and 350 fs.

The time-resolved emission experiment was performed with the fluorescence up-conversion technique. (For details see Ref. 11). Excitation parameters were: λ_{exc} = 865 nm (Rb. sphaeroides), $t_p \simeq$ 200 fs, less than 10 % of the RC absorb a photon. Up-conversion process: collinear type II phase matching in a 1mm BBO crystal; up-converted fluorescence emission 910-930 nm; width of the instrumental response function $\simeq$ 400 fs.

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. 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 mathematical description of the absorbance change in the non-adiabatic theory is described in detail in Ref. 9. It has been shown in the literature [5] that non-adiabatic theory is justified at least at room temperature. As a consequence we analyse the
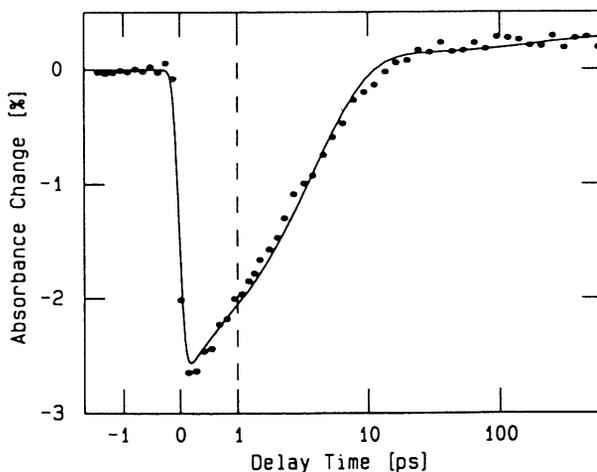
Figure 1. Transient absorption data from RC of Rps. viridis in the gain region (λ probe = 1050nm)

room temperature data by exponential functions. Due to the lack of a more reasonable theory we apply the same procedure - as a first order approximation - to the low-temperature data.

RESULTS AND DISCUSSIONS

Transient Absorption Spectroscopy of Reaction Centers of Rps. Viridis

The general features of transient absorption spectroscopy on Rps. viridis are similar to those found previously for Rb. sphaeroides [8, 9]. The decay of the excited electronic state P* of the special pair is seen at 1050 nm (Fig. 1). Here stimulated emission (gain) occurs which decays at later delay times with a time constant of approximately 3.5 ps. At early times the absorption changes more rapidly suggesting the existence of a faster kinetic component (See systematic deviations of data points from the model curve). This component is evident at measurements in the Q_x and Q_y absorption bands of the monomeric bacteriochlorophylls (Fig. 2a and b). Here a fast process with a time constant of 0.65 ± 0.3 ps occurs within a narrow spectral range. The other components observed have time constants of 3.5 ps and 200 ps.

The observation of three kinetic components with two time constants below 5 ps parallels the findings for Rb. sphaeroides. The experimental data strongly support the idea that the primary ET reactions of Rb. sphaeroides and Rps. viridis proceed via similar reaction models. 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.

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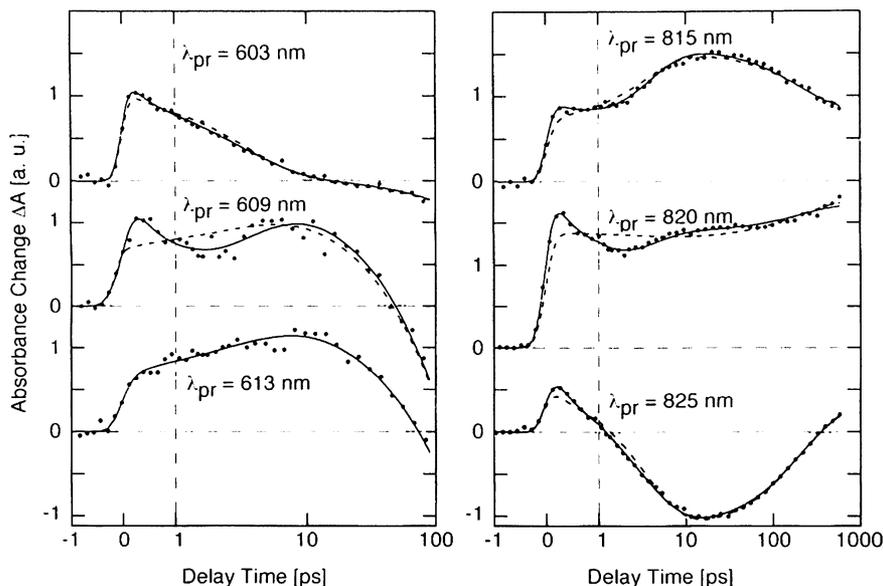
Figure 2. Transient absorption data (points) for RC from Rps. viridis recorded in the Q_x (left) and Q_y (right) absorption band of the accessory bacteriochlorophyll. The solid curves are calculated for a four component (0.65 ps, 3.5 ps, 200 ps, ∞) the broken curve for a three component model (3.5 ps,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

Reaction Centers at Low Temperatures

A first set of experiments investigated the decay of the excited state P* via stimulated emission. At the low temperature of 25 K the signal points were close to a monoexponential model function with a time constant of $\tau_1 = 1.4 \pm 0.3$ ps. This transient was followed up to room temperature where the value of 3.5 ps was reached as discussed above. The data are in agreement with previous experimental studies [13-15]. Most interesting is the investigation of the temperature dependence of the fast kinetic component [12]. To this end we studied the transient absorption changes at probing wavelengths around 795 nm in the spectral range of the Q_y band of the bacteriochlorophyll at 25 K. The transient absorption data yielded the following results: One finds a complex time dependence of the absorbance change which excludes the possibility that there is only one, namely the 1.4 ps kinetic component. There exists an additional faster kinetic process with a time constant of 0.3 ± 0.15 ps. In addition there appear some weak oscillations similar to those reported recently by Voss et al. [15]. In a set of measurements we have recorded the temperature dependence of the absorbance change in a broader spectral range. We observe qualitatively similar transient absorption features at all temperatures.

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Reaction Centers with Exchanged Bacteriochlorophyll a

In another set of experiments RC of Rb. sphaeroides were studied where the bacteriochlorophyll a molecule at the accessory position B_A and B_B were exchanged by [3-vinyl]-13^2-OH-bacteriochlorophyll a molecules [16]. The modification due to the 3-vinyl group is expected to change the redox potential of the BChl and as a consequence the energy of the radical pair state $P^+B_A^-$. This change should have pronounced consequences on the ET when the accessory BChl B_A is involved as an intermediate electron carrier. Indeed, one finds a strong change of the transient absorption data. The experimental data indicate that the RC's containing [3-vinyl]-13^2-OH-BChl a have a decay time of the excited electronic state P* of the special pair of 32 ps. On the other hand a long-lasting bleaching of the special pair absorption band shows that the exchange leads to RC's which are still photochemically active. In the [3-vinyl]-13^2-OH-BChl a containing RC's the 0.9 ps component is not visible. However, there are some indications that a related process exists which would have a longer time constant in the 5 ps domain.

REACTION MODELS

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 (Model A of Fig. 3). The transient experimental data presented here do not give any contradiction against this reaction model. Far from it the analysis of the transient data using reaction model A yields exactly the spectra of the intermediates and would expect from in vitro measurements of the chromophores [17, 18]. This finding can be illustrated by 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 (which was not seen in the short wavelength side of the gain). Data analysis using reaction model A indicates that the second intermediate I_2 must have 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 bacteriochlorophyll b anion show a distinct absorption band around 1050 nm [17, 18].

However, most transient absorption data also fit to the two models B_1 and B_2 where the subpicosecond reaction is assumed to precede the 3.5 ps process: Here the intermediate I_2 is formed very fast. It decays with 3.5 ps in a second step. Calculating the absorption spectrum of I_2 for model B_1 and B_2 leads to the following characteristics: I_2 is similar to the electronically excited state P*. It also exhibits gain; thus it should be another excited electronic state of the special pair - we call it P**. Its further absorption properties differ only slightly from those of P*. The most straightforward interpretation of P** would be that P** is a vibrationally relaxed P* state (Model B_1). Here the electron will be transferred directly in a super-
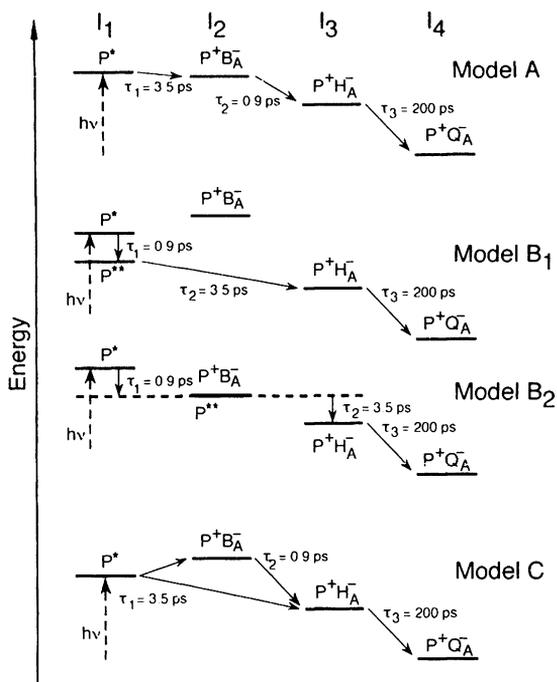
Figure 3. Schematic representation of possible reaction models for the primary photosynthetic ET. The time constants shown in the Figure represent the values for Rb. sphaeroides at room temperature.

exchange step from the special pair P to H_A. Somewhat different is the molecular interpretation for Model B_2, which is related to considerations given by H. Kuhn [19]. Model B_2 is based on the existence of an intermediate state $I_2 = P^{**}$ where the electron is delocalized over the special pair, the accessory BChl and the BPh. According to the experimental observations state $I_2 = P^{**}$ must be populated in the first 0.9 ps process. The slower 3.5 ps process is thought to be related to the trapping of the electron at the bacteriopheophytin H_A. Due to the delocalization of the electron in state P^{**} there is no need for a long- range superexchange ET in Model B_2.

In model C the energy of state $P^+B_A^-$ is the relevant parameter. Model C represents a combination of the stepwise ET (Model A) and the superexchange Model B_1 according to Bixon et al. [20, 5]. For an energy of state $P^+B_A^-$ close to the energy of P^* both reaction pathways may occur in parallel. As I_2 must have the spectral properties of a radical pair state $P^+B_A^-$ one obtains restrictions for the model parameters. We find a limit for the maximum yield of the direct superexchange transfer at room temperature of 10 %; the energy of state $P^+B_A^-$ is about 200 cm^{-1} (Rps. viridis) below that of P^*.

The experimental data obtained for RC at low temperatures and with exchanged bacteriochlorophylls allow to restrict furtheron the number of reaction models: The discussion of the two reaction Models B_1 and B_2 requires a subtile consideration of the experimental observations: In the pure superexchange picture of Model B_1 the fast kinetic component is

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related to vibrational relaxation in the excited state. From the theory of vibrational relaxation of polyatomic molecules and from a number of experiments (e. g. on amino acids [21]) it is well known that vibrational relaxation slows down at low temperatures. However, the fast reaction becomes considerably faster at low temperatures. This observation is incompatible with the interpretation of Model B_1. Additional arguments against vibrational relaxation come from experiments on modified RCs; e. g. on RCs where the monomeric BChl are exchanged by [3 vinyl]-13^2 OH-BChl and where the 3.5 ps time constant is increased to 32 ps. The molecular substitution leaves the special pair unaffected; as a consequence a P* vibrational process according to Model B_1 should be present and observable. However, the experiments do not exhibit the related 0.9 ps transient component.

The observed transient absorption data alone are not able to eliminate Model B_2. Additional information comes from hole-burning experiments (Johnson et al., [22]). In these experiments performed at very low temperatures narrow zero phonon holes were observed with a spectral width corresponding to a time constant of approximately 1 ps. From these data one can deduce that the first reaction process starting from the lowest vibrational level of P* is the slower, the 1.4 ps process. The faster 0.3 ps component must be (as it is not related with vibrational relaxation, see above) the second process in the reaction scheme. Since the important features of the reaction processes do not change strongly with temperature one may discard Model B_2 at room temperature as well.

The stepwise reaction Model A with the radical pair state $P^+B_A^-$ as a real intermediate with only a small contribution of a superexchange reaction is compatible with the extensive time resolved absorption data available today. At room temperature the stepwise ET is well described by theoretical studies giving reasonable values for the energetics in the RCs. However, the discussion of ET and absorption at low temperatures within the framework of adiabatic theory remains to be done.

NON-MONOEXPONENTIALITY OF THE FLUORESCENCE EMISSION

The experimental observations presented above fitted well into the reaction model A (or C) where only functionally necessary reaction steps are involved. We will show now that more detailed investigations exhibit an additonal kinetic component : Time resolved emission spectroscopy by fluorescence up conversion gives a valuable tool to study the properties of electronically excited emitting states without any interference of no emitting product states [11] allowing high sensitivity. An experimental result is given for RC from Rb. sphaeroides in Fig. 4 [11]. Here the decay of the emission is displayed in a semilogarithmic plot. The experimental data demonstrate that the decay of the fluorescence is not monoexponential: Apparently the 3.5 ps process deduced from the gain experiments must be split into a 2.2 ps and a 7 ps reaction. The amplitude ratio $\eta = A(7 \text{ ps})/A(2.2 \text{ ps})$ is small, $\eta \simeq 0.25$. (Indications of such a biexponential decay of P* have been seen before. [9, 15]) Of special interest is the finding that the emission experiment does not give any indication for a subpico-
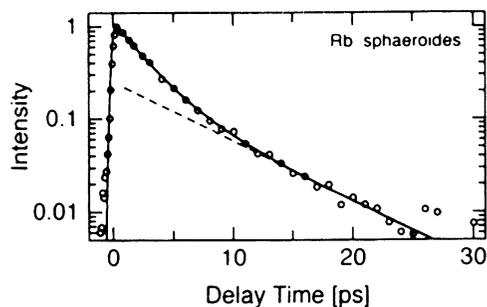
Figure 4. Time dependence of the fluorescence emission (910-930 nm) of RC from Rb. sphaeroides.

second kinetic component which one would expect, if the subpicosecond kinetic component is related to vibrational relaxation (Model B_1).

The observation of an additional time constant requires an extension of the reaction models: Trivial is the assumption of a functional heterogeneity of the sample. In this case one would deal with two components having a different speed of the primary ET reaction. For a homogeneous sample one has to assume that the longer emission decay time is related 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. We only want to discuss here the simplified situation where N is a not emitting state coupled only to P* while model A applies for the further reaction. In this case one can calculate the reaction rates to and from N (via the emission experiment) and the spectral properties of intermediate N from previously measured transient absorption data. This evaluation yields: The reaction from P* to N is slow with a rate of 1/13 ps while the reactive rate from P* to $P^+B_A^-$ is four times faster. The back reaction from N to P* is fast with a rate of 1/4.8 ps. The difference spectrum of state N shows spectral properties which are similar to those of $P^+B_A^-$. As a consequence one could speculate that N is the radical pair state $P^+B_B^-$ where the electron is transiently brought to the B branch. The further evaluation of the transient data shows that the spectra of the other intermediate states remain very similar to those obtained with the simplified reaction model A.

In conclusion, time resolved spectroscopy on reaction centers of the purple bacteria Rb. sphaeroides and Rps. viridis indicate that electron transfer of native RC occurs stepwise using the different chromophores of the A branch as real intermediate electron carriers. The observation of the biexponentiality could be taken as an indication that there is a transient population of the radical pair state $P^+B_B^-$ on the "inactive" B chromophore branch. Under this assumption the high asymmetry of the charge transfer would require a very slow electron transfer step from the monomeric bacteriochlorophyll B_B to the neighbouring bacteriopheophytin H_B.

Acknowledgements

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References

1. J. Deisenhofer, H. Michel, EMBO J. 8, 2149 (1989).

2. C.H. Chang, D. Tiede, J. Tang, U. Smith, J. Norris, M. Schiffer, FEBS Letters 205, 82 (1986).

3. M.E. Michel-Beyerle, M. Plato, J. Deisenhofer, H. Michel, M. Bixon, J. Jortner, Biochim. et Biophys. Acta 932, 52-70 (1988).

4. M.E. Michel-Beyerle, M. Bixon, J. Jortner, Chem. Phys. Lett. 151, 188-194 (1988).

5. M. Bixon, J. Jortner, M.E. Michel-Beyerle, Biochim. et Biophys. Acta 1056, 301 (1991).

6. J.L. Martin, J. Breton, A.J. Hoff, A. Migus, A. Antonetti, Proc. Natl. Acad. Sci. US 83, 957 (1986).

7. J. Breton, J.L. Martin, A. Migus, A. Antonetti, A. Orszag, Proc. Natl. Acad. Sci. US 83, 5121 (1986).

8. W. Holzapfel, U. Finkele, W. Kaiser, D. Oesterhelt, H.Scheer, H.U. Stilz, W. Zinth, Chem. Phys. Letters 160, 1 (1989).

9. W. Holzapfel, U. Finkele, W. Kaiser, D. Oesterhelt, H.Scheer, H.U. Stilz, W. Zinth, Proc. Natl. Acad. Sci. US 87, 5168 (1990).

10. K. Dressler, E. Umlauf, S. Schmidt, P. Hamm, W. Zinth, S. Buchanan, H. Michel, Chem. Phys. Letters 183, 270 (1991).

11. P. Hamm, D. Oesterhelt, R. Feick, H. Scheer, W. Zinth, to be published (1992).

12. C. Lauterwasser, U. Finkele, H. Scheer, W. Zinth; Chem. Phys. Letters 183, 471 (1991).

13. G.R. Fleming, J.L. Martin, J. Breton, Nature 333, 190 (1988).

14. N.W. Woodbury, M.Becker, D. Middendorf, W.W. Parson, Biochem. 24, 7516 (1985).

15. M.H. Voss, J.-C. Lambry, S.J. Robles, D.C. Youvan, J. Breton, J.-L. Martin, Proc. Natl. Acad. Sci. USA 88, 8885-8889 (1991).

16. U. Finkele, C. Lauterwasser, A. Struck, H. Scheer, W. Zinth, to be published in Proc. Natl. Acad. Sci. US (1992)

17. J. Fajer, D.C. Borg, A. Forman, D. Dolphin, R.H. Felton, J. Am Chem. Soc. 95, 2739-2741 (1973).

18. J. Fajer, M.S. Davis, D.C. Brune, L.D. Spaulding, D.C. Borg, A. Forman, Brookhaven Symp. Biol. 28, 74-104 (1976).

19. H. Kuhn, Phys. Rev. A 34, 3409-3425 (1986).

20. J. Bixon, J. Jortner, M.E. Michel-Beyerle, A. Ogrodnik, Biochim. et Biophys. Acta 977, 273-286 (1989).

21. T.J. Kosic, R.E.(Jr) Cline, D.D. Dlott, J. Chem. Phys. 81, 4932-4949 (1984).

22. S.G. Johnson, D. Tang, R. Jankowiak, J.M. Hayes, G. J. Small, J. Phys. Chem. 93, 5953 (1989) .