SEQUENTIAL ELECTRON TRANSFER IN THE PRIMARY PHOTOSYNTHETIC REACTION OF RHODOBACTER SPHAEROIDES.

W. Zinth, W. Holzapfel and U. Finkele

Physik Department E11 der Technischen Universität München, Arcisstrasse 21, D-8000 München 2, FRG

The primary photochemistry in the photosynthesis of bacteriochlorophyll containing organisms is a light-induced charge separation within a protein complex called reaction center (RC). Recently, the crystal structures of the reaction centers could be determined for the first time /1,2/. For Rhodopseudomonas (Rps.) viridis and Rhodobacter (Rb.) sphaeroides the structure analysis gave very similar arrangements of the prosthetic groups /3/. They are disposed as two branches (A, B) in an approximate C-2 symmetry. Two bacteriochlorophyll molecules from the so-called special pair (P) located on the symmetry axis. Starting at the special pair each branch consists of an accessory bacteriochlorophyll (B_A, B_B), a bacteriopheophytin (H_A, H_B), and a quinone (Q_A, Q_B).

A number of femtosecond spectroscopic experiments have been undertaken in order to resolve the primary electron transfer reaction. Most recent data for Rb. sphaeroides (as well as for Rps. viridis) give the following picture /4-6/: Light absorption excites the primary donor, the special pair P, to the electronically excited state P*. With a time constant of 2.8 ps an electron is transferred along the A-branch directly to the bacteriopheophytin H_A forming the state P^+H^-. It was shown in the literature that after the decay of P* and prior to the formation of state P^+H^- there is no intermediate electron carrying state or, if such a state exists, its lifetime must be shorter than 100 fs /5,6/. However, in the context of earlier experiments - not performed under optimum conditions - evidence for such an intermediate was discussed, based on the finding of rapid transient absorption changes with approximate time constants of 1 ps /7-9/. In sight of this controverse discussion we decided to reinvestigate the kinetics of the primary processes of Rb. sphaeroides under optimized experimental conditions.

Reaction centers from Rb. sphaeroides R26.1 and ATCC 17023 were prepared according to the procedure in Ref./10/. In the time-resolved absorption measurements we used femtosecond exciting pulses (t_p = 60 fs, repetition rate 10 Hz) at 860 nm in the lowest energy absorption band of the special pair (see inset in Fig.1). Probing of the light-induced absorption changes at various wavelengths λ_{pr} was performed with synchronized pulses as a function of time delay t_D. The excitation energy density was kept low, only 13% of the reaction centers were excited by each excitation pulse.

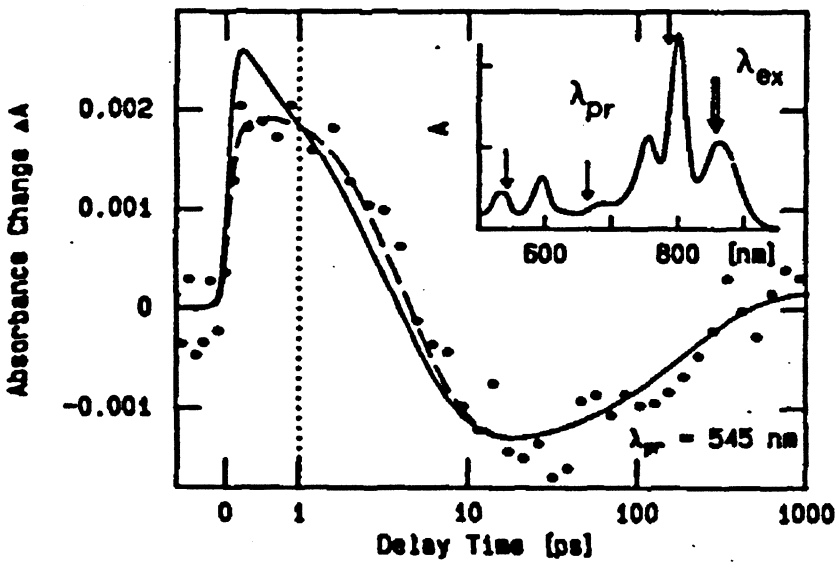

Fig.1. Time-resolved absorption data (full circles) at a probing wavelength of 545 nm for reaction centers of Rb. sphaeroides R26.1 (excitation wavelength 860 nm). The curves are calculated according to two models given in the text. Inset: absorption spectrum of the sample.

The time resolved absorption measurements are shown in Fig.1 and 2 /10/. The experimental data points are indicated as full circles. The curves are calculated according to two kinetic models. The first model (solid curve) is directly deduced from the presently accepted electron transfer scheme; i.e. electron transfer occurs from P^* directly to state P^+H^-. Consequently, the complete reaction kinetics until the formation of state P^+Q^- involves three intermediates connected by two time constants. The second model (dashed curve, schematic shown in Fig.3) considers an additional intermediate state with the lifetime of 0.9 ps. The experimental results of Fig.1 and 2 were taken at λ_{pr} = 545 nm (H_A absorption band), 785 nm (H,B absorption), and 665 nm (H^-,B^- absorption range). All three measurements indicate that the model of Fig.3 (dashed curve, four intermediates, three time constants) is required to explain the experimental data. Especially at λ = 665 nm and 785 nm the 0.9 ps kinetic is outstanding, while the 3.5 ps and the 200 ps kinetics also appear.

The amplitudes of the absorption changes connected with states I_1 to I_4 can be used to relate the various intermediate states to molecular configurations. The molecular interpretation in Fig.3 is based on the following arguments: The first state I_1 is the excited electronic state. Its monoexponential decay with a time constant of 3.5 ps is observed around 920 nm. This time constant is the first one (τ_1) in the sequence of our model. The subsequent states are intermediates of the electron transfer process. The next state is I_2 decaying rapidly with a time constant of 0.9 ps. The most obvious assignment of I_2 is the configuration P^+B^- containing

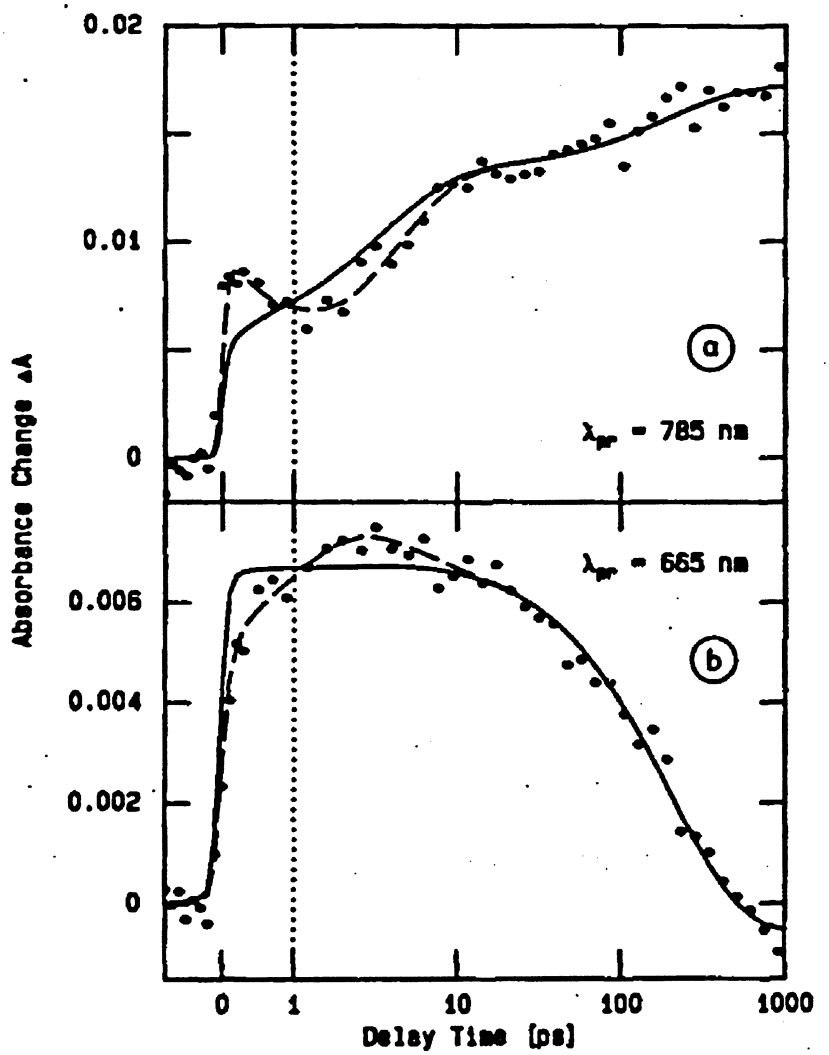

Fig.2. Time-resolved absorption data (full circles) for probing wavelengths of (a) 785 nm and (b) 665 nm for reaction centers of wild-type Rb. sphearoides ATCC 17023 (excitation 860 nm).

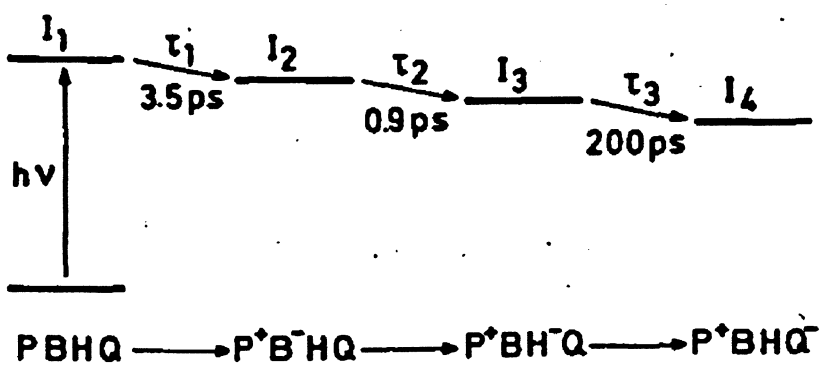

Fig.3. Scheme of the sequence of states involved in the primary electron transfer in reaction centers.

a bacteriochlorophyll anion radical. This interpretation is supported by the following observation /10/. (i) I_2 shows strongly reduced absorption at the wavelength of 797 nm, where B^- (in vitro) has strongly reduced absorption. (ii) During the rapid decay of I_2 this absorption recovers, and (iii) I_2 does not show absorption decrease in the wavelength range where bacteriopheophytin absorbs. State I_3 represents the next intermediate where the electron has reached the bacteriopheophytin H forming the configuration to P^+BH^-Q. The related decrease of the H absorption at 545 nm is clearly seen after the decay of state I_2 and prior to the formation of I_4. Finally, with a time constant of 200 ps, the electron arrives in state I_4 at the quinone Q_A (state P^+BHQ^-).

In conclusion: We have performed an experimental study of the primary charge transfer process in the reaction centers of Rb. sphaeroides. The analysis of the kinetic data (in a linear model) strongly suggests that the primary charge transfer to the bacteriopheophytin proceeds via the accessory bacteriochlorophyll as a true intermediate. The following reaction scheme results: After excitation of the special pair P an electron is transferred with a time constants of 3.5 ps to the accessory bacteriochlorophyll. Here the electron resides for the short time of 0.9 ps before it reduces the bacteriopheophytin.

Acknowledgement
The authors acknowledge valuable collaboration with and supply of the Rb. sphaeroides samples by D. Oesterhelt, H. Scheer, and H.U. Stilz.
The work was supported by the Deutsche Forschungsgemeinschaft, SFB143.

REFERENCES

1. J. Deisenhofer, O. Epp, K. Miki, R. Huber, H. Michel, J. Mol. Biol. 180, 385 (1984)
2. H. Michel, O. Epp, J. Deisenhofer, EMBO J. 5, 2445 (1986)
3. J.P. Allen, G. Feher, T.O. Yeates, H. Komiya, D.C. Rees, Proc. Natl. Acad. Sci. USA 84, 5730 (1987)
4. J.-L. Martin, J. Breton, A.J. Hoff, A. Migus, A. Antonetti, Proc. Natl. Acad. Sci. USA 83, 957 (1986)
5. G.R. Fleming, J.-L. Martin, J. Breton, Nature 333, 190 (1988)
6. J. Breton, J.-L. Martin, A. Migus, A. Antonetti, A. Orszag, Proc. Natl. Acad. Sci. USA 83, 5121 (1986)
7. D. Holten, C. Hoganson, M.W. Windsor, C.C. Schenck, W.W. Parson, A. Migus, R.L. Fork, C.V. Shank, Biochim. Biophys. Acta 592, 461 (1980)
8. W. Zinth, M.C. Nuss, M.A. Franz, W. Kaiser, H. Michel, in "Antenna and Reaction Centers of Photosynthetic Bacteria", Springer Series in Chemical Physics, Vol. 42, Ed. M.E. Michel-Beyerle, Springer, Heidelberg 1985, p. 286
9. V.A. Shuvalov, A.V. Klevanik, FEBS Lett. 160, 51 (1983) V.A. Shuvalov, J. Amesz, L.N.M. Duysens, Biochim. Biophys. Acta 851, 327 (1986)
10. W. Holzapfel, U. Finkele, W. Kaiser, D. Oesterhelt, H. Scheer, H.U. Stilz, W. Zinth, Chem. Phys. Lett., to be published (1989)