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# Ultrafast Phenomena VII

Proceedings of the 7th International Conference,  
Monterey, CA, May 14–17, 1990

Editors: C. B. Harris, E. P. Ippen,  
G. A. Mourou, and A. H. Zewail

With 435 Figures

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*Managing Editor:* Dr. Helmut K. V. Lotsch  
Springer-Verlag, Tiergartenstrasse 17,  
D-6900 Heidelberg, Fed. Rep. of Germany

ISBN 3-540-53049-5 Springer-Verlag Berlin Heidelberg New York  
ISBN 0-387-53049-5 Springer-Verlag New York Berlin Heidelberg

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Printed in Germany

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Printing: Weihert-Druck GmbH, D-6100 Darmstadt  
Binding: J. Schäffer GmbH & Co. KG, D-6718 Grünstadt  
2154/3140-543210 – Printed on acid-free paper

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# Primary Charge Separation in the Photosynthesis of Bacterial Reaction Centers

W. Zinth, W. Holzappel, U. Finkle, C. Lauterwasser, K. Dressler, and P. Hamm

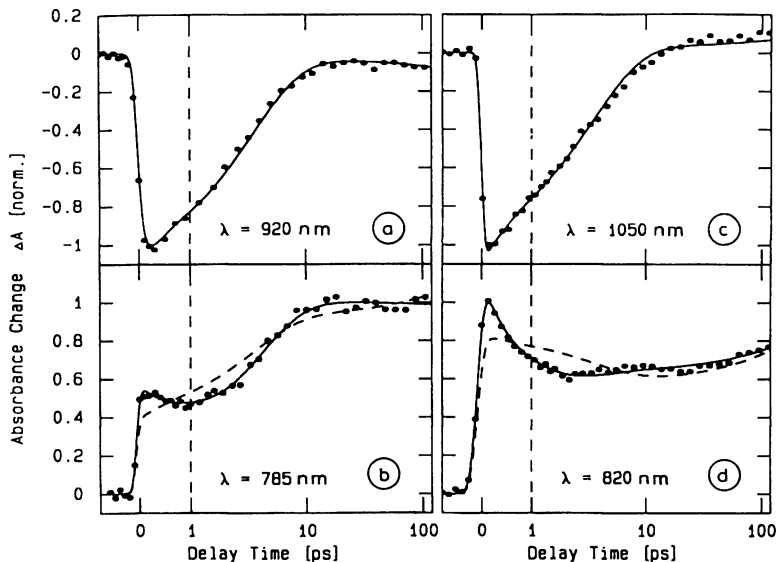
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**Abstract.** The very rapid initial electron transfer is studied for two purple bacteria. The results favor a model of electron transfer involving a bacteriochlorophyll anion radical.

Photosynthesis is the most important photochemical reaction in nature, where light energy is converted to chemical energy. Most photosynthetic systems act according to a common principle: the absorption of a photon is followed by a first charge separation and the build-up of a potential gradient. Finally, in complex biochemical processes the synthesis of energy storing molecules takes place. The crucial part of the photosynthetic energy conversion is the primary charge separation, which occurs in so-called reaction centers (RC).

It is the purpose of this paper to show that the same reaction model well describes the primary charge separation for different purple bacteria. This fact is not obvious, since different tetrapyrrols, bacteriochlorophylls (BChl) and bacterio-pheophytins (BPh) are present in various reaction centers, e.g. BChl a and BPh a are essential pigments in the reaction centers of Rhodobacter (Rb.) sphaeroides while BChl b and BPh b operate in the reaction centers of Rhodospseudomonas (Rps.) viridis. For both reaction centers x-ray structure analysis has revealed the molecular arrangement /1/. It was shown that the prosthetic groups and related aminoacids are in a very similar arrangement in both reaction centers: two BChl molecules are in close contact acting as a 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 an accessory bacteriochlorophyll (B), a bacteriopheophytin (H), and a quinon (Q) on each branch. It was shown that the electron transfer occurs via the A-branch and after about 3 - 4 ps a radical pair  $P^+H_A^-$  is formed. Another 200 ps later the electron reaches the quinon  $Q_A$  building the intermediate  $P^+Q_A^-$ . The role of the accessory bacteriochlorophyll  $B_A$  is still in debate /2,3/. Recent experiments on Rb. sphaeroides have proven the existence of a previously unresolved 0.9 ps kinetic which was interpreted to be related with a radical pair  $P^+B_A^-$  as the real intermediate formed prior to  $P^+H_A^-$  /4,5/.

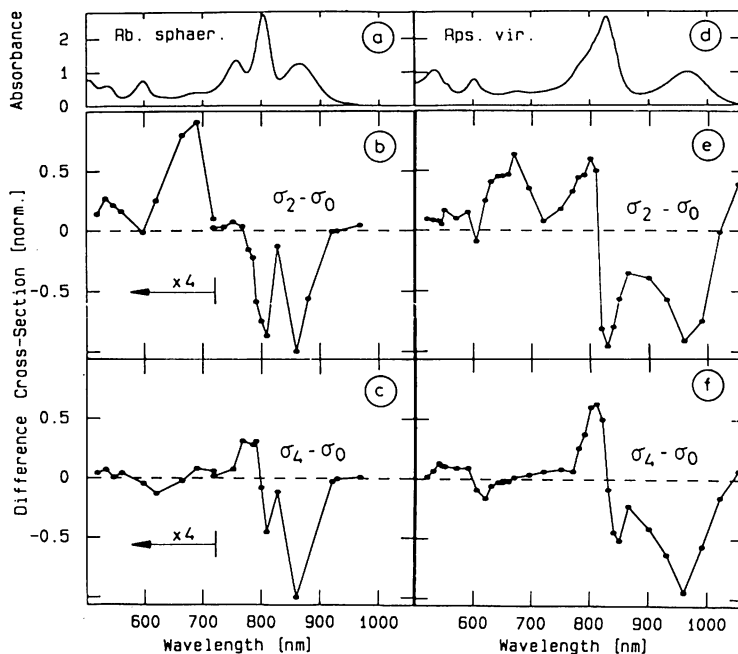
The present experiments were performed using the excite and probe technique with weak subpicosecond pulses from two different laser-amplifier systems with repetition rates of 10 Hz. Excitation was made of the lowest energy band of P (at 860 nm for Rb. sphaeroides and at 950 nm for Rps. viridis). Probing was performed by a 5 nm to 20 nm wide fraction of a femtosecond light continuum. Parallel polarisation of exciting and probing pulses was used in the experiments presented here. The reaction centers were kept at room temperature under stirring.



**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 follow model functions using the time constants given in the text.

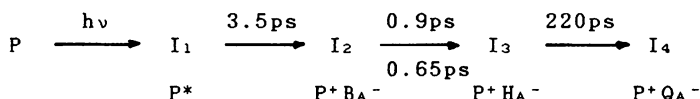
Time-resolved absorption data are shown in Fig.1 for different probing wavelengths. The decay of the excited electronic level of the special pair is seen at  $\lambda_{pr} = 920$  nm for *Rb. sphaeroides* (Fig.1a) and  $\lambda_{pr} = 1050$  nm for *Rps. viridis* (Fig.1c). Both probing wavelengths are located in the long-wave wing of the P absorption band (see Fig.2a and 2d), where the excited electronic level is visible via its stimulated emission. In Fig.1a and 1c the population of  $P^*$  decays exponentially with a time constant around 3.5 ps. The situation is drastically different at some wavelengths close to the absorption band of the accessory bacteriochlorophyll. An additional fast kinetic component becomes evident. In *Rb. sphaeroides* at 785 nm (Fig.1b) a first absorption increase at time zero is followed by a fast relative absorption decrease before the 3.5 ps process takes over, again increasing the absorption. For *Rps. viridis* one finds the additional fast kinetic component quite clearly at 820 nm (Fig.1d). Extensive studies at different wavelengths gave numbers for this fast process of  $0.9 \pm 0.4$  ps in *Rb. sphaeroides* and  $0.65 \pm 0.3$  ps in *Rps. viridis*. In addition they supplied amplitudes of the various kinetic components which can be used to calculate difference spectra of the intermediate states for specific sequential reaction models.

When the fast process is assumed to precede the 3.5 ps decay, the spectral data require the existence of two excited electronic states and the electron should be transferred directly from the special pair P to the bacteriopheophytin  $H_A$ . However, this direct electron transfer gives serious difficulties for the microscopic theoretical description of the electron transfer process. In addition, recent results on mutants of reaction centers of *Rb. sphaeroides* are in contradiction to this reaction



**Fig.2** Spectral data for *Rb. sphaeroides* (a,b,c) and *Rps.viridis* (d,e,f). (a,d) give the absorption spectra, (b,c,e,f) show difference spectra calculated according to the second model for the intermediates  $I_2$  and  $I_4$ .

tion model /6/. The second possibility is a model, where the 3.5 ps process precedes the 0.9ps/0.65ps decay.



Of interest are the difference spectra of intermediate  $I_2$  and  $I_4$  which are shown in Fig.2. The spectrum  $\sigma_4 - \sigma_0$  of the final picosecond product  $I_4$  ( $P^*Q_A^-$ ) does not depend on the reaction model used. The absorption changes observed are well known. They reflect the disappearance of the absorption of the special pair P and small contributions from  $P^+$  and from electrochromic shifts. The spectrum  $\sigma_2 - \sigma_0$  of intermediate  $I_2$  again exhibits some of the features known for  $P^+$  formation. In addition, there are strong absorption changes of the  $Q_y$  band of the accessory bacteriochlorophyll (at 800 nm for *Rb. sphaeroides*, at 820 nm for *Rps. viridis*) and around 660 nm (in the bacteriochlorophyll anion band) indicative of the formation of a bacteriochlorophyll anion radical. Consequently, the calculated difference spectra give strong evidence of the existence of  $P^*B_A^-$  as a short-lived intermediate in the primary electron transfer in the reaction centers of the two purple bacteria. The notion of  $P^*B_A^-$  being the second intermediate is also strongly supported by additional experiments on the transient dichroism /5/.

In conclusion: We have shown that the primary electron transfer in different bacterial reaction centers proceeds according

to a common reaction scheme, where a subpicosecond - previously undetected - reaction is involved. The present data support the model of sequential electron transfer. From the excited electronic state of the special pair P\* an electron proceeds to the accessory bacteriochlorophyll within 3.5 ps forming the radical pair state P<sup>+</sup>B<sub>A</sub><sup>-</sup>, which decays more rapidly with 0.9ps/0.65ps to the radical pair state P<sup>+</sup>H<sub>A</sub><sup>-</sup> where the electron has been transferred over a distance of approximately 17Å to the bacteriopheophytin.

#### Acknowledgement

Experiments were performed in collaboration with W. Kaiser, H. Scheer, D. Oesterhelt, U. Stolz, S. Buchanan, and H. Michel.

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