# FRONTIERS OF PHOTOBIOLOGY

Proceedings of the 11th International Congress on Photobiology, Kyoto, Japan, 7–12 September 1992

*Editors:*

**AKIHIRO SHIMA**
University of Tokyo, Laboratory of Radiation Biology,
Tokyo, Japan

**MASAMITSU ICHIHASHI**
Kobe University, Department of Dermatology,
Kobe, Japan

**YOSHISADA FUJIWARA**
Kobe University, Department of Radiation Biophysics and Genetics,
Kobe, Japan

**HIRAKU TAKEBE**
Kyoto University, Department of Experimental Radiology,
Kyoto, Japan


**1993**

**EXCERPTA MEDICA**
**AMSTERDAM - LONDON - NEW YORK - TOKYO**

International Congress Series No. 1021
ISBN 0 444 89721 6

*This book is printed on acid-free paper.*

*Published by:*
Elsevier Science Publishers B.V.
P.O. Box 211
1000 AE Amsterdam
The Netherlands



*Library of Congress Cataloging in Publication Data:*

International Congress on Photobiology (11th : 1992 : Kyoto, Japan)
Frontiers of photobiology : proceedings of the 11th International Congress on Photobiology, the 11th International Congress on Photobiology, Kyoto, Japan, 7-11 September 1992 / editors, Akihiro Shima ... [et al.].
p. cm. -- (International congress series ; no. 1021)
"EM 1992."
Includes index.
ISBN 0-444-89721-6 (alk. paper)
1. Photobiochemistry--Congresses. 2. Photobiology--Congresses.
I. Shima, Akihiro, 1941- . II. Title. III. Series.
[DNLM: 1. Chronobiology--congresses. 2. Photochemistry--congresses. 3. Photoreceptors--physiology--congresses. 4. Photosensitivity Disorders--congresses. 5. Phototherapy--congresses. 6. Retinal Pigments--physiology--congresses. W3 EX89 no.1021 1992 / QH 515 I61 1992f]
QP517.P45I58 1992
574.19'15--dc20
DNLM/DLC
for Library of Congress 92-48400
CIP



Printed in The Netherlands

**Sponsored by:**

Science Council of Japan
Photobiology Association of Japan

**Supported by:**

The Commemorative Association for the Japan World Exposition (1970)
Japanese Dermatological Association
Japanese Society for Investigative Dermatology

# CONTENTS

**Furocoumarines**



**Synchrotron-radiation photobiology**



**Rhodopsins and light receptor molecules**

**Visual pigments and transduction**



**Ultrafast reaction by laser light and spectroscopy**

**Bio- and chemi-luminescence**



## PHOTOBIOLOGY AND PHOTOIMMUNOLOGY

**Chronobiology**

**Molecular approaches to plant development**



**DNA damage, repair and UV mutagenesis**

**Photoimmunology**

## PHOTOMEDICINE

### Photocarcinogenesis



### Advances in photodermatology

**Photosensitizers and photodynamic therapy**



## SOLAR UV LIGHT AND ENVIRONMENT

**Environment and solar UV light**

**Biological and medical consequences of ozone depletion**



**Effects of solar UV light on plants**

Frontiers of Photobiology.
A. Shima et al., editors.


# Femtosecond Spectroscopy of the Primary Electron Transfer in Photosynthetic Reaction Centers

C. Lauterwasser[a], U. Finkele[b], A. Struck[c], H. Scheer[c], W. Zinth[a]

[a] Institut für Medizinische Optik der Ludwig-Maximilians-Universität München, Barbarastr. 16, 8000 München 40, Germany

[b] Physik Department E 11 der Technischen Universität München, James-Franck-Str., 8046 Garching/München, Germany

[c] Botanisches Institut der Ludwig-Maximilians-Universität München, Menzinger Str. 67, 8000 München 19, Germany

## INTRODUCTION

The primary photochemical event during photosynthesis of bacteriochlorophyll (BChl) containing organisms is a light induced charge separation within a transmembrane protein called the reaction center (RC). The crystal structures of the RCs from purple bacteria [1-3] show that the chromophores are arranged to two branches (named A and B) forming a pseudo $C_2$-symmetry. On the symmetry axis lies the primary electron donor P, a pair of strongly interacting BChl molecules. Along each branch a monomeric BChl molecule ($B_A$ and $B_B$) is located adjacent to P. Each branch is completed by a bacteriopheophytin ($H_A$ and $H_B$) and a quinone ($Q_A$ and $Q_B$). Following excitation of the special pair P an electron is rapidly transferred to the quinone $Q_A$. It has been shown that this process occurs predominantly along the A branch. There is general agreement that after 3-4 ps the electron reaches the intermediate acceptor $H_A$ from where it is transferred to $Q_A$ within 200 ps. While it is accepted that spectral changes occur on a time scale shorter than $P^+H_A^-$ formation [4-10], two different models are discussed for the multiexponential kinetics. In the superexchange electron transfer (ET) model the electron is transferred directly from the special pair P to the bacteriopheophytin $H_A$ on the A branch. The monomeric BChl is only used as a virtual electron carrier [11-13]. Here the fast kinetic component is related most likely to rapid vibrational motion or relaxation in the excited state of P. 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 suggested by recent experimental results on RCs 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) [4, 5].

In this paper we give additional information on the primary ET reaction obtained by transient absorption experiments on native RCs at various temperatures and on RCs where the monomeric bacteriochlorophylls $B_A$ and $B_B$ have been modified.

## MATERIAL AND METHODS

Native RCs from *Rb. sphaeroides* R26.1 were prepared as described in Ref. 14. Measurements at low temperatures were performed on quinone depleted RC from *Rb. sphaeroides* strain R26.1 desolved in glycerol (56 % v/v). RCs containing [3-vinyl]-13$^2$-hydroxy-bacteriochlorophyll a were prepared after Struck et al. [14, 15]. The BChl a exchange yielded values of 40 ± 5 %. Since the two BChl a-molecules of the primary donor P do not exchange, this value corresponds to an average exchange of 80 % at sites $B_A$ and $B_B$.

The time resolved absorption experiments used the excite-and probe technique. Details of the experimental set-up are described elsewhere [4, 16]. 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 875 nm, 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 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 300 fs.

The signal points (full circles) were modelled (solid and broken curves) by a sum of exponentials convoluted with the instrumental response function (for details see [5]).

## RESULTS

### The Primary Reaction at Room Temperature

Investigations on the light induced kinetics in wild type reaction centers from *Rb. sphaeroides* have been published in detail in Ref. 5. In summary we have found: (i) The excited electronic level P* of the primary donor P decays with a time constant of about 3.5 ps. (ii) At various wavelengths in the visible and near infrared spectral range an additional faster kinetic component is needed to account for the experimental data. The amplitude of this kinetic is largest in spectral ranges where BChl ($Q_X$, $Q_Y$) or BChl anions (640-670 nm) are known to have a strong absorption. From a series of measurements the time constant was determined to be 0.9 ± 0.3 ps. As an example three measurements are shown in Fig. 1A at probing wavelengths in the $Q_Y$ (B) band. From these data the existence of a fast process is evident.

### Reaction Centers at Low Temperatures

A first set of experiments investigated the decay of the excited state P* via stimulated emission (not shown). At the low temperature of 25 K the absorbance change can be described by 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 [17, 18]. Most interesting is the investigation of the temperature dependence of the fast kinetic component. To this end we studied the transient absorption changes at 25 K for 30 probing wavelengths ranging from 640 nm to 920 nm. The transient absorption data yielded the following results: One finds a complex time dependence of the absorbance changes which excludes the possibility that there is only one, namely the 1.4 ps kinetic component. The data can be well fit assuming an additional faster kinetic process with a time constant of 0.3 ± 0.15 ps.

Besides this acceleration of the electron transfer processes we find a close similarity in the transient spectral features between low temperature and room temperature data, if we take into account differences due to the narrowing of the absorption bands at low temperatures. Fig. 1B shows kinetic traces at 25 K in the $Q_Y$ (B) band (peak absorption at 802 nm). The qualitative agreement with the room temperature traces (Fig. 1A) is striking. In addition, there appear some weak oscillations at low temperature similar to those reported by Vos et al. [9] (probing wavelength 795 nm, Fig. 1B). In a set of measurements we have recorded the temperature dependence of the fast kinetic. We observe a smooth temperature dependence [16].

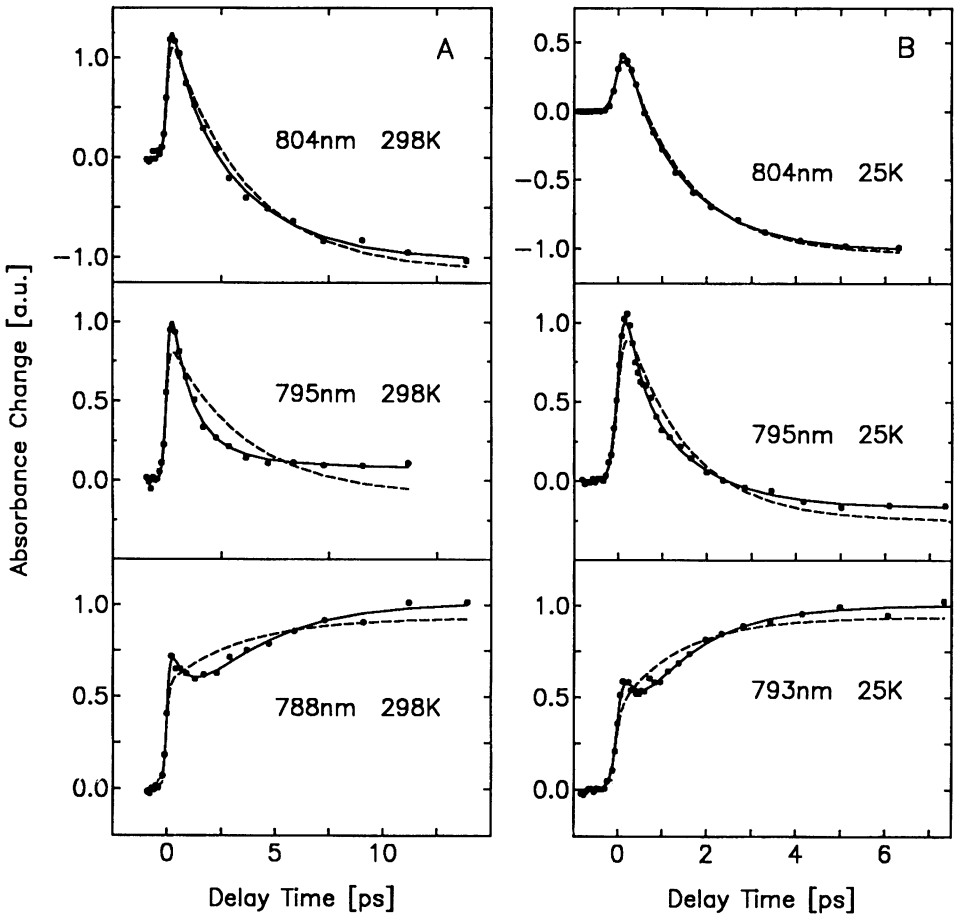


Fig. 1. Transient absorption data for RCs from *Rb. sphaeroides* recorded in the $Q_Y$ absorption band of the monomeric bacteriochloropylls. A: room temperature data, B: data at 25 K. The solid curves are calculated for a three component (3.5 ps, 0.9 ps, ∞ at 298 K; 1.4 ps, 0.3 ps, ∞ at 25 K), the broken curves for a two component model (3.5, ∞ at 298 K; 1.4 ps, ∞ at 25 K). The data are normalized (peak values of ΔA between 0.03 and 0.08).

**Reaction Centers with Exchanged Bacteriochlorophyll a**

In another set of experiments, RC of *Rb. sphaeroides* were studied where the bacteriochlorophyll a molecule at the monomeric position $B_A$ and $B_B$ were exchanged by [3-vinyl]-13$^2$-OH-BChl a molecules [19]. 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. 2). The transient experimental data presented here do not give any contradiction against this reaction model. In fact, the analysis of the transient data using reaction model A yields the spectra of the intermediates one would expect from in vitro measurements of the chromophores [20, 21].

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 superexchange 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 [22]. 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 $B_2$.

The experimental data obtained for RC at low temperatures and with exchanged bacteriochlorophylls allow to restrict further on the number of reaction models: The discussion of the two reaction Models $B_1$ and $B_2$ requires a subtle consideration of the experimental observations: In the pure superexchange picture of Model $B_1$ the fast kinetic component is 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 [23]) it is well known that vibrational relaxation slows down at low temperatures. However, the fast reaction observed in our experiment 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 spectrally unaffected in the singlet and doublet (= radical cation) states [14, 15]; 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., [24]). 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. As the faster 0.3 ps component is not related with vibrational relaxation (see above) it must then be the second process in the reaction scheme. Since the important features of the reaction processes do not change strongly with temperature it is likely that Model $B_2$ is not operative at room temperature either.

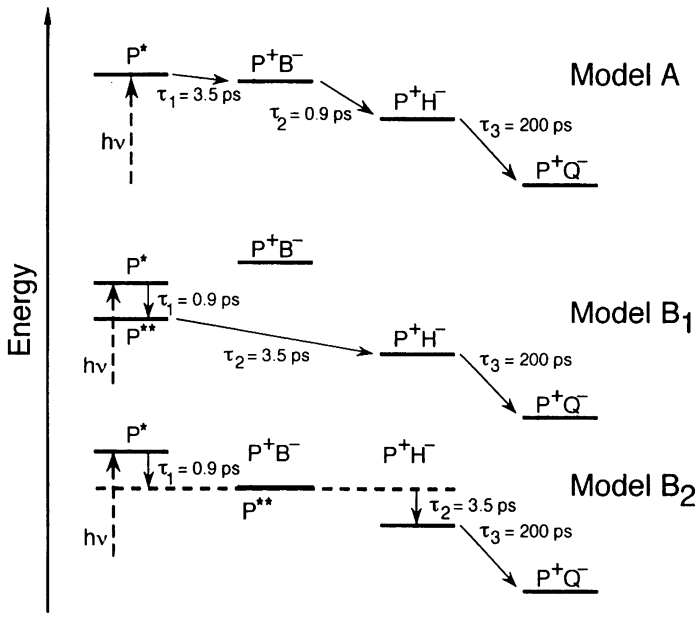


Figure 2. Schematic representation of possible reaction models for the primary photosynthetic ET. The time constants shown in the Figure represent the values for Rb. sphaeroides at room temperature.

Oscillatory features in wild type RC have been observed recently under special experimental conditions [9]: at low temperatures and with a spectrally narrow pump pulse. It is not expected that they are relevant under physiological conditions at room temperature. In our view the stepwise reaction Model A with the radical pair state $P^+B_A^-$ as a real intermediate 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.

## REFERENCES

1. J. Deisenhofer, H. Michel, EMBO J. 8, 2149 (1989).
2. C.H. Chang, O. El-Kabbani, D. Tiede, J. Norris, M. Schiffer, Biochemistry 30, 5352 (1990)
3. J.P. Allen, G. Feher, T.O. Yeates, H. Komiya, D.C. Rees, Proc. Natl. Acad. Sci. USA 84, 5730 (1987)
4. W. Holzapfel, U. Finkele, W. Kaiser, D. Oesterhelt, H.Scheer, H.U. Stilz, W. Zinth, Chem. Phys. Letters 160, 1 (1989).

5. W. Holzapfel, U. Finkele, W. Kaiser, D. Oesterhelt, H.Scheer, H.U. Stilz, W. Zinth, Proc. Natl. Acad. Sci. USA 87, 5168 (1990).
6. C. Kirmaier, D. Holten, Proc. Natl. Sci. USA 87, 3552 (1990)
7. K. Dressler, E. Umlauf, S. Schmidt, P. Hamm, W. Zinth, S. Buchanan, H. Michel, Chem. Phys. Lett. 183, 270 (1991).
8. C.-K. Chan, T.J. DiMagno, L.X.-Q. Chen, J.R. Norris, G.R. Fleming, Proc. Natl. Acad. Sci. USA 88, 11202 (1991)
9. M.H. Vos, J.-C. Lambry, S.J. Robles, D.C. Youvan, J. Breton, J.-L. Martin, Proc. Natl. Acad. Sci. USA 88, 8885-8889 (1991).
10. M.H. Vos, J.-C. Lambry, S.J. Robles, D.C. Youvan, J. Breton, J.-L. Martin, Proc. Natl. Sci USA 89, 613 (1992)
11. M.E. Michel-Beyerle, M. Plato, J. Deisenhofer, H. Michel, M. Bixon, J. Jortner, Biochim. et Biophys. Acta 932, 52 (1988).
12. M.E. Michel-Beyerle, M. Bixon, J. Jortner, Chem. Phys. Lett. 151, 188 (1988).
13. M. Bixon, J. Jortner, M.E. Michel-Beyerle, Biochim. et Biophys. Acta 1056, 301 (1991).
14. A. Struck, H. Scheer, FEBS Lett. 261, 385 (1990)
15. A. Struck, E. Cmiel, I. Katheder, H. Scheer, FEBS Lett. 268, 180 (1990)
16. C. Lauterwasser, U. Finkele, H. Scheer, W. Zinth, Chem. Phys. Letters 183, 471 (1991).
17. N.W. Woodbury, M.Becker, D. Middendorf, W.W. Parson, Biochemistry. 24, 7516 (1985).
18. G.R. Fleming, J.L. Martin, J. Breton, Nature 333, 190 (1988).
19. U. Finkele, C. Lauterwasser, A. Struck, H. Scheer, W. Zinth, Proc. Natl. Acad. Sci. USA (1992), in press.
20. J. Fajer, D.C. Borg, A. Forman, D. Dolphin, R.H. Felton, J. Am Chem. Soc. 95, 2739 (1973).
21. J. Fajer, M.S. Davis, D.C. Brune, L.D. Spaulding, D.C. Borg, A. Forman, Brookhaven Symp. Biol. 28, 74-104 (1976).
22. H. Kuhn, Phys. Rev. A 34, 3409 (1986).
23. T.J. Kosic, R.E.(Jr) Cline, D.D. Dlott, J. Chem. Phys. 81, 4932 (1984).
24. S.G. Johnson, D. Tang, R. Jankowiak, J.M. Hayes, G. J. Small, J. Phys. Chem. 93, 5953 (1989) .

**Index of Authors**