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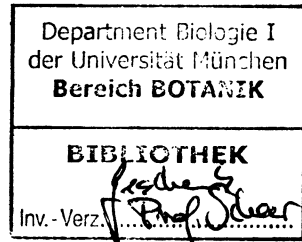
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CONTENTS

CONGRESS LECTURE: Japan as a theatre state

T. Yano

xxiii

PHOTOSYNTHESIS

Molecular organization of the photochemical apparatus of oxygenic photosynthesis

K. Satoh

3

Photosystem I can control photosystem II in leaves

U. Heber, E. Katona, G. Schönknecht and K. Asada

13

Fucoxanthin-chlorophyll *a/c* protein in brown algae: Their molecular assembly and energy transfer

T. Katoh

21

Location and relaxation paths of the carotenoid S1 state and its role in energy transfer

T. Gillbro, P.-O. Andersson, R.S.H. Liu, A.E. Asato and R.J. Cogdell

25

Phycocyanin: A photoreceptor pigment with two faces

K.-H. Zhao, Q. Hong, S. Siebzehrübl and H. Scheer

31

Blue-light regulation of Cab gene expression in the apical buds of peas and the cotyledons of Arabidopsis

L.S. Kaufman, J. Gao, K. Bhattacharya, J. Tilghman, J. Marsh, K.A. Marrs and K.M.F. Warpeha

37

Molecular-genetic analysis of Mg-tetrapyrrole biosynthesis in bacteria and algae

C.E. Bauer, D. Bollivar, J. Dobrowolski and J. Suzuki

41

Phosphoenolpyruvate carboxylase for C4 photosynthesis in maize

K. Izui, S. Yanagisawa, A. Morishima and N. Ogawa

43

PHOTOPHYSICS, PHOTOCHEMISTRY AND PHOTOBIOCHEMISTRY

Photochemistry of nucleic acids

Photooxidation reactions of nucleic acids

J. Cadet, M. Berger, G. Buchko, J.-L. Ravanat and H. Kasai

49

Photoinduced reactions of organic and inorganic substrates with DNA

H. Morrison, W.M. Baird, S. Farrow, T. Mohammad and L. Paredes

55

Photofootprinting of DNA	
<i>P.E. Nielsen</i>	61
Chemical aspects of DNA-protein cross-linking by UV light	
<i>M.D. Shetlar</i>	67
Photochemistry of 5-halouracil-containing DNA	
<i>H. Sugiyama, Y. Tsutsumi, E. Yamaguchi and I. Saito</i>	73
Furocoumarines	
The solution structures of psoralen cross-linked and monoadducted DNA oligomers by NMR spectroscopy and restrained molecular dynamics	
<i>H.P. Spielmann, T.J. Dwyer, J.E. Hearst and D.E. Wemmer</i>	79
C ₄ -cycloaddition reactions between furocoumarins and unsaturated fatty acids or lecithins	
<i>S. Caffieri, Z. Zarebska and F. Dall'Acqua</i>	85
Photochemistry of furocoumarins	
<i>Sang Chul Shim and Mi Hong Yun</i>	91
Psoralen photochemotherapy and its action mechanisms	
<i>K. Danno</i>	97
An animal model and new photosensitizers for extracorporeal photochemotherapy	
<i>H.P. van Iperen and G.M.J. Beijersbergen van Henegouwen</i>	101
Synchrotron-radiation photobiology	
Structural analysis of antigenic variation in <i>Borrelia burgdorferi</i> using ultraviolet synchrotron radiation	
<i>L.L. France, B. McGrath, J.J. Dunn, B.J. Luft, J. Kieleczawa, G. Hind and J.C. Sutherland</i>	107
DNA damage induced by monochromatic vacuum-UV radiation in solids	
<i>K. Hieda</i>	115
Rhodopsins and light receptor molecules	
25 years of bacterial rhodopsins	
<i>W. Stoeckenius</i>	123
Structure changes of bacteriorhodopsin in the L-to-M and M-to-N conversions	
<i>A. Maeda</i>	135

Ring demethylated 6-s locked methano-bacteriorhodopsins <i>M. Groesbeek and J. Lugtenburg</i>	139
Photochemistry and functions of bilirubin <i>A.F. McDonagh</i>	145
Hydrophobic interaction between retinal analogues and aporetinochrome in the pigment formation <i>K. Tsujimoto, K. Iida, M. Miyazaki, T. Kinumi, Y. Shirasaka, M. Ohashi, M. Sheves, R. Hara, T. Hara and K. Ozaki</i>	147
Structure and function of the ciliate photoreceptors <i>Pill-Soon Song</i>	153
Visual pigments and transduction	
Vision in photobiology <i>T. Yoshizawa</i>	159
¹⁹ F-NMR studies of fluorinated visual pigment analogs <i>R.S.H. Liu, L.U. Colmenares and W. Niemczura</i>	171
Excited state dynamics of retinal proteins by FTOA method <i>T. Kakitani, Y. Hatano, Y. Shichida, Y. Imamoto, F. Tokunaga and H. Kakitani</i>	173
Primary processes in rhodopsin and iodopsin <i>Y. Shichida</i>	179
N-terminal heterogeneous acylation of phototransduction proteins <i>J.B. Hurley, T.A. Neubert, A.M. Dizhoor, Ching-Kang Chen, E. Olshevskaya, R.S. Johnson and K.A. Walsh</i>	183
Regulation of signal coupling proteins in octopus photoreceptors <i>M. Tsuda, T. Iwasa, M. Nakagawa, S. Kikkawa and T. Tsuda</i>	189
Roles of lipids linked to α - and γ -subunits of photoreceptor G protein <i>Y. Fukada</i>	195
Role of rhodopsin kinase and arrestin in the quenching of phototransduction <i>K. Palczewski</i>	201
Ultrafast reaction by laser light and spectroscopy	
Recombination dynamics of photodissociated CO of myoglobin and its E7 mutants studied by time-resolved resonance Raman spectroscopy <i>T. Kitagawa, Y. Sakan, T. Ogura, F.A. Fraunfelder, R. Mattera and M. Ikeda-Saito</i>	205
Femtosecond spectroscopy of the primary electron transfer in photosynthetic reaction centers <i>C. Lauterwasser, U. Finkeler, A. Struck, H. Scheer and W. Zinth</i>	209

DNA-protein interaction imagery using high-intensity lasers <i>D. Angelov and I. Pashev</i>	215
Recent solid state NMR studies of bacteriorhodopsin <i>J. Herzfeld, M. Auger, M.R. Farrar, K.V. Lakshmi, A.E. McDermott, J. Raap, L.K. Thompson, C.M. van der Wielen, J. Lugtenburg and R.G. Griffin</i>	221
Femtosecond studies of primary photoprocesses in octopus rhodopsin <i>T. Kobayashi, M. Taiji, K. Bryl, M. Nakagawa and M. Tsuda</i>	227
Structure and dynamics of the charge separated state P^+Q^- of photosynthetic reaction centres from transient EPR spectroscopy <i>D. Stehlik, I. Sieckmann and A. van der Est</i>	233

Bio- and chemi-luminescence

Bioluminescence of the Ca^{2+} -binding photoprotein aequorin <i>F.I. Tsuji, S. Inouye, Y. Ohmiya and M. Ohashi</i>	239
Recent advances of chemiluminescent and bioluminescent enzyme immunoassay <i>A. Tsuji, M. Maeda, H. Arakawa, K. Itoh, M. Kitamura and N. Murakami</i>	243
The role of superoxide anion in bioluminescence <i>O. Shimomura</i>	249

PHOTOBIOLOGY AND PHOTOIMMUNOLOGY

Chronobiology

A circadian pacemaker in the suprachiasmatic nucleus and its interaction with light <i>S.-I.T. Inouye, K. Shinohara, K. Tominaga, Y. Otori, C. Fukuhara and J. Yang</i>	257
Circadian organization in birds: Different pacemakers for feeding and locomotor activity rhythms? <i>S. Ebihara and E. Gwinner</i>	263
Circadian photoreception in reptiles and mammals <i>R.G. Foster, S. Argamaso, W.J. DeGrip, J.M. Garcia-Fernandez and I. Provencio</i>	267

Molecular approaches to plant development

Phytochrome-mediated light signal transduction in plants: Structure function and possible involvement of G-proteins <i>Pill-Soon Song</i>	273
Genetic engineering of phytochrome molecules <i>M. Furuya</i>	279
Control of gene expression by light, nitrate and a plastidic factor <i>H. Mohr, A. Neininger and B. Seith</i>	283
Molecular genetic approaches to plant photomorphogenesis <i>A. Pepper, T. Delaney, P. Nagpal, J. Reed, D. Poole, M. Furuya and J. Chory</i>	285
Cytoskeletal rearrangement during photomorphogenesis of fern protonemata <i>M. Wada</i>	291

DNA damage, repair and UV mutagenesis

Analysis of DNA excision repair genes in XP <i>K. Tanaka</i>	293
DNA repair genes and proteins of yeast and human <i>S. Prakash and L. Prakash</i>	303
Recent biochemical studies of the xeroderma pigmentosum group A complementing protein involved in nucleotide excision repair of DNA <i>C.J. Jones and R.D. Wood</i>	309
Symposium: Human DNA repair diseases <i>J.E. Cleaver, L.H.F. Mullenders, M. Stefanini, A.R. Lehmann and H. Takebe</i>	315
Isolation of <i>Arabidopsis thaliana</i> mutants hypersensitive to UV-B light or ionizing radiation <i>G.R. Harlow, M.E. Jenkins, C. Davies and D.W. Mount</i>	319
SOS-inducible recombination repair genes: Products of the <i>ruvA</i> , <i>ruvB</i> , and <i>ruvC</i> genes are Holliday junction specific enzymes <i>H. Shinagawa, H. Iwasaki, M. Takahagi, T. Shiba and A. Nakata</i>	323
Regulation of DNA repair genes in <i>Saccharomyces cerevisiae</i> <i>G.B. Sancar and J. Sebastian</i>	329
Two types of photoreactivation enzyme identified in <i>Drosophila melanogaster</i> <i>T. Todo, H. Takemori and H. Ryo</i>	333

Biological role of (6-4) photoproducts and cyclobutane dimers <i>D.L. Mitchell, G.P. Pfeifer, J.-S. Taylor, M.Z. Zdzienicka and O. Nikaido</i>	337
Photoreactivation in human skin in situ <i>B.M. Sutherland, H. Hacham, R.W. Gange and J.C. Sutherland</i>	345
Photoreactivation: Perspectives and dimensions <i>B.M. Sutherland and T. Ohnishi</i>	347
<i>Neurospora crassa</i> photolyase: Diversity in photoreactivation action spectra <i>A.P.M. Eker, H. Yajima and A. Yasui</i>	349
Regulation of photorepair in fish cells <i>H. Mitani, S. Yasuhira, T. Funayama, M. Kondo and A. Shima</i>	351
Functional analysis of <i>Escherichia coli</i> DNA photolyase <i>K. Yamamoto, M. Ihara and T. Ohnishi</i>	353
Primary structures of photolyases from microorganisms <i>A. Yasui</i>	355
Understanding spectra of UV-induced mutations: Studies with individual photoproducts <i>P. Gibbs, M. Horsfall, A. Borden, B.J. Kilbey and C.W. Lawrence</i>	357
Molecular analysis of UV and EMS induced mutations in a human tumor cell line <i>M. Meuth and A. Tachibana</i>	363
Two <i>umuDC</i> -like operons in <i>Salmonella typhimurium</i> : Their roles in UV mutagenesis <i>T. Nohmi, M. Yamada, M. Watanabe, M. Matsui, S. Y. Murayama and T. Sofuni</i>	369
Gene specific DNA repair and molecular mutation spectra <i>A.A. van Zeeland, L.F.H. Mullenders, M.Z. Zdzienicka and H. Vrieling</i>	375
Symposium - Historical background of DNA repair <i>C.S. Rupert, R.B. Setlow, K.C. Smith, J.E. Cleaver, S. Kondo and J. Jagger</i>	379
Photoimmunology	
The role of Interleukin-10 in the induction of systemic immunosuppression following UV exposure <i>S.E. Ullrich and J.M. Rivas</i>	389
Effects of ultraviolet radiation on granuloma formation <i>H. Okamoto, K. Danno, Z.-P. Guo and S. Imamura</i>	395

PHOTOMEDICINE

Photocarcinogenesis

- Photocarcinogenesis: Past, present and future
F. Urbach 403
- Sunlight exposure and melanoma - Is timing and character of exposure important?
R.P. Gallagher 415
- Action spectrum for melanoma induction
R.B. Setlow and E. Grist 421

Advances in photodermatology

- Boundary between UVA and UVB
M.J. Peak and J.C. van der Leun 425
- Human skin photoprotection and advances in sunscreens
M.A. Pathak 429
- Changes of elastic fiber according to ageing process in human skin
C.H. Oh 437
- Oxidative stress in cutaneous photoaging
Y. Miyachi 443
- The pathogenesis of solar urticaria
T. Horio 447
- The porphyrias
M. Kondo, Y. Yano, G. Urata and M. Shirataka 449
- Skin photosensitivity reactions in porphyrias
M.A. Pathak 455
- Biological significance of photosensitized lipid peroxidation in drug-induced phototoxicity
I. Matsuo, M. Ohkido, H. Fujita and M. Sasaki 461
- Recent advances in polymorphous light eruption
E. Hölzle 463
- Chronic actinic dermatitis
Y. Funasaka, M. Kondoh, A. Itoh, M. Ueda and M. Ichihashi 469
- Drug-induced photosensitive skin diseases
R. Kamide 475
- Protective effect of $1\alpha,25$ -dihydroxyvitamin D_3 against UVB injury - Possible role of the vitamin D_3 -induced metallothionein
K. Hanada, T. Sugawara, Y. Ohishi and I. Hashimoto 479

Photoprotection: Possible role of metallothionein against UVB injury <i>K. Hanada</i>	483
Topical PUVA for psoriasis <i>S. Kawara and T. Hirone</i>	487
Photosensitizers and photodynamic therapy	
Photo-chlorin (ATX-S10) as a new photosensitizer for PDT <i>S. Nakajima, I. Sakata, T. Takemura and H. Hayashi</i>	493
Detection of bronchial dysplasia and carcinoma <i>in-situ</i> using laser induced fluorescence <i>S. Lam, C. MacAulay, J.C. Leriche, J. Hung and B. Palcic</i>	497
Optimizing the photodetection of early cancer <i>G. Wagnières, D. Braichotte, Ph. Monnier, R. Bays, J.-M. Calmes, J.-C. Givel, S. Folli, A. Pèlegrin, J.-P. Mach and H. van den Bergh</i>	499
Modes of biodistribution of photosensitizing agents <i>D. Kessel</i>	501
Mechanism of photodynamic therapy: Exploration by photophysicochemical study <i>T. Takemura, S. Nakajima and I. Sakata</i>	503
Integral laser-photodynamic treatment of refractory multifocal bladder tumors with special reference to carcinoma <i>in situ</i> <i>K. Naito and H. Hisazumi</i>	507
Photodynamic therapy and early response gene induction <i>C.J. Gomer, M. Luna, S. Wong, P. Ziolkowski and A. Ferrario</i>	511

SOLAR UV LIGHT AND ENVIRONMENT

Environment and solar UV light

UV-B observation network in the Japan Meteorological Agency <i>T. Ito</i>	515
UV-exposure and ozone monitoring with a dual bandpass solar UVA-UVB meter in Stockholm since 1989 <i>U. Wester</i>	519
UV radiation in the tropics (1979-1989) <i>M. Ilyas</i>	523
UV solar spectral irradiance in New Zealand <i>R.L. McKenzie</i>	527

Biological and medical consequences of ozone depletion

Biological consequences of stratospheric ozone depletion <i>T.P. Coohill</i>	531
Immunological effects of UV-B radiation <i>M.L. Kripke and A. Jeevan</i>	537
Effects of elevated ultraviolet-B-radiation, temperature and CO ₂ on growth and function of sunflower and corn seedlings <i>M. Tevini and U. Mark</i>	541
UV-B effects on phytoplankton <i>D.-P. Häder</i>	547
Effects of solar UV light on plants	
Harmful and beneficial effects of solar UV light on plant growth <i>T. Hashimoto, N. Kondo and T. Tezuka</i>	551
UV-induced events at plant plasma membranes <i>T.M. Murphy, Y.C. Qian, C.K. Auh and C. Verhoeven</i>	555
Solar UV and oxidative stress in algal-animal symbioses <i>J.M. Shick</i>	561
Index of authors	565

Femtosecond Spectroscopy of the Primary Electron Transfer in Photosynthetic Reaction Centers

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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]-13²-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].

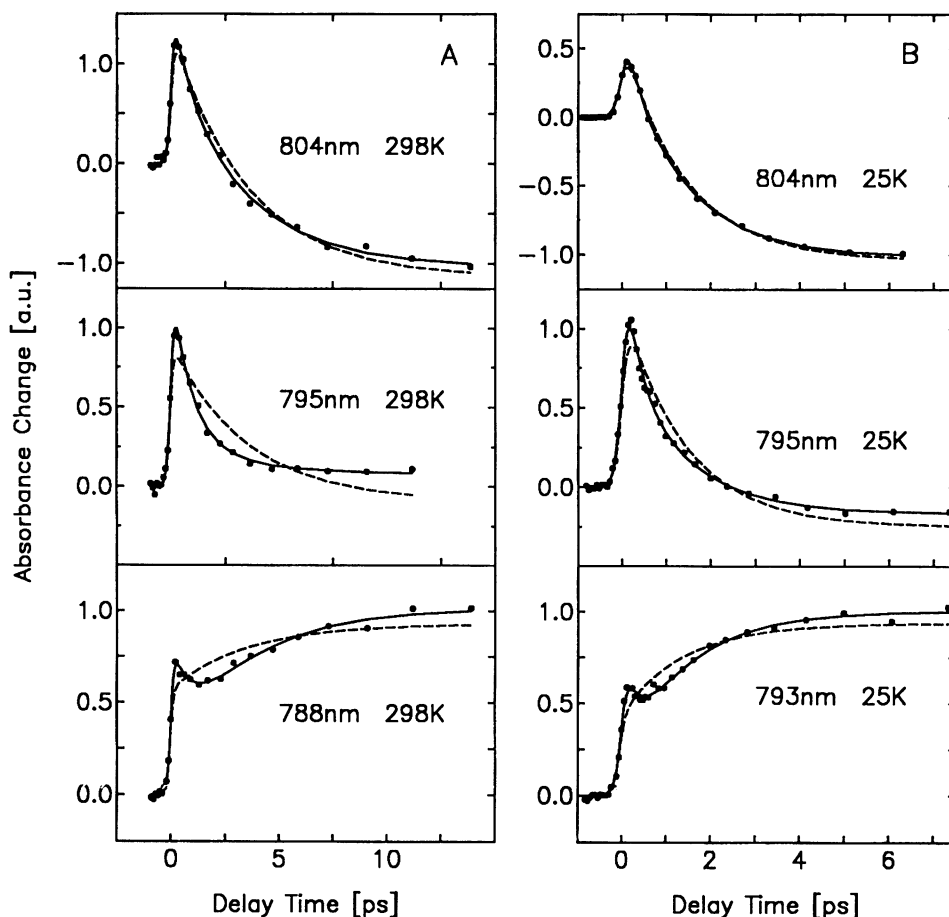


Fig. 1. Transient absorption data for RCs from *Rb. sphaeroides* recorded in the Q_y absorption band of the monomeric bacteriochlorophylls. A: room temperature data, B: data at 25 K. The solid curves are calculated for a three component (3.5 ps, 0.9 ps, ∞ at 298 K; 1.4 ps, 0.3 ps, ∞ at 25 K), the broken 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 B₁ and B₂ 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 B₁ and B₂ 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 B₁). 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 B₂, which is related to considerations given by H. Kuhn [22]. Model B₂ 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 B₂.

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 B₁ and B₂ requires a subtle consideration of the experimental observations: In the pure superexchange picture of Model B₁ 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 B₁. 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 B₁ 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 B₂. 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 B₂ is not operative at room temperature either.

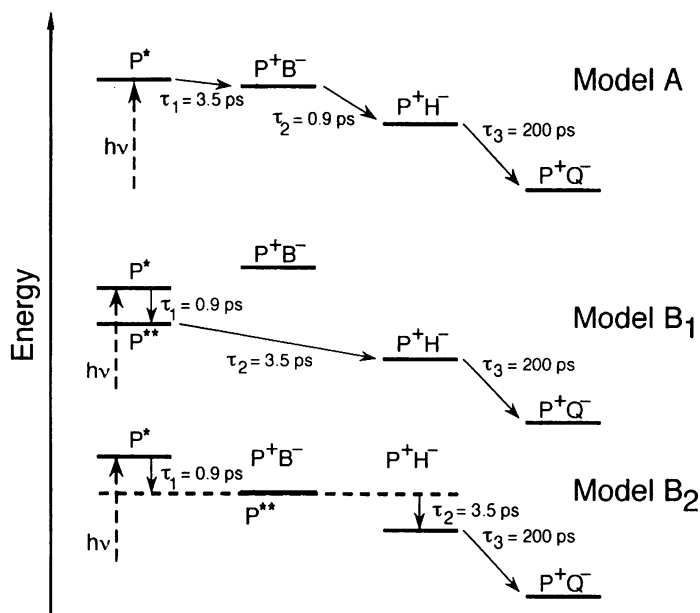


Figure 2. Schematic representation of possible reaction models for the primary photosynthetic ET. The time constants shown in the Figure represent the values for *Rb. sphaeroides* at room temperature.

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.

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Index of Authors

- Andersson, P.-O., 25
 Angelov, D., 215
 Arakawa, H., 243
 Argamaso, S., 267
 Asada, K., 13
 Asato, A.E., 25
 Auger, M., 221
 Auh, C.K., 555

 Baird, W.M., 55
 Bauer, C.E., 41
 Bays, R., 499
 Beijersbergen van Henegouwen, G.M.J.,
 101
 Berger, M., 49
 Bhattacharya, K., 37
 Bollivar, D., 41
 Borden, A., 357
 Braichotte, D., 499
 Bryl, K., 227
 Buchko, G., 49

 Cadet, J., 49
 Caffieri, S., 85
 Calmes, J.-M., 499
 Ching-Kang Chen, 183
 Chory, J., 285
 Cleaver, J.E., 315, 379
 Cogdell, R.J., 25
 Colmenares, L.U., 171
 Coohill, T.P., 531

 Dall'Acqua, F., 85
 Danno, K., 97, 395
 Davies, C., 319
 DeGrip, W.J., 267
 Delaney, T., 285
 Dizhoor, A.M., 183
 Dobrowolski, J., 41
 Dunn, J.J., 107
 Dwyer, T.J., 79

 Ebihara, S., 263
 Eker, A.P.M., 349

 Farrar, M.R., 221
 Farrow, S., 55
 Ferrario, A., 511
 Finkele, U., 209
 Folli, S., 499
 Foster, R.G., 267
 France, L.L., 107
 Fraunfelter, F.A., 205
 Fujita, H., 461
 Fukada, Y., 195
 Fukuhara, C., 257
 Funasaka, Y., 469
 Funayama, T., 351
 Furuya, M., 279, 285

 Gallagher, R.P., 415
 Gange, R.W., 345
 Gao, J., 37
 Garcia-Fernandez, J.M., 267
 Gibbs, P., 357
 Gillbro, T., 25
 Givel, J.-C., 499
 Gomer, C.J., 511
 Griffin, R.G., 221
 Grist, E., 421
 Groesbeek, M., 139
 Guo, Z.-P., 395
 Gwinner, E., 263

 Hacham, H., 345
 Häder, D.-P., 547
 Hanada, K., 479, 483
 Hara, R., 147
 Hara, T., 147
 Harlow, G.R., 319
 Hashimoto, I., 479
 Hashimoto, T., 551
 Hatano, Y., 173
 Hayashi, H., 493
 Hearst, J.E., 79
 Heber, U., 13
 Herzfeld, J., 221
 Hieda, K., 115
 Hind, G., 107
 Hirone, T., 487

- Hisazumi, H., 507
Hölzle, E., 463
Hong, Q., 31
Horio, T., 447
Horsfall, M., 357
Hung, J., 497
Hurley, J.B., 183
- Ichihashi, M., 469
Ihara, M., 353
Iida, K., 147
Ikeda-Saito, M., 205
Ilyas, M., 523
Imamoto, Y., 173
Imamura, S., 395
Inouye, S., 239
Inouye, S.-I.T., 257
Ito, T., 515
Itoh, A., 469
Itoh, K., 243
Iwasa, T., 189
Iwasaki, H., 323
Izui, K., 43
- Jagger, J., 379
Jeevan, A., 537
Jenkins, M.E., 319
Johnson, R.S., 183
Jones, C.J., 309
- Kakitani, H., 173
Kakitani, T., 173
Kamide, R., 475
Kasai, H., 49
Kato, T., 21
Katona, E., 13
Kaufman, L.S., 37
Kawara, S., 487
Kessel, D., 501
Kieleczawa, J., 107
Kikkawa, S., 189
Kilbey, B.J., 357
Kinumi, T., 147
Kitagawa, T., 205
Kitamura, M., 243
Kobayashi, T., 227
Kondo, M., 351, 449
Kondo, N., 551
Kondo, S., 379
- Kondoh, M., 469
Kripke, M.L., 537
- Lakshmi, K.V., 221
Lam, S., 497
Lauterwasser, C., 209
Lawrence, C.W., 357
Lehmann, A.R., 315
Leriche, J.C., 497
Liu, R.S.H., 25, 171
Luft, B.J., 107
Lugtenburg, J., 139, 221
Luna, M., 511
- MacAulay, C., 497
Mach, J.-P., 499
Maeda, A., 135
Maeda, M., 243
Mark, U., 541
Marrs, K.A., 37
Marsh, J., 37
Matsui, M., 369
Matsuo, I., 461
Mattera, R., 205
McDermott, A.E., 221
McDonagh, A.F., 145
McGrath, B., 107
McKenzie, R.L., 527
Meuth, M., 363
Mi Hong Yun, 91
Mitani, H., 351
Mitchell, D.L., 337
Miyachi, Y., 443
Miyazaki, M., 147
Mohammad, T., 55
Mohr, H., 283
Monnier, Ph., 499
Morishima, A., 43
Morrison, H., 55
Mount, D.W., 319
Mullenders, L.F.H., 315, 375
Murakami, N., 243
Murayama, S.Y., 369
Murphy, T.M., 555
- Nagpal, P., 285
Naito, K., 507
Nakagawa, M., 189, 227
Nakajima, S., 493, 503

- Nakata, A., 323
 Neininger, A., 283
 Neubert, T.A., 183
 Nielsen, P.E., 61
 Niemczura, W., 171
 Nikaido, O., 337
 Nohmi, T., 369
- Ogawa, N., 43
 Ogura, T., 205
 Oh, C.H., 437
 Ohashi, M., 147, 239
 Ohishi, Y., 479
 Ohkido, M., 461
 Ohmiya, Y., 239
 Ohnishi, T., 347, 353
 Okamoto, H., 395
 Olshevskaya, E., 183
 Otori, Y., 257
 Ozaki, K., 147
- Palcic, B., 497
 Palczewski, K., 201
 Paredes, L., 55
 Pashev, I., 215
 Pathak, M.A., 429, 455
 Peak, M.J., 425
 Pèlerin, A., 499
 Pepper, A., 285
 Pfeifer, G.P., 337
 Pill-Soon Song, 153, 273
 Poole, D., 285
 Prakash, L., 303
 Prakash, S., 303
 Provencio, I., 267
- Qian, Y.C., 555
- Raap, J., 221
 Ravanat, J.-L., 49
 Reed, J., 285
 Rivas, J.M., 389
 Rupert, C.S., 379
 Ryo, H., 333
- Saito, I., 73
 Sakan, Y., 205
 Sakata, I., 493, 503
 Sancar, G.B., 329
- Sang Chul Shim, 91
 Sasaki, M., 461
 Satoh, K., 3
 Scheer, H., 31, 209
 Schönknecht, G., 13
 Sebastian, J., 329
 Seith, B., 283
 Setlow, R.B., 379, 421
 Shetlar, M.D., 67
 Sheves, M., 147
 Shiba, T., 323
 Shichida, Y., 173, 179
 Shick, J.M., 561
 Shima, A., 351
 Shimomura, O., 249
 Shinagawa, H., 323
 Shinohara, K., 257
 Shirasaka, Y., 147
 Shirataka, M., 449
 Siebzehnriibl, S., 31
 Sieckmann, I., 233
 Smith, K.C., 379
 Sofuni, T., 369
 Spielmann, H.P., 79
 Stefanini, M., 315
 Stehlik, D., 233
 Stoeckenhuis, W., 123
 Struck, A., 209
 Sugawara, T., 479
 Sugiyama, H., 73
 Sutherland, B.M., 345, 347
 Sutherland, J.C., 107, 345
 Suzuki, J., 41
- Tachibana, A., 363
 Taiji, M., 227
 Takahagi, M., 323
 Takebe, H., 315
 Takemori, H., 333
 Takemura, T., 493, 503
 Tanaka, K., 293
 Taylor, J.-S., 337
 Tevini, M., 541
 Tezuka, T., 551
 Thompson, L.K., 221
 Tilghman, J., 37
 Todo, T., 333
 Tokunaga, F., 173
 Tominaga, K., 257

Tsuda, M., 189, 227
Tsuda, T., 189
Tsuji, A., 243
Tsuji, F.I., 239
Tsujimoto, K., 147
Tsutsumi, Y., 73

Ueda, M., 469
Ullrich, S.E., 389
Urata, G., 449
Urbach, F., 403

Van den Bergh, H., 499
Van der Est, A., 233
Van der Leun, J.C., 425
Van der Wielen, C.M., 221
Van Iperen, H.P., 101
Van Zeeland, A.A., 375
Verhoeven, C., 555
Vrieling, H., 375

Wada, M., 291
Wagnières, G., 499
Walsh, K.A., 183
Warpeha, K.M.F., 37
Watanabe, M., 369
Wemmer, D.E., 79
Wester, U., 519
Wong, S., 511
Wood, R.D., 309

Yajima, H., 349
Yamada, M., 369
Yamaguchi, E., 73
Yamamoto, K., 353
Yanagisawa, S., 43
Yang, J., 257
Yano, Y., 449
Yasuhira, S., 351
Yasui, A., 349, 355
Yoshizawa, T., 159

Zarebska, Z., 85
Zdzienicka, M.Z., 337, 375
Zhao, K. -H., 31
Zinth, W., 209
Ziolkowski, P., 511