

SPECTROSCOPY OF BIOLOGICAL MOLECULES NEW ADVANCES

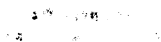
Proceedings of the
Second European Conference on the
Spectroscopy of Biological Molecules
Freiburg, West Germany, 1987

Editors

E. D. Schmid, F. W. Schneider, F. Siebert

John Wiley & Sons

Chichester · New York · Brisbane · Toronto · Singapore



CONTENTS

<u>General Survey</u>	1
Current Highlights in Spectroscopic Studies of Biological Systems	3
R.E. Hester and J.C. Austin	
How Vibrational Spectroscopists can acquire some of Biotechnology's Billions	11
P.R. Carey	
<u>Theoretical Methods</u>	17
Determination of Polypeptide Chain Conformation by Normal mode analysis of vibrational spectra	19
S. Krimm	
Attempting to simulate water as a liquid and as a solvent on supercomputers	25
E. Clementi	
Side Chain rotational isomerism of protein in different viscous solutions - A theoretical study	39
I. Ghosh	
Magnetic field effects in CARS of some biological molecules	43
R. Brakel, H. Spiegel and F.W. Schneider	
<u>Protein Structure and Enzyme Mechanism</u>	49
Determination of the secondary structure of proteins: from X-Ray crystallography to Raman spectroscopy	51
A.J.P. Alix, M. Berjot, J. Marx and G. Pedanou	
Infrared vibrational circular dichroism	57
M. Diem	
Raman optical activity	63
L.D. Barron	

Secondary structure analysis of Tubulin and Microtubule Protein using Raman spectroscopy	69
R. Audenaert, L. Heremans, K. Heremans and Y Engelborghs	
Vibrational circular dichroism of Biopolymers	73
T.A. Keiderling, S.C. Yasui, U. Narayanan, A. Annamalai, P. Malon, R. Kobrinskaya and L. Yang	
Infrared vibrational circular dichroism of small peptide models	77
G-M. L. Roberts and M. Diem	
Raman spectroscopic study of conformational changes in chymotrypsin induced by pH and pressure	81
L. Heremans and K. Heremans	
Conformation of oxytocin in solution determined by 2D NMR and distance geometry algorithm - Structural refinement with stereospecific constraints	85
Y. Kuroda, S. Endo, A. Wada and K. Nagayama	
Raman spectrographic studies and normal coordinate analyses of biologically active acylcholines	89
P. Derreumaux, K.J. Wilson, G. Vergoten and W.L. Peticolas	
Vibrational spectroscopy of atomic interactions in enzyme-substrate intermediates	95
C.W. Wharton, R.S. Chittock, J. Austin and R.E. Hester	
Ultraviolet resonance Raman study of calcium-modulated proteins: Troponin C and Calmodulin	101
P. Hildebrandt, R.A. Copeland, J.R. Perno, T.G. Spiro, and F.G. Prendergast	
Spectroscopic and properties of Vu-9 Calmodulin, an engineered Calmodulin	105
M.C. Kilhoffer, D.M. Watterson, D. Haiech and J. Haiech	
Conformational changes in Calmodulin upon calcium binding: a study by time-resolved fluorescence	109
M. Chabbert, M.C. Kilhoffer, J. Haiech and H. Lami	

- Evidence for two types of cadmium-binding sites in Calmodulin by perturbed angular correlation spectroscopy 113
J.N. Rimbart, F. Adnet, F. Dumas, C. Wolf and G. Bereziat
- How Resonance Raman spectroscopy contributes to the great 'Enzyme-induced Red Shift' debate 117
P.J. Tonge and P.R. Carey
- Structure and Mechanistic studies of glyceraldehyde-3-phosphate dehydrogenase by Raman spectroscopy 121
J.C. Austin, R.S Chittock, R.E. Hester and C.W. Wharton
- A study of triose phosphate isomerase by FT-IR 125
J. Castresana, A. Muga, F.M. Goni and J.L.R. Arrondo
- Determination of Hemoglobin derivatives after freeze-drying of carbonmonoxyhemoglobin by multicomponent analysis of molecular absorption spectra 129
B. Chaillot, D. Larcher, C. Thirion, P. Labrude and C. Vigneron
- Contribution of circular dichroism to the research of Hemoglobin structural changes after spray drying 133
C.Thirion, P. Labrude, M. Rasolomanana and C. Vigneron
- Temperature dependence of soret band of oxy- and carbonmonoxy-myoglobin in the range 300-20 K. 137
A. Cupane, M. Leone, E. Vitrano and L. Cordone
- Functional activity of haemoglobins adsorbed at a colloidal silver surface 141
J. de Groot, R.E. Hester, T. Kitagawa and S. Kaminika
- Resonance Raman spectra of CCP expressed in Escherichia Coli and its ASN-235 mutant protein 145
G. Smulevich, J.M. Mauro, J. Kraut, A.M. English and T.G. Spiro

<u>Biomembranes</u>	149
Vibrational spectroscopic studies of membranes at high pressures	151
P.T.T. Wong, D.J. Siminovitch and H.H. Mantsch	
FT-IR spectroscopic investigations of C-labelled phospholipids	157
A. Blume and W. Hübner	
FT-IR- and ² H-NMR- spectroscopic investigations of ² H-labelled phospholipids with ω-cyclohexyl fatty acids	161
W. Hübner and A. Blume	
Raman spectroscopic and calorimetric studies of lipid-chromatin component interactions in model membranes	165
A. Bertoluzza, S. Bonora, G. Fini and M.A. Morelli	
Polarized Raman spectra of Langmuir-Blodgett ultra-thin films: conformational analysis	169
M. Harrand and M. Masson	
Molecular interactions and thermotropic behaviour of Grisorixin/dipalmitoylphosphatidylcholine/D ₂ O multibilayers. An infrared study.	173
A. Mellier	
Conformational aspects of brain proteolipid apoprotein studied by infrared spectroscopy	177
M. Cózar, P. Carmona and J. Monreal	
Vibrational spectroscopic study of the DMPC-α-lacatalbumin complex	181
J.P. Lafaut and H. Van Dael	
Raman conformational analysis of human platelet carotenes in different physiological states	185
J. Marx, M.A. Boisseau, M. Berjot, A.J.P. Alix, G. Potron and M. Harrand	

<u>Chromophore containing Systems</u>	189
On the protonation of Schiff bases	191
S. Badilescu, L.S. Lussier, C. Sandorfy, H. Le Thanh and D. Vocelle	
On the potential surface regulating the motions of a proton in a $N^+ \cdots H \cdots O$ bond: relation to visual pigments.	199
H. Le Thanh and D. Vocelle	
Tetrafluoroborates NH^+ and ND^+ of N-2-methyl-propylidene 2-propanamine assignment of the ν, δ and γ modes of the 'free' NH^+ and ND^+ groups. Solvent effect on iminium tetrafluoroborate and chloride.	205
A. Goypiron, D. Baron, M.H. Baron, J. Belloc, M.J. Coulange, J. Favrot and H. Zine	
Photochemical cycle of bacteriorhodopsin studied by Resonance Raman spectroscopy	209
R. Diller and M. Stockburger	
Determination of retinal chromophore structure in rhodopsins with resonance Raman spectroscopy	215
R.A. Mathies, S.P.A. Fodor, S.O. Smith, E.M.M. van den Berg, R. Gebhard and J. Lugtenburg	
The Photochemistry of rhodopsin investigated by FTIR spectroscopy	225
U. Ganter and F. Siebert	
Slow intermediates in the photocycle of bacteriorhodopsin	233
R. Uhl	
Investigation of the blue membrane photocycle by FTIR- difference spectroscopy	239
K. Fahmy and F. Siebert	
Functionally relevant amino acids in bacteriorhodopsin	243
M. Engelhard, B. Hess, D. Emeis, K. Gerwert and F. Siebert	
Photochemistry of retinal analogues in bacteriorhodopsin	247
W. Gärtner	

Kinetic resonance Raman studies of the bacteriorhodopsin retinal chromophore as a function of pH	253
P. Dupuis and R. Fournier	
Light induced polarized Fourier Transform infrared spectroscopy of bacteriorhodopsin - A study of the M_{412} intermediate by photoselection	257
J. Breton and E. Navedryk	
Investigation of the M to BR back reaction in bacteriorhodopsin by time resolved FTIR difference spectroscopy	261
K. Gerwert and B. Hess	
Molecular dynamics of the primary photochemical event in bacteriorhodopsin. Theoretical evidence for an excited singlet state assignment for the J intermediate	265
R.R. Birge, L.A. Findsen and B.M. Pierce	
The nature of the protein binding site of rhodopsin based on two-photon spectroscopy and molecular orbital theory	267
R.R. Birge, L.P. Murray, L.A. Findsen and B.M. Pierce	
The primary steps of photosynthesis in bacteriorhodopsin	269
W. Zinth, J. Dobier, M.A. Franz and W. Kaiser	
Kinetic analysis of the interaction between rhodopsin and G-protein on rod disc-membranes	275
K.P. Hofmann	
Resonance Raman spectra of hexatriene model polyenes in ground and excited triplet states	279
R. Wilbrandt, F.W. Langkilde, A.M. Brouwer and H.J.C. Jacobs	
Protein-prosthetic group interactions in photosynthetic complexes: resonance Raman spectroscopy	285
M. Lutz, B. Robert, Z. Qing, J.M. Neumann, W. Szponarski, G. Berger and G. van Brakel	
Resonance Raman and fluorescence studies on the energy transfer between carotenoids and bacteriochlorophylls in the light-harvesting pigment-protein complexes	291
T. Noguchi, H. Hayashi and M. Tasumi	

FTIR spectroscopy of primary photosynthetic reactions in the two photosystems of green plants. Comparison with spectroelectrochemistry of chlorophyll models	297
B.A. Tavitian, E. Nabedryk, A. Wollenweber, W. Mänteles and J. Breton	
FTIR spectroscopy on crystals of photosynthetic reaction centers from <i>rhodospseudomonas viridis</i> using an IR microscope	301
K. Gerwert, S. Buchanan, B. Hess and H. Michel	
Infrared spectroscopy of the photosystem II - water splitting complex	305
R. Hienerwadel, W. Kreutz and W. Mänteles	
Spectroscopic studies of crystallized pigment-protein complexes from purple photosynthetic bacteria	309
T. Wacker, K. Steck, A. Becker, W. Kreutz, W. Mänteles W. Welte	
Purification and spectroscopic characterization on photosynthetic membrane proteins	313
M.E. Schafheutle, W. Welte, W. Mänteles and W. Kreutz	
Infrared spectroelectrochemistry of chlorophylls: models for their interaction in vivo	317
W. Mänteles, A. Wollenweber, E. Nabedryk and J. Breton	
Spectroelectrochemistry of cytochrome c	323
D.A. Moss and W. Mänteles	
Chromophore modifications in c-phycoyanin from <i>mastigocladus laminosus</i>	327
G. Schmidt, S. Siebzehnrubl, R. Fischer and H. Scheer	
Resonance CARS and time-resolved fluorescence studies of native and chemically modified phycocyanin trimers	331
S. Schneider, F. Baumann, P. Geiselhart, S. Siebzehnrubl, R. Fischer and H. Scheer	
A spectroscopic study of the active site of an enzyme: cytochrome P-450	335
P. Anzenbacher, J. Hudeček, V. Fidler and V. Baumruk	

Redox reactions of cytochrome c on the AG electrode probed by surface enhanced resonance Raman spectroscopy	339
P. Hildebrandt and M. Stockburger	
The alkaline isomerization of cytochrome c observed with surface enhanced resonance Raman spectroscopy	343
F. Vanhecke and K. Heremans	
Identification and dynamics of active site Fe(IV)=O groups in heme enzyme activated intermediates observed by resonance Raman spectroscopy	347
J. Turner, A.J. Sitter, C.M. Reczek and J.R. Shifflet	
Resonance Raman investigations of flavins and flavo-proteins	351
C.R. Lively, W. G. Gustafson and J.T. McFarland	
A surface enhanced resonance Raman study of avidin-dye interactions	355
T.M. Cotton and F. Ni	
<u>Nucleic Acids</u>	359
Z form of poly d(A-T) in solution	361
E. Taillandier, P. Bourtayre, M. Ghomi, J-P. Ridoux and J. Liquier	
Raman, fluorescence and NMR studies on the Aclacinomycin-DNA complex ---- A use of a new rotating cell	367
M. Tsuboi	
Spectroscopic and thermodynamic investigations of order/order transitions in helical polynucleotides	373
H. Klump, T. Jovin, M. Wosgien and E.D. Schmid	
Viral genome structures determined from Raman spectra	383
G.J. Thomas, Jr.	
Nucleic acids and DNA-drug interactions in resonance Raman spectroscopy	389
P.Y. Turpin, L. Chinsky, A. Laigle, M. Tsuboi, K. Nakamoto and J.H. Schneider	

SERS of derivatives of adenine	395
C. Otto, F.F.M. de Mul, A. Huizinga and J. Greve	
UV Photoelectron studies of 5'-dCMP: electronic influences on DNA alkylation patterns	399
P.R. LeBreton and S. Urano	
Spectroscopic investigation of the structure of complexes between nucleotide bases and amino acid carboxylic groups: nucleic acid-protein recognition aspect	403
N.V. Zheltovsky, S.A. Samoilenko, I.N. Kolomeits, M.I. Gubaidullin and I.V. Kondratyuk	
Molecular interaction between polyamines (putrescine) and nucleic acid constituents (dGMPH ₂) by vibrational spectroscopy	407
A. Bertoluzza, C. Fagnano, P. Filippetti, M.A. Morelli, A. Tinti and M.R. Tosi	
Raman and NMR spectroscopies of interactions of carcinogenic and anticarcinogenic substances with nucleic acids constituents	411
A. Bertoluzza, C. Fagnano, M.R. Tosi, V. Tugnoli, M.A. Morelli and G. Barbarella	
Fluorescence studies of reversible interactions between benzo[a]pyrene metabolites and DNA: relationship of physical binding to epoxide reactivity, and physical binding to viral single-stranded M13 data	415
P.R. LeBreton and H.L. Price	
Electronic and vibrational transitions of some anthra-cyclines and their complexes with DNA	419
G. Smulevich, A. Feis and M.P. Marzocchi	
Sequence-dependent luminescence from ApC and CpA. Resolution by emission anisotropy.	423
C.S. Shaar, J.P. Morgan and M. Daniels	
Comparison of the reactions of OH and SO ₄ radicals with pyrimidine nucleosides and nucleotides. ESR studies in aqueous solution.	427
K. Hildenbrand and D. Schulte-Frohlinde	

Medical and Pharmaceutical Applications and in-vivo studies

- Metal binding and conformational changes in nucleic acids 433
T. Theophanides and J. Anastassopoulou
- Raman spectroscopy in biomedical field 439
A. Bertoluzza
- New perspectives of the microspectrofluormetry on living cells 445
M. Manfait, M. Gigli, J.-M. Millot, T. Rasoanaivo, C. Perchard, S. Nocentini, P.-Y. Turpin, P. Vigny and S. Doglia
- Vibrational spectroscopy of an oxidised iron-porphyrin 451
A. Bertoluzza, G. Bottura and M.A. Pavesi
- An UV microspectrophotofluorometric study of the uptake and the photoreactions of furocoumarins in human culture cells 455
C. Amirand-Perchard, S. Nocentini, P. Vigny, J.F. Angiboust and M. Manfait
- Improvements in the detection of very weak fluorescences from biological molecules by the use of microspectrofluorometry 459
C. Amirand-Perchard, P. Vigny, A. Moysan, J.-P. Ballini, J.-F. Angiboust and M. Manfait
- Cellular uptake and metabolization of polycyclic aromatic hydrocarbons: A microspectrofluorometric study on single living cells 463
F. Sureau, M. Duquesne, P.Y. Turpin, L. Chinsky, G. Zuppiroli, S. Nocentini, M. Manfait and J.F. Angiboust
- Quantitative microspectrofluorometry of doxorubicin in living cell nuclei 467
M. Gigli, S.M. Doglia, J.-M. Millot and M. Manfait
- Raman spectroscopy of a new soft hydrophilic intraocular lens (IOL) 471
A. Bertoluzza, R. Caramazza, C. Fagnano, P. Monti and R. Simoni

Multichannel Raman detection of 'in-vivo' rabbit lens	475
A. Bertoluzza, C. Fagnano, P. Monti, R. Caramazza, E. Barbaresi and S. Mancini	
Raman spectroscopic study of the human and rabbit eye lens: determination of relative water content with high spatial resolution	479
A. Huizinga, A.A.C. Bot, F.F.M. de Mul, G.F.J.M. Vrensen and J. Greve	
In situ investigation of the amplification mechanism in retinal rods	483
M. Kahlert and K.P. Hofmann	
Structural changes on pathological and healthy articular human cartilage revealed by FT-IR spectroscopy	487
S. Lefebvre, J.P. Eschard, C. Guillaumie, J.C. Etienne and M. Manfait	
Mossbauer and X-Ray absorption spectroscopy studies of the iron storage proteins in iron overloaded livers	491
J.N. Rimbart, F. Dumas, G. Richardot, S. Pin and R. Cortés	
Ultraviolet-excited resonance Raman spectroscopy: A potential means for the rapid identification of bacteria	495
K.A. Britton, R.A. Dalterio, M. Baek, W.H. Nelson, D. Britt and J.F. Sperry	
Author Index	499
Subject Index	503

THE PRIMARY STEPS OF PHOTOSYNTHESIS IN BACTERIORHODOPSIN

W. Zinth, J. Dobler, M.A. Franz, W. Kaiser

Physik Department Ell, Technische Universität München,
München, Germany

During the evolution of biological species on earth nature developed very elaborated systems, which convert the energy of sun light into chemical energy. Ideal systems for the study of photosynthesis are found in some bacteria, where the photosynthetic units can be separated from the rest of the bacterium and prepared as pure samples. Single, functionally active proteins are obtained, which allow to study the primary steps of photosynthesis by spectroscopic techniques.

In this paper we concentrate on bacteriorhodopsin, a special retinal containing photosynthetic system used in halobacterium halobium. Bacteriorhodopsin (BR) acts as a light-driven proton pump generating a proton gradient across the cell membrane, which drives important physiological processes of the bacterium, e.g. the ATP synthesis. Here we study the primary photochemical reactions of bacteriorhodopsin, where the light energy is initially stored. These primary processes proceed on a very rapid time scale, and picosecond and femtosecond optical techniques are required.

Bacteriorhodopsin has a number of interesting properties/1/: it is a trans-membrane protein with known amino acid sequence. It uses the dye retinal as a functional pigment in the course of its photoreaction. The retinal molecule is bound via a protonated Schiff's base to lysine 217 of the amino acid sequence. In bacteriorhodopsin the retinal can be chemically removed and replaced by the same or a chemically modified retinal. BR samples kept in the dark contain retinal molecules to 50% in the all-trans and to 50% in the 13-cis,15-syn configuration /2/. Only the all-trans retinal containing BR is able to pump protons, while the other BR molecules must be converted by light to the pump-active all-trans form. After strong illumination by light the BR sample contains 100% all-trans retinals (light-adapted bacteriorhodopsin BR_{1a}) /3/.

Light-adapted bacteriorhodopsin:

In a number of publications the primary processes in light-adapted bacteriorhodopsin have been studied /4-6/. Here we give a brief summary of the results: The absorption of a photon by the retinal molecule brings the molecule to the potential surface of the excited electronic state (BR_{S1}) A first photochemical movement along

the reaction coordinates takes place. After the very short time of 430 fs the system returns to the ground-state potential surface and generates product state J, which exhibits a red-shifted absorption spectrum. With a slower time constant of approximately 3 ps /7/ the system again changes the spectral properties going to the product state K, which is stable for at least 1000 ps. During these steps the peak of the absorption spectrum of BR changes from 568 nm (ground-state BR) via 600 nm (J) to 590 nm (K) /6/. Experiments on BR preparations containing modified retinal molecules suggest that the very rapid primary reaction, which leads to the formation of the intermediate J, is an all-trans to 13-cis (presumably to 13-cis,14s-cis) isomerisation /8,9,14/. The changes of the absorption spectrum may be understood by movements of the protonated Schiff's base, away from a counter-ion during the isomerisation process /9/.

Bacteriorhodopsin containing 13-cis retinals:

Dark-adapted BR contains 50% 13-cis,15-syn retinals. It has been shown that upon excitation the cis retinal forms a red-shifted intermediate, which decays with 35 ms (cis cycle) /3/. Very recently, the formation of the red-shifted intermediate of dark-adapted BR was studied by Petrich et al. /10/, who found the same time constants for both light- and dark-adapted samples. Here we point to the differences between the cis and trans cycles by experiments on dark-adapted BR and BR containing a modified retinal. In the experiments on dark-adapted BR two reactions of the all-trans and 13-cis containing BR samples proceed in parallel and kinetics of the two species are superimposed. As a consequence the analysis of the experimental data is difficult. Nevertheless, interesting results are obtained at certain probing wavelengths. In order to obtain more convincing experimental data we studied a BR sample with increased 13-cis retinal concentration by femtosecond excite and probe measurements. The higher 13-cis concentration is obtained when the retinal molecule of native BR is replaced by a 13-demethyl retinal /11/. These samples (BR_{13-dmr}) contain 85% 13-cis retinals. We assume that the 13-cis form of both the BR_{13-dmr} sample and the dark-adapted BR sample have very similar properties /12/. The results from the femtosecond time-resolved measurements on the BR_{13-dmr} samples are shown in Fig.1 and 2 for four wavelengths of the probing light pulse (excitation wavelength $\lambda = 620$ nm). The experimental data are given by the circles, the solid lines are calculated fitting curves using exponential functions. Straight-forward is the analysis of the experimental data at 800 nm (Fig.1c). At this wavelength there is neither absorption of BR nor absorption of a ground-state photoproduct. The transmission changes found at this wavelength are due to the gain induced by the excited electronic state of the BR molecules. The observed curve is predominantly determined by one exponential with the time constant of 1.2 ps. We assign this time constant to the lifetime of the excited electronic state of the 13-cis retinal molecules. In the fit of the experimental data of Fig.1c only a very weak component with a time constant of 0.4 ps was necessary to optimize the fit around time zero. The latter component with the relative amplitude of 15% is assigned to the remaining 15% of all-trans molecules in the sample. The other measurements (Fig.1a and

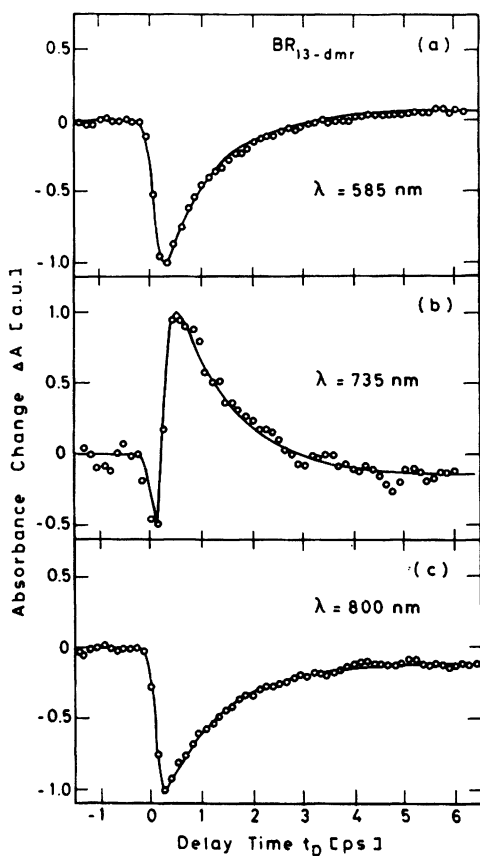


Fig.1 Absorption changes induced by femtosecond light pulses ($\lambda_{ex} = 620$ nm) in bacteriorhodopsin containing 13-demethylretinal at different probing wavelengths. Curve 1c shows a clear 1.2 ps kinetic assigned to the cis photocycle.

1b at $\lambda = 585$ nm and 735 nm, respectively) also display the fast 0.4 ps and the slower 1.2 ps kinetic together with a 3 ps kinetic (see below); e.g. at 585 nm the 0.4 ps all-trans kinetic shows up most clearly.

Of special interest is the question, if a J to K transition is also found in the cycle of the 13-cis containing BR molecules. Fig.2 shows the experimental results at the probing wavelength of $\lambda = 660$ nm. At this wavelength the J to K transition is clearly seen in light-adapted BR samples. Also in the BR_{13-dmr} samples the "J" to "K" transitions is evident. The 3 ps kinetic found here has the same amplitude as in the light-adapted BR samples, demonstrating that similar processes occur at that time in the cis and trans cycle.

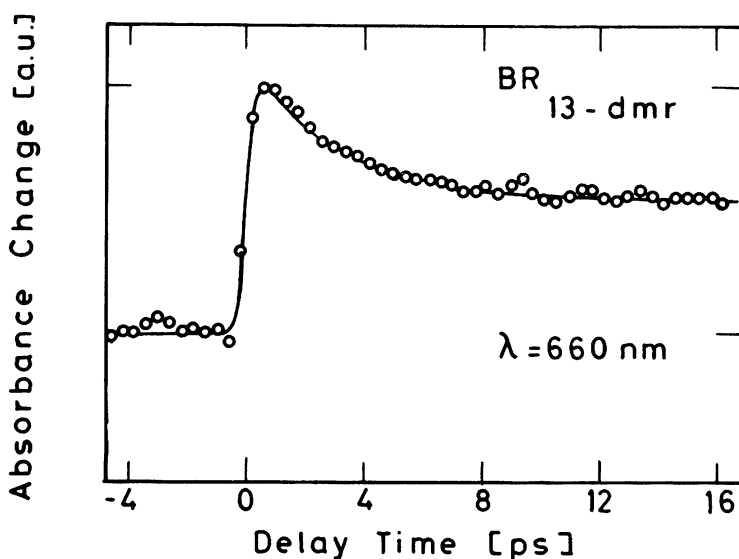


Fig.2 Time-resolved absorption measurement showing the existence of the "J" to "K" transition in the cis cycle.

We give a brief summary on other observations. The absorption spectra of the initial ground state are only slightly different in BR_{1a} and BR_{13-dmr}. The fluorescence spectra are similar. The product states K exhibit similar absorption spectra. Considerable differences are found for the absorption of the excited electronic state, which is much stronger for the 13-cis retinal than for the all-trans retinal samples in the near IR at 735 nm, while at 490 nm, in the blue, the 13-cis retinal containing BR samples absorb less.

A comparison of the experimental data of the primary processes of the trans and cis cycles of BR indicates:

- i) the excited states of the two cycles have different absorption spectra and different decay kinetics,
- ii) the final picosecond products show similar absorption properties,
- iii) there is in both cycles a 3 ps ground-state kinetic relating two red-shifted products (J to K transition).

These findings are explained within the frame of a recently published model of the trans-cis cycles /10,12/. In Fig.3a, the retinal lysine, part of BR, is shown schematically. Also shown are four residues A₁ to A₄ (presumably from aspartic acids /13,14/), which are within reach of the NH group of the Schiff's base during the isomerisation motions of the retinal molecules (circles shown in Fig.3b-e). It was suggested in recent publications /10,12/ that during the primary processes in the cis and trans cycle the NH group is brought into contact with the residue A₃H, see Fig.3b and c (starting from residue A₁- and A₂- in the trans and cis cycle, respectively). This interpretation is strongly supported by our experimental data (point

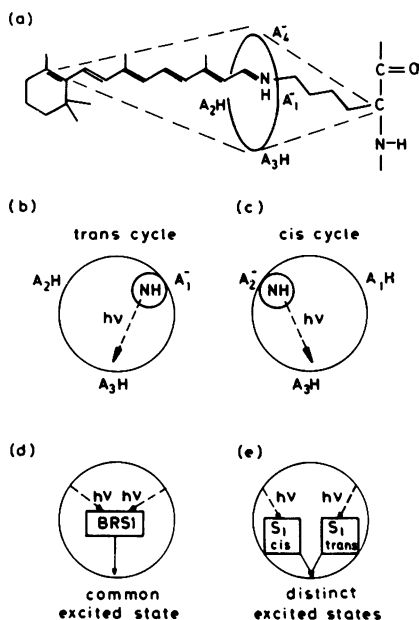


Fig.3 Model for the primary reactions in the cis and trans photocycle of bacteriorhodopsin. (a) Schematic of the retinal and residues A₁ to A₄ in the neighborhood of the Schiff's base. (b) and (c) Formation of the final picosecond photoproduct during the trans and the cis cycle, respectively, by the transfer of the NH-group during the isomerisation motion. (d) and (e) Model for the transfer pathways. Only the transfer via two distinct excited states (e) is in agreement with the experimental data.

2 and 3). Additional information on the transfer path of the primary reaction originates from our results on the properties of the excited electronic states. The different absorption properties together with the difference in the decay kinetics indicate that the transfer to the ground-state product does not go via one excited state BRS₁, common for the cis and trans cycle (as would be suggested by model of Fig.3d). It rather shows that two distinct excited states, S₁-cis and S₁-trans are involved in the course of the two primary photoreactions (model shown in Fig.3e). While the reaction pathways are different in the cis and trans photocycle, the primary reactions in both cases are rapid photoisomerisations of the retinal molecule.

Acknowledgement: This work was performed in collaboration with Prof. Oesterhelt and E. Kölling. The authors gratefully acknowledge the valuable contributions from the group of Prof. Oesterhelt.

1. J.K. Lanyi, in Bioenergetics, ed. L. Ernster, Elsevier, Amsterdam 1984, p. 315
2. G.S. Harbison, S.O. Smith, J.A. Pardo, C. Winkel, J. Lugtenburg, J. Herzfeld, R.A. Mathies, R.G. Griffin, Proc. Natl. Acad. Sci. US 81, 1706 (1984)
3. W. Sperling, P. Carl, C.N. Rafferty, N. Dencher, Biophys. Struct. Mech. 3, 79 (1977)
4. M.C. Nuss, W. Zinth, W. Kaiser, E. Kölling, D. Oesterhelt, Chem. Phys. Lett. 1, 117 (1985)
5. A.V. Sharkov, A.V. Pakulev, S.V. Chekalin, Y.A. Matveetz, Biochim. Biophys. Acta 808, 94 (1985)
6. H.J. Polland, M.A. Franz, W. Zinth, W. Kaiser, E. Kölling, D. Oesterhelt, Biophys. J. 49, 651 (1986)
7. In Ref.6 we published a time constant of ≈ 5 ps for the J to K transition. More recent femtosecond experiments indicate a somewhat shorter time constant of 3 ± 1 ps.
8. H.J. Polland, M.A. Franz, W. Zinth, W. Kaiser, E. Kölling, D. Oesterhelt, Biochim. Biophys. Acta 767, 635 (1984)
9. P. Tavan, K. Schulten, D. Oesterhelt, Biophys. J. 47, 415 (1985)
10. J.W. Petrich, J. Breton, J.L. Martin, A. Antonetti, Chem. Phys. Lett. 137, 369 (1987)
11. W. Gärtner, P. Towner, H. Hopf, D. Oesterhelt, Biochem. 22, 2637 (1983)
12. H.W. Trissl, W. Gärtner, Biochem. 26, 751 (1987)
13. M. Engelhard, K. Gerwert, B. Hess, W. Kreutz, F. Siebert, Biochem. 24, 400 (1985)
14. K. Gerwert and F. Siebert The EMBO Journal 5, 805 (1986)