PHOTOBIOLOGY
The Science and Its Applications

Edited by

Emanuel Riklis

Israel Atomic Energy Commission
Beer-Sheva, Israel

PLENUM PRESS • NEW YORK AND LONDON

Contents

UV Light and DNA Damage, Repair and Mutagenesis

The Primary Photochemical Process in Bacteriorhodopsin

W. ZINTH* AND D. OESTERHELT+

**Physik Department*
Technische Universität München
D-8000 München
+Max-Planck-Institut für Biochemie
D-8033 Martinsried, FRG

Introduction

In the halobacterial branch of archaebacteria a special kind of retinal-based photosynthesis is found. Two light-driven ion pumps, bacteriorhodopsin (BR) as a proton pump and halorhodopsion (HR) as a chloride pump occur in the cell membrane and mediate phototrophic growth of halobacterial cells (for review see Lanyi et al., 1984).

Similar basic principles of ion transport are effective in BR and HR: In the active state the proteins contain retinal in the all-*trans* configuration linked via a protonated Schiff's base to a lysine residue of the amino acid sequence. After the absorption of a photon the chromophore retinal changes its configuration and acquired the 13-*cis* form (Nuss et al. 1985; Polland et al., 1984a, 1984b, 1986; Dobler et al., 1988; Zinth et al., 1988; Mathies et al., 1988). This isomerization induces the ion transport which must proceed via polar side groups of the protein structure (Oesterhelt and Tittor, 1989). After about 10 ms ion transport is finished and the chromoprotein has returned to its initial state. In this paper we focus on the very early steps of the photosynthetic reaction in bacteriorhodopsin prior to and during the isomerization of the retinal chromophore. We describe absorption changes seen in spectroscopic experiments with femtosecond time-resolution and discuss the related molecular processes.

Materials and Methods

Bacteriorhodopsin (purple membrane) was prepared according to the procedure published by Oesterhelt and Stoeckenius (1974). The samples were kept in the light-adapted form by appropriate background illumination. Time-resolved excite and probe experiments were performed using 80 fs pulses from a colliding pulse mode-locked (CPM) laser-amplifier system operated at a repetition rate of 7 kHz. Part of the output (10%) of the laser-amplifier system served as the exciting pulse. The excitation wavelength was $\lambda = 620$ nm. The residual of the laser output produced the probe pulses via femtosecond continuum generation. The change of transmission of the sample

Photobiology, Edited by E. Riklis
Plenum Press, New York, 1991

induced by the exciting pulses was measured with high precision as a function of time delay. The time resolution of the experiment depended on the width $\Delta t = 90$ fs were obtained from the system permitting the investigation of dynamic processes faster than 50 fs.

The choice of the probing wavelength is of major importance for the interpretation of the observed absorption transients. At short probing wavelengths, in the region of the 0-0 transition of the molecule and below, the absorption changes may be related to different processes, e.g. to cross relaxation of an inhomogeneous ground-state distribution, to excited-state processes, and to the formation of photoproducts. Working at longer wavelengths, i.e. in the fluorescent region of the molecule, the ground-state processes may be neglected, i.e. a more straight-forward interpretation of the experiment is possible (Dobler et al., 1988; Zinth et al., 1988).

Results

Time-resolved changes of absorption observed on light-adapted bacteriorhodopsin samples at room temperature are shown in Fig. 1 (circles) for three probing wavelengths in the gain region of BR. Only the very rapid processes occurring within 1.5 ps after excitation are shown. At long probing wavelengths ($\lambda = 850$ nm, Fig. 1a) a pronounced gain is found, i.e. the transmitted pulse is more intense than the incident pulse. The gain decays at later times with a time-constant of approximately 500 fs. A more careful inspection of the data points shows that a faster process ($\tau = 180$ fs, see below) also contributes to the absorption change. With decreasing probing wavelengths the 500 fs contribution diminishes and at 735 nm (Fig. 1b) the faster (180 fs) process dominates the decay of the gain. Fig. 1c shows the absorption changes at a still shorter probing wavelength of 660 nm, where, on the other hand, the S_0-S_1 absorption of BR may still be neglected. An induced absorption due to the intermediate J is built up with 500 fs. Around time zero a very short-lived ($\tau < 100$ fs) gain is found.

Discussion

The experimental data *per se* suggest a qualitative view of the primary reactions. To obtain a more quantitative picture of the ultrafast molecular processes the observed absorption changes have to be compared with the predictions from a simplifying mathematical model (Polland et al., 1984b). We assume that the reaction proceeds via several intermediate levels /i > which are characterized by their absorption spectra. The occupation of the product levels decays exponentially with decay times τ_i. While this description is well justified on the time scale of picoseconds, one should keep in mind that this model may fail when coherent motions with large amplitude along special normal coordinates take place.

A detailed analysis of the experimental data together with a precise determination of the experimental response function now reveals an interesting rapid sequence of events: Three intermediate levels appear during the first picosecond. Their decay times are $\tau_1 = 50$

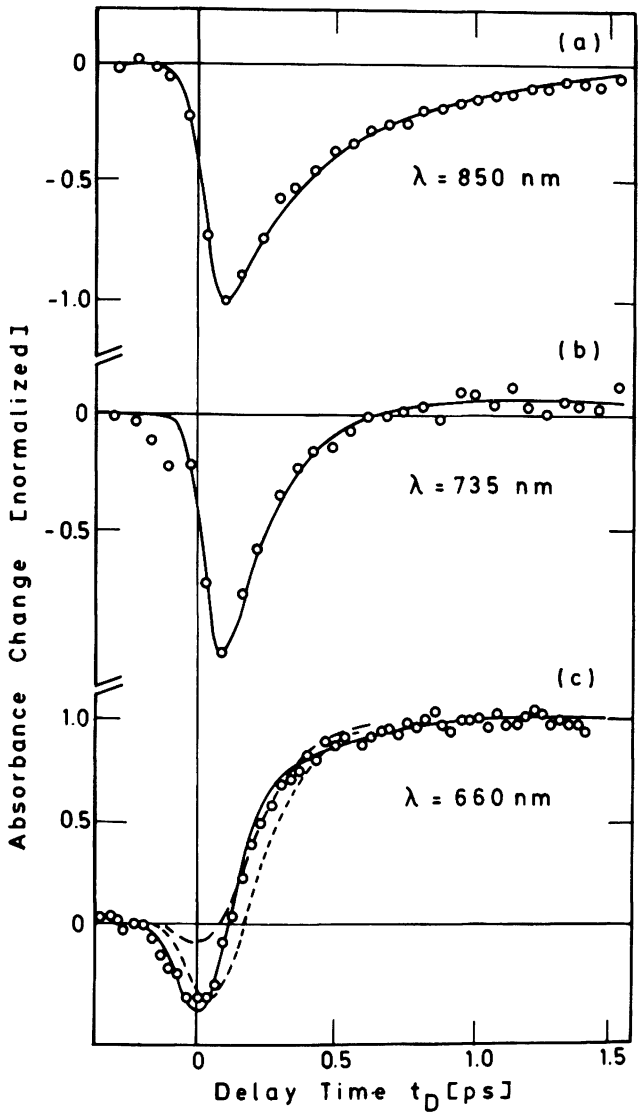

Figure 1. Time-dependent changes of absorption (negative values correspond to gain) induced by exciting femtosecond pulses at λ = 850 nm (a), λ = 735 nm (b), λ = 660 nm (c). The solid curves are calculated using the decay kinetics discussed in the text. The broken curves are calculated for two different sets of amplitude parameters excluding the 50 fs kinetics.

fs ± 30 fs, τ_2 = 180 fs ± 70 fs and τ_3 = 500 fs ± 100 fs. The gain related to all three levels proves that they exist in the electronically excited (S_1) state of BR.

Taking into account the spectral properties of the transient signal and the known molecular data of retinal the following microscopic picture of the very early reactions is suggested (see Fig. 2). The incident photons promote the retinal to the Franck-Condon

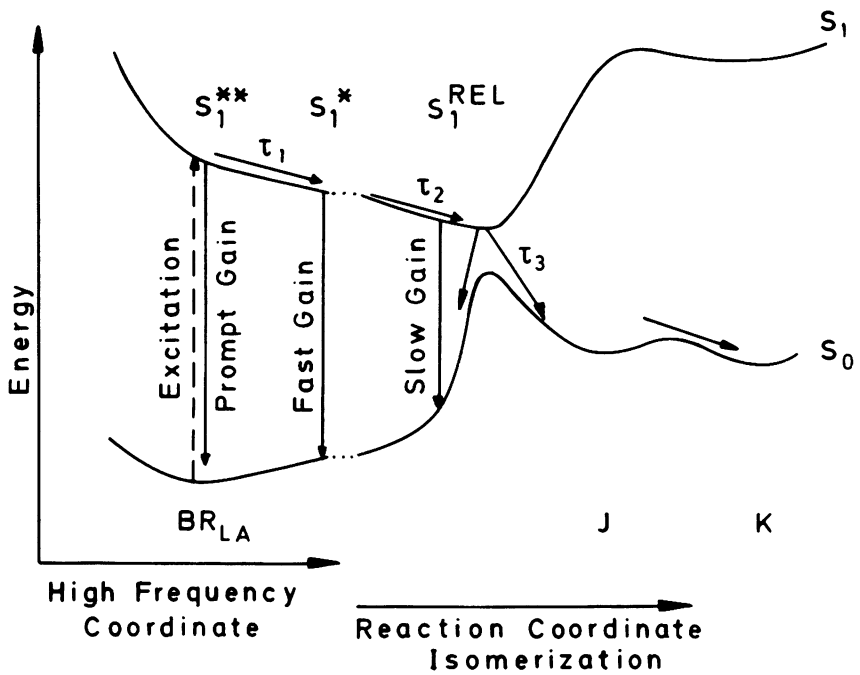

Figure 2. Scheme of the ground-state (S_0) and excited-state (S_1) potential energy surfaces as a function of the high-frequency vibrational and low-frequency reactive coordinates.

state S_1^{**} on the S_1 potential energy surface. Here a number of vibrational modes are displaced relative to the S_1 equilibrium position (Heller, 1981). Within 50 fs after light absorption an equilibration of high-frequency vibrational modes to the state S_1^* occurs. During this first reaction the molecule does not have the time to move along the coordinates of the low-frequency (reactive) modes. The following slower reactive motion of the retinal is related to the 180 fs gain kinetics. In this process, part of the isomerization (presumably a rotation by 60 to 90 degrees around the C_{13}–C_{14} double bond) takes place and the molecules arrive at the bottom of the S_1 potential surface (level S_1^{Rel}). The system leaves this area via internal conversion with a time constant $\tau_2 = 500$ fs. Two decay pathways are possible: more than 60% of the molecules form the intermediate photoproduct J, while the rest returns to the original ground state of BR. The following reaction in the active branch proceeds from J with a 3 ps time constant leading to the intermediate K which is stable on the picosecond time scale.

Conclusions

Time resolved absorption experiments on bacteriorhodopsin performed on the femtosecond time scale show strong absorption and gain dynamics which are related to the excited-state reaction of the retinal chromophore. Extremely rapid (50 fs) absorption

changes reflect the relaxation of high-frequency modes. The slower kinetics are related to the isomerization of the retinal: Isomerization starts in the excited electronic state and is finished directly after the 500 fs internal conversion process during the formation of the first ground state photoproduct J.

Acknowledgements

The authors acknowledge valuable contributions by W. Kaiser, J. Dobler and K. Dressler.

References

Dobler, J. Zinth, W. Kaiser, W. Oesterhelt, D. *Chem. Phys. Lett.* 144 (1988) 215.
Heller, E.J. *Acc. Chem. Res.* 14 (1981) 368.
Lanyi, J.K. in Bioenergetics, ed. Ernster, L. (Elsevier, Amsterdam) 1984, p. 315.
Mathies, R.A. BritoCruz, C.H. Pollard, W.T. Shank, C.V.*Science* 240 (1988) 777.
Nuss, M.C. Zinth, W. Kaiser, W. Kölling, E. Oesterhelt, D. *Chem. Phys. Lett.* 117, (1985) 1.
Oesterhelt, D. Stoeckenius, W. *Meth. Enzymol.* 31A (1974) 667.
Oesterhelt, D. Tittor, J. TIBS 14 (1989) 57.
Polland, H.J. Franz, M.A. Zinth, W. Kaiser, W. Kölling, E. Oesterhelt, D. *Biochem. Biophys. Acta* 767 (1984) p. 635.
Polland, H.J. Franz, M.A. Zinth, W. Kaiser, W. Kölling, E. Oesterhelt, D. *Biophys. J.* 49 (1986). 651.
Polland, H.J. Zinth, W. Kaiser, W. in Ultrashort Phenomena, vol. 4, eds. Auston, D.H. Eisenthal, K.B. (Springer, Heidelberg, 1984) p. 456.
Zinth, W. *Naturwiss.* 751 (1988) 173.