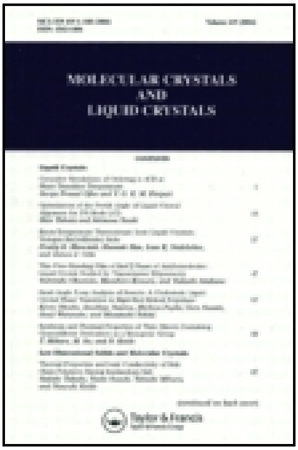

Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl19

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D. Zeisel [a] & N. Hampp [a]

[a] Institute for Physical Chemistry, University of Munich, Sophienstr. 11, D-80333, München, Germany

Version of record first published: 24 Sep 2006.

To cite this article: D. Zeisel & N. Hampp (1994): Bacteriorhodopsin Films Containing the Variant D96N as Media for Dynamic Holography and Interferometry, Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals, 246:1, 371-374

To link to this article: http://dx.doi.org/10.1080/10587259408037846

Mol. Cryst. Liq. Cryst. 1994, Vol. 246, pp. 371–374
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Printed in the United States of America

BACTERIORHODOPSIN FILMS CONTAINING THE VARIANT D96N AS MEDIA FOR DYNAMIC HOLOGRAPHY AND INTERFEROMETRY

D. ZEISEL, N. HAMPP
Institute for Physical Chemistry, University of Munich, Sophienstr. 11, D-80333 München, Germany

Abstract The photochromic retinal protein bacteriorhodopsin which was obtained from *Halobacterium salinarium* has been studied as the photoactive component of recording materials for different dynamic holographic applications like degenerate-four-wave-mixing (DFWM) and interferometry. With the mutated BR-variant D96N resonantly induced reflectivities of up to 20% are observed at 647 nm in DFWM. The excellent reversibility of the BR-materials and the extended thermal decay time of this particular BR-variant support its application in real-time interferometry.

INTRODUCTION

The biological photochrome bacteriorhodopsin (BR) is the key protein in the halobacterial photosynthesis. The 248 amino acids of BR are arranged as seven transmembrane α-helices in the halobacterial cell membrane. The chromophore is formed by a retinylidene residue which is attached to the protein moiety via a Schiff base linkage to Lys216 and an inner shell of amino acids[1]. The biological function of BR is that of a light-driven proton pump. Under illumination BR transports protons from the innner of the cell to the outer medium. Thereby light energy is converted into chemical energy which can be utilized by the halobacterial cell. About 20 years after its discovery BR is one of the best investigated membrane proteins[2-3]. Light-driven proton transport is coupled to the photochromic properties of BR. In fig. 1 a sketch of the photochemical and thermal conversions of BR is shown.

The photocycle of BR is initiated by the absorption of a photon (phototransition B → J). From there the molecule cycles thermally (thin arrows) through a sequence of intermediates which are distinguished by their absorption maxima (subscripts in fig. 1). The retinal configuration is indicated by upright letters for all-*trans*, right slanted italics for 13-*cis* and left slanted italics for 9-*cis*[4-5]. From each intermediate BR can be converted back to the initial B-state photochemically (grey arrows). For dynamic recording the transition M → B with blue light is important[6] since the M-state shows the longest lifetime of all intermediates and therefore the highest population density. The lifetime of the M-intermediate can be further prolonged by several orders of magnitude by exchange of aspartic acid (Asp=D) in position 96 for *e.g.* asparagine (Asn=N)[7].

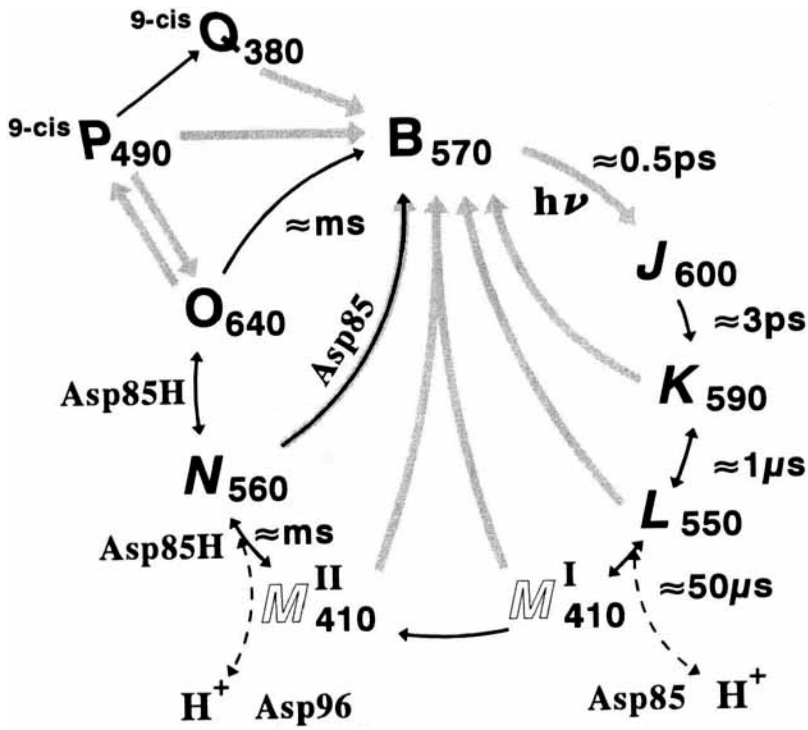

Fig. 1 : Photocycle of bacteriorhodopsin

Since Asp96 acts as a proton donor in the thermal relaxation of the M-intermediate the loss of this function (Asp96 → Asn96) leads to a stabilization of the M-state. Thus the photochromic properties of BR are enhanced since the thermal component is less important in this BR-variant BR-D96N. For the realization of such single amino acid exchanges gene-technology is a valuable tool.

APPLICATIONS OF BR IN DYNAMIC HOLOGRAPHY

For applications in dynamic holography the high quantum efficiency ($\Phi_{B\rightarrow J}$ = 0.64) and fast transition times between B ⟷ M are attractive. But the most important reason to use this biological material as a photochromic compound in dynamic holography is its excellent reversibility. At least 10^6 write/erase cycles can be performed without noticable degradation of the material. Since the M-lifetime of the variant D96N can be easily controlled by the pH-value and the proton availability[8] in the films made from BR, recording media with increased light sensitivity can be obtained. It was observed that the light-induced changes of the absorption coefficient and the correlated refractive index changes can be described in good approximation by the *Kramers-Kronig* relation[9]. Therefore the wavelength-dependent diffraction efficienies (up tp 7%) can be calculated from the easily available absorption changes.
Two applications where the latter hologram type is employed shall be described briefly, *i.e.* phase-conjugation (PC) by degenerate-four-wave-mixing (DFWM) and interferometry.

DFWM: Optical phase conjugation with BR-films is preferably realized in an DFWM-setup[10]. For the wavelength 647 nm rise and decay of the phase-conjugated signal are observed on the millisecond timescale. The dependence of the reflectivity, *i.e.*, the ratio of the intensities of the incoming probe beam and the outgoing phase-conjugated signal on the intensity of the pump beams is shown in fig. 2. For BR-films with initial optical densities of iOD_{647nm} = 0.5 and 0.75 maximal reflectivities of about 20% are obtained at 10 mW/cm^2 and 30 mW/cm^2 (intensities of the pump beams). The intensity of the probe beam was 1 mW/cm^2. The rise and decay times of the phase-conjugation signal is remarkable in comparison to the widely used photorefractive materials[11], however, the obtainable reflectivities do not enable any 'gain' which is required in various phase-conjugation experiments for optical data processing.

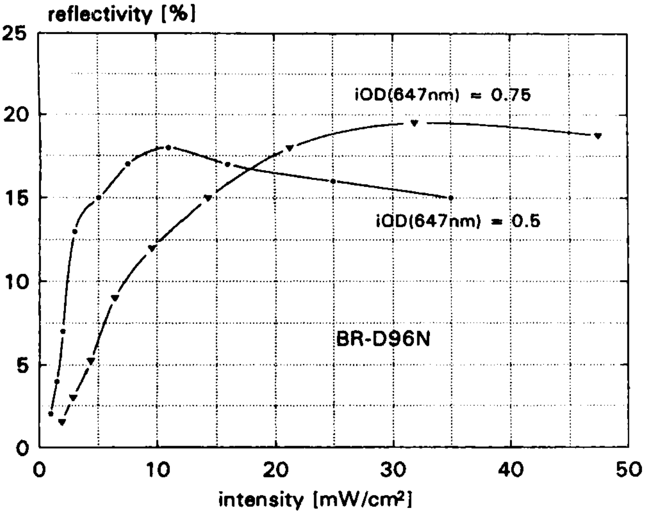

Fig. 2 : Intensity-dependence of the reflectivity in DFWM-PC

Real-time interferometry: Holographic interferometry is an important tool in non-destructive testing. In fig. 3 a typical setup is shown. Dynamic time-averaging holography with heterodyne detection have been successfully demonstrated with bacteriorhodopsin films for the analysis of periodically vibrating objects[12]. The setup (see fig.3) is a homodyne implementation for real-time interferometry with BR-films where reflection type holograms are used. An expanded laser

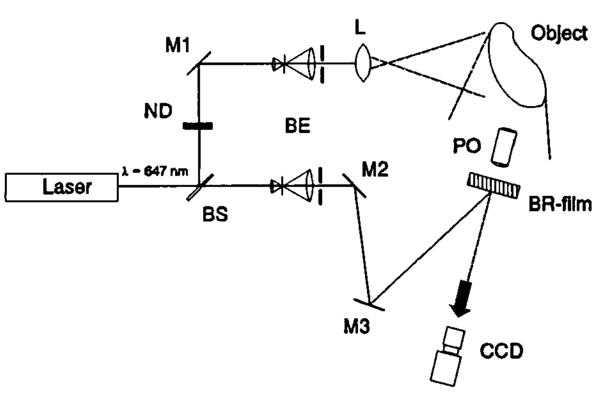

Fig. 3 : Interferometric setup

beam (BE) of 647 nm is used for the illumination of the object. A few percent of the intensity are decoupled from the laser beam by means of the beamsplitter (BS) and directed via mirrors M2 and M3 to the sample where it serves as the reference beam. The wave reflected from the object and transmitted through the BR-film together with the reconstructed hologram form the interferogram and are monitored by a CCD camera. After holographic recording of the "initial state" of the object at the time $t=t_0$ a neutral density filter (ND) is switched into the object beam in order to obtain a low intensity object wave which interferes with the simultaneously reconstructed "initial state" hologram for all times $t>t_0$. An example of such an interferogram is shown in fig. 4.

Fig. 4 : Real-time interferogram of a steel plate with applied torsional force.

CONCLUSIONS

Modification of the optical properties of a biological photochrome like BR and adaptation to the demands of optical techniques like DFWM-PC and interferometry demonstrate, that biological material can be successfully used in technical systems and may replace synthetic materials in selected applications.

ACKNOWLEDGEMENT: This work was supported by the "Bundesministerium für Forschung und Technologie" (FKZ 0319231 B).

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