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Biophysical Chemistry 56 (1995) 189–192

Biophysical Chemistry

Bacteriorhodopsin variants as versatile media in optical processing

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Abstract

The photochromic properties of bacteriorhodopsin (BR), in addition to its longevity and excellent reversibility, are attractive features for the construction of light-sensitive media for optical information processing. However, the various optical techniques require media with specifically adapted and widely differing properties. Genetic engineering of BR and biotechnological production of mutated BRs is the key for the utilization of this photochromic compound in optical applications. Mutated BRs, generated by single and double amino acid exchanges, have been used as recording media for optical applications such as phase conjugation or long-term data storage at room temperature.

Keywords: Bacteriorhodopsin; Optical processing

1. Introduction

The photochromic retinal protein bacteriorhodopsin (BR) is the only protein in the purple membrane (PM) fragments from the bacterium *Halobacterium salinarium* (former *Halobacterium halobium*). BR is the key protein in halobacterial photosynthesis [1,2] and shows interesting features for optical applications, e.g. its efficient photochemistry [3] and its excellent reversibility [4]. Neither these properties nor its excellent stability against chemical, thermal and photochemical degradation [5] is affected by isolation of the PM patches from the bacterial cell. Thus BR is an interesting candidate for optical information processing and data recording.

In Fig. 1 the simplified model of the wild-type BR photocycle, with the recently discovered long-term stable P- and Q-intermediates [6] is shown. The intermediates are represented by a single letter and the absorption maxima are given as subscripts. Thick arrows indicate photochemical transitions and thin black arrows stand for thermal reactions. Small superscript letters indicate all-*trans* (t) and 13-*cis* (c) retinal configurations. The uptake and the release of protons is referred to by broken lines.

2. Optical applications with modified BR-systems

Optical applications require media with specific characteristics which cannot be achieved by the wild-type form alone. For that reason the properties of BR have been modified by physical methods using e.g. low temperature, resulting in a stabilization of early intermediates [7]. Reducing either the proton concentration or the proton mobility, e.g. by replacing water by glycerol, leads to a hindered

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SSDI 0301-4622(95)00032-1

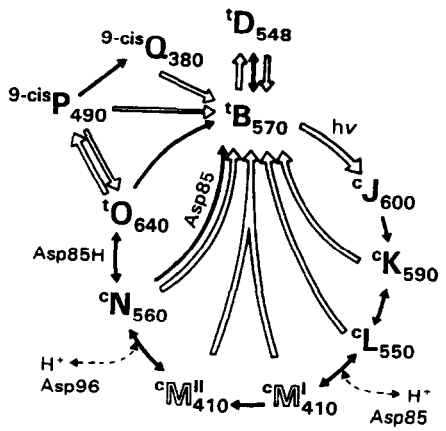


Fig. 1. Photocycle of bacteriorhodopsin (BR). The aspartic acids in positions 85 and 96 (Asp85 and Asp96) act as proton acceptor and proton donor for the reversible protonation of the retinal Schiff base linkage during the photocycle. BR returns to the initial B-state either from the N-state directly or it cycles through the O-state. The first pathway is used if Asp85 is deprotonated when the BR molecule leaves the N-state and the second pathway is used if Asp85 is still protonated (= Asp85H).

reprotonation of the Schiff base from aspartic acid 96 (Asp96) and results in an increased lifetime of the M-intermediate [8]. Also chemical modification of the retinal chromophore can result in new photochemical properties [9], but sometimes other valuable features are compromised.

Modifying the photochemical properties of BR in a very specific way became possible by genetic engineering [10,11]. The replacement of functionally important amino acids leads to altered photochemical behaviour of the mutants. One of the best investigated mutants is BR_{D96N} in which aspartate in position 96 is replaced by asparagine which cannot function as a proton donor. In the $BR_{D85,96N}$ (BR_{D2N}) variant both the proton donor and the proton acceptor (Asp85) are replaced by asparagine residues which leads to a red shifted pigment with totally different photochemistry [12].

For most applications, suspensions of BR are not feasible because thermal diffusion and aggregation of the BR-suspension within the sample cannot be controlled. The use of films formed, e.g. by drying [4] or other methods [13], is a possible way to overcome this disadvantage. Further important features of BR-films which satisfy the requirements for optical applications are their great homogeneity in both thick and thin films and their excellent mechanical stability. Furthermore BR-films show properties which cannot be found in suspension. A prominent example is the prolonged lifetime of the M-intermediate in dried films of BR_{D96N} [14]. In Table 1 the molecular properties of BR and specific features of BR-films for holographic experiments are compared.

During the last few years, new ideas utilizing the photochromism of BR have arisen e.g. [15,16]. In our group mutated BRs have been used for real time pattern recognition [17–19], real time interferometry [20], optical phase conjugation [21] and long-term data storage at room temperature [22].

3. Optical phase conjugation with BR-films

Optical phase conjugation is an important method in nonlinear optical data processing [23]. Phase con-

Table 1
Relation between the molecular properties of BR and the holographic properties of BR-films

Molecular properties of BR	Properties of BR-films
Mol. absorption and quantum efficiency (B: $\Phi \geq 64\%$, $\epsilon = 63.000\ l\ mol^{-1}\ cm^{-1}$)	Light sensitivity (B: type 1, 80 mJ/cm^2, M type: 30 mJ/cm^2)
Transition times B → M and M → B ($\approx 50\ \mu s$)	Hologram rise and decay times (< 40 ms)
Anisotropic chromophore (linear anisotropy 20:1)	Polarization recording (possible)
2-Dimensional crystal lattice of BR and BR variants (purple membrane)	Reversibility (> 10^6), thermal stability
Absorption range of the B (570 ± 60 nm), M (410 ± 50 nm) and P (490 ± 50 nm) state	Spectral range (400–700 nm)
Packing density of BR molecules (crystalline)	Thickness of films (10 μm–40 μm), optical density (1–20 OD_{570})
Size of BR (5 nm)	Spatial resolution (5000 lines/mm)
Independent reaction of individual chromophores	

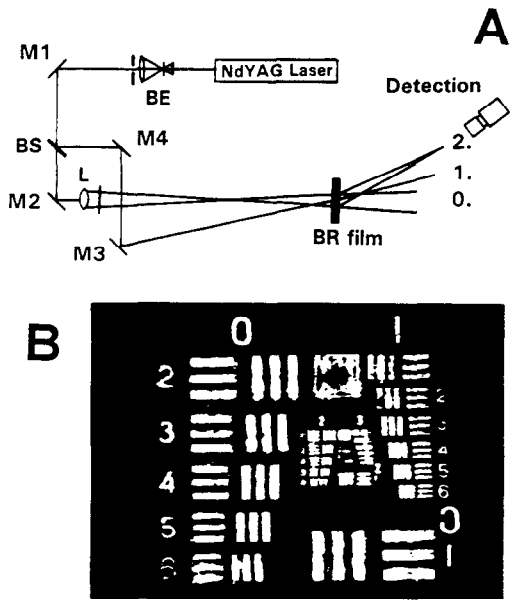


Fig. 2. (A) Setup for three-wave mixing experiments with BR-films. (B) Image of the test-pattern observed in the second diffraction order. Abbreviations: BE = beam expander; M1, M2, M3 and M4 = mirrors; BS = beam splitter; L = lens.

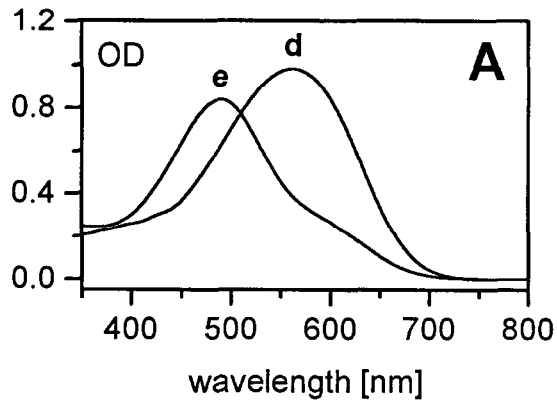
jugation via degenerate four-wave mixing (DFWM) is interesting due to its promising technical applications, e.g. correction of distortions in optical resonators and its use as amplifier in optical memories. We have carried out DFWM experiments which will be published elsewhere [21].

A further example of phase conjugation with BR-films is three-wave mixing (forward oriented phase conjugation) [24]. Fig. 2A shows the simplified experimental setup. Two coherent laser beams (wavelength 532 nm) of identical polarisation are incident at a small angle of 0.4 on the BR_{D96N}-film. The signal beam passes through an achromatic lens and is detected by a CCD-camera.

A superimposed USAF-test pattern was analyzed in the second diffraction order and is shown in Fig. 2B. The pattern was detected with an efficiency of 3% after 100 ms. The mechanism responsible for the three-wave mixing effect in BR-films is a resonant process which has the advantage of e.g. short reaction times and low intensity requirements.

4. Long-term data storage with mutated BRs

BR-films containing wild-type BR at pH 6.5 in the presence of glycerol show the formation of 9-*cis*-retinal [6]. The key role in this reaction involves the O-intermediate which can be photochemically converted to the so called P-intermediate consisting of thermally stable 9-*cis*-retinal (see Fig. 1). This side-reaction in the wild-type system is the main photochemical step in the mutant BR_{D2N} [22]. The 'blue form' of BR_{D2N} exists in a pH range from 3 to 5 and has its maximum absorption at 600 nm. At pH 4 a 13-*cis*/all-*trans* ratio of 20:80 is found in suspensions which cannot be changed by broad band illumination. The all-*trans* isomer, however, can be enriched in BR_{D2N}-films at reduced relative humidity (rh). A further parameter controlling the formation of all-*trans*-retinal is the amount of glycerol added during preparation. Fig. 3A shows the absorption spec-



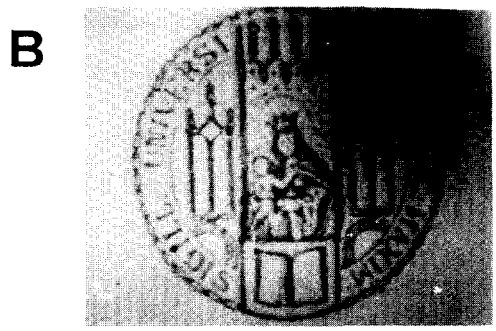


Fig. 3. (A) Absorption spectra of a BR_{D2N} sample before exposure (d = dark) and after exposure (e = exposed). (B) Long-term stable image stored in a BR_{D2N}-film.

tra of a BR_{D2N} sample with more than 90% all-*trans*-retinal containing 4% glycerol at pH 4 and 9% rh (curve d). Bleaching of the sample with red light (6 min with 400 mW/cm^2 at 647 nm) shifts the absorption maximum from 600 nm to 490 nm and about 50% of 9-*cis*-retinal is formed from all-*trans*-retinal (curve e). The photoproducts are stable for months if stored in darkness, but can be reconverted to all-*trans*-retinal with blue light. This allows reversible long-term data storage, demonstrated in Fig. 3B, where the signet of the University of Munich was written into a BR_{D2N}-film. Read-out of information is possible by making use of the chromophore anisotropy.

5. Conclusion

Optical applications of specifically adapted BR-films have shown that genetic engineering of the evolutionary optimized chromophoric protein system BR is the key technology for the design of BR-based optical media with a realistic potential for application.

Acknowledgements

Fruitful discussions with D. Oesterhelt and C. Bräuchle are gratefully acknowledged. This work was supported by the 'Bundesministerium für Forschung und Technologie' (FKZ 0319231B).

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