Mechanism of Nonlinear Photoinduced Anisotropy in Bacteriorhodopsin and its Derivatives

E.YA. KORCHEMSKAYA^{1*}, D.A. STEPANCHIKOV¹, A.B. DRUZHKO² and T.V. DYUKOVA²

¹ Institute of Physics, National Academy of Sciences, Prospect Nauki 46, Kiev-22, 252650 Ukraine

² Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Moscow Region, 142292, Russia

(* Author for correspondence, e-mail: elkorch@iop.kiev.ua)

Abstract. Polymer films made with photosensitive chromophore protein bacteriorhodopsin (BR) from the extreme halophile *Halobacterium salinarium* as well as films made with BR derivatives exhibit a nonlinear photoinduced anisotropy. Two different methods can be used to induce anisotropy in polymer BR films. The first method is based on the anisotropic properties of the initial form of the photocycle, BR570 (B-type anisotropy). Another method is based on the anisotropic properties of the longest-lived photocycle intermediate M412 (M-type anisotropy). CW gas lasers were employed to induce a reversible anisotropy in polymer BR films. Nonlinear photoinduced anisotropy is discussed in the context of a model for the anisotropic photoselection of BR molecules under linearly polarized light. A comparison of the experimental dependencies of nonlinear photoinduced anisotropy on laser intensity with similar calculated dependencies enables one to determine the molecular dichroism of BR and its derivatives not only for the initial form of the photocycle, B but also for the longest-lived intermediate M. Here we present the data showing the correlation between the laser induced nonlinear anisotropic properties and chromophore/protein interactions in BR. The effect of polymer binder on the nonlinear photoanisotropic properties of polymer BR films is also described.

Key words: Bacteriorhodopsin, chromophore/protein interaction, molecular dichroism, photoinduced anisotropy, photoselection, polymer films

Introduction

The biological photoreceptor bacteriorhodopsin (BR), a transmembrane chromophore protein, is embedded in the purple membranes of the extreme halophilic micro-organism *Halobacterium salinarium*. Its name is derived from the fact that the BR molecule is similar to visual pigment rhodopsin inherent in the human eye. Upon absorbing visual light, the BR molecule undergoes a photochemical cycle of several distinct intermediates accompanied by conformation changes [1]. A transfer of one proton across the purple membrane also takes place during the photocycle. For present purposes we consider a simplified photocycle of BR (Figure 1). Not only is BR an important natural solar energy converter, but it might also be used as ‘eyes of robots’ [2]. Consequently, real time optical methods in

Figure 1. A simplified photocycle of BR: BR570 is the initial form of the photocycle, B; M412 is a longest-lived (in films) intermediate, M.

studies of photoinduced changes in BR are of great importance for both getting a deeper insight into the function of photosensitive protein BR and for the advent of an artificial retina.

Polymer films made with BR as well as films made with BR derivatives possess very interesting nonlinear optical properties. It is well known that some of the largest optical nonlinearities are those due to the saturable absorption in materials with a small value of a saturation intensity [3]. Polymer BR films show very small saturation intensity (of about 10 mW/cm^{-2}). It is also very important that the rate of absorption saturation depends on the polarization state of both exciting and testing beams [4]. Thus, polymer films fabricated with BR and its derivatives exhibit a nonlinear photoinduced anisotropy [5]. On the other hand, the reversible photoinduced anisotropy can be used to study any photosensitive proteins and photoreceptors. An important advantage of this technique is a zero background in the absence of an exciting beam, whereas in commonly used methods the dichroic ratio is nonzero even in the absence of an exciting beam [6]. Thus reversible photoinduced anisotropy can be used to follow changes that are too small to be detected by any other means. It is important to note that the reversible photoinduced anisotropy is a non-destructive method.

Photoinduced anisotropy can be interpreted in the following way. A sample is a randomly-oriented rigidly-bound domain of anisotropically-absorbing molecules (Figure 2a). It is isotropic in its initial state. The angular distribution of molecules in their initial form is schematically shown in Figure 2d, circle 1. Under linearly polarized light a photoselection of molecules takes place. The molecules whose long axes are aligned close to the direction of light polarization do absorb light, whereas those with perpendicular orientation to this direction do not absorb light (Figure 2b). Current angular distribution of molecules in the initial form is shown in Figure 2d, ellipse 2. By this means a macroscopic optical axis is induced by linearly polarized exciting light. At near-saturation light intensity all molecules convert to

Figure 2. An illustration interpreting a photoselection of molecules under linearly polarized light.

a phototransformed state, and a sample appears to be in a transient newly isotropic state (Figure 2c).

In this paper the correlation between the laser induced nonlinear anisotropic properties and chromophore/protein interaction in BR will be discussed. Also it will be shown that a combination of photoinduced anisotropy method and some biochemical approaches is a very promising research strategy.

Materials and Methods

Gelatin films made with the wild-type (WT) BR, BR genetic mutants D96E and D96N (all with maximum absorption at around 570 nm for their initial forms B) were prepared as previously described [7, 8]. These BR genetic mutants show considerable promise as optical materials for image processing. Novel BR pigments were also studied wherein an artificially synthesized chromophore, 4-keto retinal is reconstituted with the WT and D96N BR apoproteins (with resulting absorption maximum at 510 nm for their initial forms B) as described in [9]. All BR proteins possess an absorption maximum at 412 nm for the longest-lived (in films) intermediate M. The samples with WT, D96E and D96N BR have an optical density

Figure 3. A schematic representation of the experimental setup to detect photoinduced anisotropy: BR – BR-film, I_{exc} – exciting laser beam, I_{test} – testing beam, A – analyzer, P – polarizer, O-O' – induced optical axis, PD – photodiode.

$D_0 = 2.3$ at 570 nm; the samples with 4-keto and 4-keto-D96N BR have an optical density $D_0 = 2.0$ at 510 nm. The WT BR was also immobilized in polyvinyl alcohol (PVA) to compare the effect of two different matrices, gelatin and PVA.

A schematic of the experimental setup for the detection of the laser-induced anisotropy is shown in Figure 3. He-Ne laser, $\lambda = 633$ nm, Cu-laser, $\lambda = 510$ nm, Ar-laser, $\lambda = 488$ nm and He-Cd laser, $\lambda = 442$ nm are used to induce the reversible dichroism and birefringence. The BR film is mounted between a crossed polarizer P and analyzer A, an optical axis O-O' induced by the exciting beam I_{exc} is inclined at 45° to the polarizer axis. Testing is performed by a collimated beam from an incandescent lamp with a monochromator tuned to an appropriate wavelength. In the absence of exciting beam the intensity of a testing beam passed through the system P-BR-A is zero. Laser-induced dichroism $D_\perp - D_\parallel$ is measured at $\lambda_{test} = 570$ nm for the WT [5], D96E and D96N BR and at $\lambda_{test} = 510$ nm for 4-keto and 4-keto-D96N BR. At maximum absorption for all these proteins, birefringence equals zero and dichroism reaches its maximum value. The value and sign of dichroism are determined by measuring the compensation angle of analyzer rotation $\Delta\theta$: $\tan^2(\pi/4 - \Delta\theta) = T_\perp/T_\parallel$, where $T_\perp$ and $T_\parallel$ are transmittancies of a sample in a testing beam polarized perpendicular and parallel to an induced optical axis, respectively; a transmittance T and optical density D are interconnected ($D = \lg(1/T)$) and laser-induced dichroism can be presented as $D_\perp - D_\parallel = -2\lg(\tan(\pi/4 - \Delta\theta))$. Measurements of laser-induced birefringence $n_\perp - n_\parallel$ are performed at $\lambda_{test} = 633$ nm for the WT, D96E and D96N BR. At 633 nm dichroism value is negligible and birefringence reaches its maximum value [5]. The value of birefringence is determined by measuring the transmittance T_{P-BR-A} of the system P-BR-A (Figure 3) using the photodiode PD. At small absorption values, T_{P-BR-A} is proportional to $(n_\perp - n_\parallel)^2$.

Table I. Effect of polymer binder on photoinduced anisotropy in WT BR Film

Matrix	Transmittance of P-BR-A system, arb.un. (birefringence)	Water content in matrix, %(w/w)
Gelatin	1 ± 0.1	31 ± 9
PVA	0.77 ± 0.11	25 ± 9

EFFECT OF POLYMER BINDER ON PHOTOINDUCED ANISOTROPY OF BR FILMS

WT BR films were fabricated using two different polymers, gelatin and PVA to study the effect of the polymer on laser-induced birefringence. As indicated in Table I, the birefringence value is somewhat higher in BR gelatin film as compared to PVA film. We associate this difference with a stronger capacity of gelatin helices to bind and hold water molecules as compared to PVA molecules so that a gelatin matrix is less dehydrated [10]. BR as a transmembrane protein gains greater conformational flexibility in a gelatin matrix resulting in a birefringence increase. Thus, gelatin films offer an additional advantage over PVA in the detection of photoinduced anisotropy.

MODEL OF REVERSIBLE ANISOTROPIC PHOTSELECTION OF BR MOLECULES IMMOBILIZED IN POLYMER MATRIX

Photoinduced anisotropy in BR films is based on photoanisotropic selective bleaching of BR molecules under linearly polarized light. BR film is a domain of randomly-oriented rigidly-bound anisotropically-absorbing BR molecules (Figure 2a) [11]. For simplicity, the BR molecule is approximated by a linear oscillator. The distribution of populations of molecules between the initial form B (N^B) and intermediate M (N^M) is described by the following balance equation:

$$\frac{dN^B}{dt} = \frac{N^M}{\tau} - \sigma^B A^{B \rightarrow M} \frac{I}{h\nu} N^B, \quad (1)$$

where σ^B is an absorption cross-section for the B -form at exciting wavelength, $A^{B \rightarrow M}$ is a quantum yield of $B \rightarrow M$ transition, $I/h\nu$ is a photon flow density and τ is a lifetime of the M -form. Depending on various conditions, a lifetime of the M -form varies from 10 milliseconds to several minutes (Figure 1). It is assumed that in all cases $N^B + N^M = N_0$, where N_0 is a total density of BR molecules. At small exciting intensity, the M -form is poorly populated ($N^M \rightarrow 0$) and N^B is determined as:

$$N^B = (1 - \sigma^B A^{B \rightarrow M} I t / h\nu) N_0 \quad (2)$$

Hence, the photoresponse linearly depends on exposition: $E = It$. As the exciting intensity increases, the molecular population N^M in the phototransformed form grows so that a photoresponse is no longer linear.

Upon excitation with linearly polarized light, an absorption cross-section depends on an angle φ between a molecular dipole vector and the electric field vector of light:

$$\sigma^B(\varphi) = \sigma_{\parallel}^B \cos^2 \varphi, \quad (3)$$

where $\sigma_{\parallel}^B$ is an absorption cross-section lengthwise of the molecular dipole moment. Thus, the probability of a $B \rightarrow M$ transition depends on the orientation of BR molecules in a sample plane (Figure 2b). As a result, the heterogeneous distribution of angular concentration of molecules occurs (Figure 2d). The photostationary angular distribution is written, in view of (1) and (3), as:

$$\frac{N^B(\varphi)}{2\pi} = \frac{N_0}{2 \left(1 + \sigma_{\parallel}^B A^{B \rightarrow M} \frac{I}{h\nu} \cos^2 \varphi \right)}. \quad (4)$$

This gives rise to a macroscopic photoinduced anisotropy. As seen, its lifetime is coincident with a lifetime of the M -form. For the red region of the spectrum wherein the absorption cross-section for the M -form at testing wavelength is equal to zero, in view of (3) and (4) the macroscopic dichroism $D_{\perp} - D_{\parallel}$ is determined as:

$$D_{\perp} - D_{\parallel} = D_0 \int_0^{2\pi} \frac{N^B(\varphi)}{2\pi} (\sin^2 \varphi - \cos^2 \varphi) d\varphi, \quad (5)$$

where $D_{\perp}$ and $D_{\parallel}$ are optical densities of a sample in a testing beam polarized perpendicular and parallel to an induced optical axis, respectively. Here $D_0 = N_0 \sigma_{\parallel}^B d_0 / \ln 10$ is the initial optical density of photocycling molecules (d_0 is a sample thickness, $\sigma_{\parallel}^B$ is an absorption cross-section at λ_{test}). The integration of (5) gives:

$$D_{\perp} - D_{\parallel} = \frac{4D_0}{a} \left(\frac{2+a}{2\sqrt{1+a}} - 1 \right), \quad (6)$$

where a is a parameter proportional to an exciting light intensity:

$$a = \sigma_{\parallel}^B A^{B \rightarrow M} \tau \frac{I_{exc}}{h\nu}. \quad (7)$$

INTERRELATION OF PHOTOINDUCED MACROSCOPIC DICHROISM AND MOLECULAR DICHROISM

In general, an anisotropically absorbing molecule can be approximated by mutually orthogonal linear oscillators, so that the dependence of an absorption cross-section on angle φ between a long absorption axis of a molecule and electric field vector of light takes the form:

$$\sigma^B(\varphi) = \sigma_{\parallel}^B \cos^2 \varphi + \sigma_{\perp}^B \sin^2 \varphi, \quad (8)$$

Figure 4. Absorption cross-section of an anisotropically absorbing molecule.

Figure 5. Calculated dependencies of the photoinduced dichroism $(D_{\perp} - D_{\parallel})/D_0$ on intensity of an exciting laser beam $I_{exc}(a = \sigma_{\parallel}^B A^{B \rightarrow M} \tau I_{exc}/h\nu)$ at $\lambda_{exc} = 633, 510, 488$ nm.

where $\sigma_{\parallel}^B$ and $\sigma_{\perp}^B$ are absorption cross-sections for the light polarized parallel and perpendicular to a long absorption axis of a molecule, respectively (Figure 4). Using Equation (8) in our estimation is correct since fabricated BR films possess random orientation of purple membrane fragments and the integrated projection of chromophore dipole moments on Z axis is zero. Anisotropic absorption of molecules is described by molecular dichroism k :

$$k^B = \frac{\sigma_{\perp}^B}{\sigma_{\parallel}^B}. \quad (9)$$

Molecular dichroism arises from both the chromophore absorption and the chromophore/protein interaction. For example, when absorbing molecules are approximated by a linear oscillator, $k = 0$, and if they present an isotropically absorbing center, then $k = 1$. Solving (1) in terms of (8) for $N^B(\varphi)/2\pi$ and integrating, we obtain, for the wavelengths activating just B -form:

$$D_{\perp} - D_{\parallel} = \frac{4D_0}{a(1+k^B)} \left(\frac{2+a+k^B a}{2\sqrt{(1+a)(1+k^B a)}} - 1 \right). \quad (10)$$

For these wavelengths, calculated dependencies of the macroscopic photoinduced dichroism on exciting intensity for different values of molecular dichroism are shown in Figure 5. For the WT BR, the experimental dependence of the photoinduced dichroism on exciting intensity at $\lambda = 633, 510$ and 488 nm (activating predominantly initial B -form) is shown in Figure 8, curve 1. It is seen that a very good agreement is observed between the experimental curve and theoretical curve 2 in Figure 5. Thus for all these wavelengths, the molecular dichroism of the B -form, k^B equals 0.04.

As is known, both B and M -forms are photoactive (Figure 1). When the laser beam simultaneously activates B and M -forms, the distribution of populations between the initial form B (N^B) and form M (N^M) is determined by the following balance equation:

$$\frac{dN^B}{dt} = \left(\sigma^M A^{M \rightarrow B} \frac{I}{h\nu} + \tau^{-1} \right) N^M - \sigma^B A^{B \rightarrow M} \frac{1}{h\nu} N^B \quad (11)$$

where σ^M is an absorption cross-section for the M -form at exciting wavelength, $A^{M \rightarrow B}$ is a quantum yield of $M \rightarrow B$ transition. Solving (11) for $N^B(\varphi)/2\pi$ and integrating, we come to:

$$D_{\perp} - D_{\parallel} = 2D_0 \frac{1-k_{test}^B}{1+k_{test}^B} \frac{EH - FG}{H^2} \left(\frac{G}{\sqrt{G^2 - H^2}} - 1 \right), \quad (12)$$

where $E = 2 + al(1+k^M)$, $F = al(1-k^M)$, $G = 2 + a(1+k^B + l(1+k^M))$, $H = a(1-k^B + l(1-k^M))$, $l = \frac{\sigma_{\parallel}^M A^{M \rightarrow B}}{\sigma_{\parallel}^B A^{B \rightarrow M}}$, k_{test}^B is molecular dichroism at λ_{test} .

The wavelength $\lambda = 458$ nm presents an isosbestic point for forms B and M . The calculation of the macroscopic photoinduced dichroism at 458 nm is of special interest (Figure 6). As is seen, the nature of the dependence of macroscopic photoinduced dichroism on exciting intensity in the region where spectra of forms B and M overlap, is conditioned by both the molecular dichroism of the B -form, k^B and the molecular dichroism of M -form, k^M . A good agreement is observed between experimental curve 2 in Figure 8 and the theoretical curve 2 in Figure 6. The calculated curves for different values of molecular dichroism k^B and k^M at

Figure 6. Calculated dependencies of the photoinduced dichroism $(D_{\perp} - D_{\parallel})/D_0$ on intensity $I_{exc}(a = \sigma_{\parallel}^B A^{B \rightarrow M} \tau I_{exc} / h\nu)$ at $\lambda_{exc} = 458$ nm.

Figure 7. Calculated dependencies of the photoinduced dichroism $(D_{\perp} - D_{\parallel})/D_0$ on intensity $I_{exc}(a = \sigma_{\parallel}^B A^{B \rightarrow M} \tau I_{exc} / h\nu)$ at $\lambda_{exc} = 442$ nm.

Figure 8. Experimental dependence of the photoinduced dichroism $(D_{\perp} - D_{\parallel}) / (D_0 - D_{sat})$ on intensity of an exciting laser beam I at 633, 510 and 488 nm (curve 1), 458 nm (curve 2) and 442 nm (curve 3) for the WT BR. D_{sat} is a residual absorption at $\lambda_{test} = 570$ nm upon saturation intensity irradiation.

442 nm (a region of a predominant absorption of M -form) are shown in Figure 7. A sufficient agreement is observed between the experimental curve 3 in Figure 8 and the theoretical curve 3 in Figure 7. We suggest that it is related to a more complicated decay of the M -form than it is assumed in the theoretical calculations. A comparison of experimentally obtained dependencies and calculated dependencies allows for the estimating the molecular dichroism of B -form, k^B and M -form, k^M at these wavelengths.

PHOTOINDUCED ANISOTROPY OF B -TYPE, M -TYPE AND B - M -TYPE

Thus, BR molecules both in B and M -forms possess anisotropic absorption. Two different methods can be applied to induce anisotropy in BR polymer films. The first method which has already been reported [4, 5, 11, 12] employs linearly polarized light with wavelengths activating predominantly the $B \rightarrow M$ transition. By analogy with hologram types [13], we designate this method of photoinduced anisotropy as ' B -type anisotropy'. We use mostly the He-Ne laser, $\lambda = 633$ nm to induce B -type anisotropy. Another method which was not previously reported, involves activation of the $M \rightarrow B$ transition with linearly polarized blue light. We designate it as ' M -type anisotropy'. To induce anisotropy of the latter type, it is

Figure 9. Calculated dependence of the transmittance T_{P-BR-A} of P-BR-A system on laser intensity $I(a_1 = \sigma_{\parallel}^B A^{B \rightarrow M} \tau I_{He-Ne}/h\nu_1, a_2 = \sigma_{\parallel}^B A^{B \rightarrow M} \tau I_{He-Cd}/h\nu_2)$: B -type anisotropy (curve 1), M -type anisotropy (curve 2), $B - M$ -type anisotropy (curve 3).

necessary to populate the M -form by illuminating the sample with light activating just the initial form B (520–670 nm) and run simultaneous anisotropic photoselection of M molecules with linearly polarized blue light. We concurrently employ the He-Cd laser, $\lambda = 442$ nm and a He-Ne laser, $\lambda = 633$ nm to induce the M -type anisotropy.

For M -type anisotropy, the distribution of populations between B -form, N^B and M -form, N^M is determined by the following balance equation:

$$\frac{dN^B}{dt} = \sum_{i=1,2} \left(\sigma_i^M A^{M \rightarrow B} \frac{I_i}{h\nu_i} N^M - \sigma_i^B A^{B \rightarrow M} \frac{I_i}{h\nu_i} N^B \right) + \frac{N^M}{\tau}, \quad (13)$$

where indices 1 and 2 refer to the red and blue light, respectively (Figure 1). The difference between B and M -types of anisotropy is the most pronounced in the characteristics of photoinduced reversible birefringence. The value of birefringence is calculated similarly to Equation (5) and measured as the transmittance T_{P-BR-A} of the system P-BR-A (Figure 3). The calculated dependence of T_{P-BR-A} on the blue light intensity at constant intensity of the circular polarized red light is shown in Figure 9, curve 2. However, the red light does not need to be circular polarized to populate the M -form. The linearly polarized red light at near-saturation intensity transfers all molecules to the M -form (Figure 2c). The sample is brought into a transient newly isotropic state, and B -type anisotropy decays at $a_1 > 100$ (Figure 9, curve 1). The calculated dependence of the T_{P-BR-A} on the blue light intensity at

Figure 10. Experimental dependence of the transmittance T_{p-BR-A} on laser intensity I for the WT BR: for B -type anisotropy (curve 1), intensity I_1 of the He-Ne laser is varied, intensity I_2 of He-Cd laser equals zero; for M -type anisotropy (curve 2) and $B - M$ -type anisotropy (curve 3), I_1 is 150 mW/cm^{-2} , I_2 is varied.

constant intensity of linearly polarized red light is shown in Figure 9, curve 3. In a strict sense, this dependence is related to mixed $B - M$ -type anisotropy since this anisotropy type is based on the anisotropic properties of both B and M -forms. The experimental dependencies for the B -type, M -type and $B - M$ -type anisotropy are shown in Figure 10 (curves 1, 2 and 3, respectively). It is seen, that $B - M$ -type anisotropy produces the highest signal. Thus, $B - M$ -type anisotropy holds much greater promise for an implementation of devices capable of controlling light with light.

ESTIMATION OF MOLECULAR DICHROISM OF B AND M -FORMS

A comparison of experimentally obtained dependencies and the calculated dependencies for B -type, M -type and $B - M$ -type anisotropy gives an additional information for the estimation of molecular dichroism of B -form, k^B and M -form, k^M at 442 nm. For the WT BR, the results of estimating molecular dichroism for B and M -forms are shown in Table II. It is seen that k^B equals 0.04 over the wide range of wavelengths. An increase of k^B in 440–460 nm range is evidently associated with short-wave absorption β -band of B -form [14], (Figure 11). In such a manner our results add considerable support for the speculation reported in [14]

Figure 11. Spectra of the initial *B*-form and the phototransformed *M*-form in the WT BR at -180°C [15].

Table II. Molecular dichroism of initial *B*-form k^B and longest-lived *M*-form k^M for WT BR

Photocycle Intermediate	Wavelength (nm)	Molecular dichroism, k^B , k^M
<i>B</i> -form, absorption peak at 570 nm	633, 510, 488	0.04 ± 0.005
	458	0.1 ± 0.02
	442	0.6 ± 0.1
<i>M</i> -form, absorption peak at 412 nm	458	0.02 ± 0.002
	442	0.11 ± 0.01

that the transition vector of the β -band is perpendicular to the vector of the main band for the *B*-form.

At the same time, in 458–442 nm range k^M drastically increases from 0.02 to 0.11. The molecular dichroism of *M*-form k^M not only reflects the retinal configuration change from all-trans in *B*-form to 13-cis in *M*-form but the change in the chromophore/protein interaction as well. This seems to be an evidence that the expansion of electron-density distribution for the *M*-form takes place, hence, indicating a weaker chromophore/protein interaction in the *M*-form as compared to *B*-form. Alternatively, molecular volume expansion for the *M*-form as compared to *B*-form has been reported [16]. Thus, both volume change technique and photoinduced anisotropy method point to a weaker chromophore/protein interaction

Table III. Molecular dichroism of initial *B*-form k^B for WT BR, genetic mutants D96E and D96N BR and reconstructed proteins 4-keto and 4-keto-D96N BR

BR Type	Wavelength (nm)	Molecular dichroism, k^B
WT	633, 510, 488	0.04 ± 0.005
D96E, D96N	633, 510, 488	0.06 ± 0.005
4-keto, 4-keto-D96N	510, 488	0.14 ± 0.01

in the *M*-form as compared to the *B*-form. However, it should be noted that the reason for such dramatic wavelength dependence of k^M is currently unclear and is a subject of further investigation.

PHOTOINDUCED ANISOTROPY OF BR MUTANTS AND DERIVATIVES

Similarly, we have determined the molecular dichroism of the initial *B*-form k^B for genetic mutants D96E and D96N BR and reconstituted proteins 4-keto and 4-keto-D96N BR (Table III). Molecular dichroism is slightly increased in D96E and D96N BR compared to the WT BR. This is an indication that site-specific mutations D96E and D96N result in insignificant weakening of chromophore/protein interaction as against the WT BR. As is known, strongly altered photochemistry of these mutants is not accompanied by any substantial change of their structure and/or any loss of stability [13]. At the same time, the molecular dichroism for reconstituted proteins increases more considerably, i.e., chromophore substitution in 4-keto and 4-keto-D96N BR causes much stronger disruption of chromophore/protein interaction. Chemical modification of purple membranes along with extended irradiation during the preparation of apoproteins leads to a noticeable change of a membrane structure, specifically, certain conformation changes take place in apoprotein which are induced by modified chromophore/protein interaction [17]. Thus, a combination of photoinduced anisotropy method and several biochemical approaches is a very promising research field.

Conclusions

1. Nonlinear photoinduced dichroism and birefringence in BR films are explained in the context of a model for reversible anisotropic photoselection of BR molecules immobilized in polymer matrix. A comparison of the experimental and calculated dependencies of nonlinear photoinduced anisotropy on laser irradiation intensity enables one to determine the molecular dichroism in any chromoproteins.

2. A correlation between the laser-induced nonlinear anisotropic properties and chromophore/protein interactions in chromoprotein BR is shown. Chromophore

substitution in 4-keto BR derivatives is shown to cause a certain modification of chromophore/protein interaction.

References

1. Birge, R.: Nature of the primary photochemical events in rhodopsin and bacteriorhodopsin, *Biochimica et Biophysica Acta* **1016** (1990), 293–327.
2. Miyasaka, T.: Design of intelligent optical sensors with organized bacteriorhodopsin films, *Jpn. J. Appl. Phys.* **34** (1995), 3920–3924.
3. Kramer, M., Tompkin, W. and Boyd, R.: Nonlinear optical interactions in fluorescein-doped boric acid glass, *Phys. Rev. A* **34** (1986), No. 3, 2026–2031.
4. Korchemskaya, E., Soskin, M., Stepanchikov, D., Druzhenko, A. and Dyukova, T.: Nonlinear polarization interaction in bacteriorhodopsin films with anisotropically saturating absorption, *SPIE Proc.* **2802** (1995), 200–210.
5. Burykin, N., Korchemskaya, E., Soskin, M., et al.: Photoinduced anisotropy in Biochrom films, *Opt. Commun.* **54** (1985), No. 2, 68–71.
6. Nagle, J., Bhattacharjee, S., Parodi, L. and Lozier, R.: Effect of photoselection upon saturation and the dichroic ratio in flash experiments upon effectively immobilized systems, *Photochem. Photobiol.* **38** (1983), 331–339.
7. Dyukova, T. and Lukashev, E.: Dehydration effects on D96N bacteriorhodopsin films, *Thin Solid Films* **283** (1996), 1–4.
8. Dyukova, T., Robertson, B. and Weetall, H.: Optical and electrical characterization of bacteriorhodopsin films, *BioSystems* **41** (1997), 91–98.
9. Druzhenko, A. and Weetall, H.: Photoinduced transformations of wild type and D96N mutant 4-keto-bacteriorhodopsin gelatin films, *Thin Solid Films* **293** (1997), No. 1–2, 281–284.
10. Dyukova, T., Vsevolodov, N. and Chekulaeva, L.: Dehydration-induced changes in photochemical activity of bacteriorhodopsin in polymer matrix, *Biofizika* **30** (1985), No. 4, 613–616.
11. Korchemskaya, E. and Soskin, M.: Application of anisotropically-saturating nonlinearity of polymer films based on bacteriorhodopsin for spatially-intensity filtration, *Optical Memory and Neural Networks* **4** (1995), No. 1, 53–59.
12. Imam, H., Lindvold, L. and Ramanujam, P.: Photoanisotropic incoherent-to-coherent converter using a bacteriorhodopsin thin film, *Optics Letters* **20** (1995), 225–227.
13. Hampp, N., Brauchle, C. and Oesterhelt, D.: Bacteriorhodopsin wildtype and variant aspartate-96'asparagine as reversible holographic media, *Biophys. J.* **58** (1990), 83–93.
14. Sperling, W. and Rafferty, C.N.: Relationship between absorption spectrum and molecular conformations of 11-cis-retinal, *Nature* **224** (1969), 591–594.
15. Balashov, S.P. and Litvin, F.F.: Photochemical transformations of bacteriorhodopsin, *Biofizika* **26** (1981), No. 3, 557–570.
16. Braslavsky, S.: Time-resolved photothermal studies with biological photoreceptors, *The Spectrum*, 1994, 10–16.
17. Tuzi, S., Yamaguchi, S., Naito, A., Needleman, R., Lanyi, J. and Saito, H.: Conformation and dynamics of [3-¹³C] Ala-labeled bacteriorhodopsin and bacterioopsin, induced by interaction with retinal and its analogs, as studied by ¹³C nuclear magnetic resonance, *Biochemistry* **35** (1996), No. 23, 7520–7527.