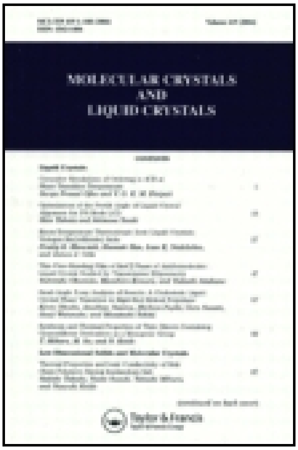

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# Linear Stark Effect in Bacteriorhodopsin-Containing Oriented Purple Membrane Films

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# LINEAR STARK EFFECT IN BACTERIORHODOPSIN-CONTAINING ORIENTED PURPLE MEMBRANE FILMS

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Abstract: By appling an oscillating 25 V electric field across an oriented purple membrane we observed a large electrochromic effect with $\Delta A = -1.46 \cdot 10^{-3}$ at 608 nm.

## INTRODUCTION

During the last years several technical applications of bacteriorhodopsin (bR) and bR-containing purple membranes (PM) have been suggested. The unique physical, chemical and biological properties of bR have stimulated activities in this direction [1,2]. It is very likely that bR is the most promising biological material for some technical applications. Several approaches to this subject exist.

First one may consider bR as a photochromic material for the recording and readout of optical information. This approach combines purely optical (optical pump - optical probe) methods like e.g. holography, interferometry, optical memory and bistability [2]. One advantage of bR-based materials is especially evident. The photochemical stability of bR leads to the extreme resistance of bR compared to other materials for multiple write/readout/erase processes.

In parallel to spectral changes a photovoltage arises over the purple membrane under illumination. This photovoltage is associated with the main biological function of bR as a light-driven proton pump. The bacteriorhodopsin molecules in PM form a two dimensional crystal . For this reason, purple membranes are excellent building blocks for different oriented systems over which the photoelectric response can be measured. From an application point of view one can speak about photoelectric or optoelectronic (optical pump - electrial probe) methods. An artifical photoacceptor comprising a 64-pixel network of the bR photocell was recently fabricated and connected to an electronic readout system [4].

The primary photochemical events in bR (J and K intermediate formation) take 0.5 and 3 ps. respectively; as measured by a femtosecond optical pump-probe method [5]. The ultrafast photoelectric response of PM has been measured using a sample head [6] or the Josephson junction [7] oscilloscope methods. Photoelectric response times of <21 ps and < 5 ps, respectively, were obtained. These findings indicate further possibilities to produce ultrafast bR-containing optoelectronic devices.

A high second order optical nonlinearity of PM-fabricated systems has been reported in several publications [2]. We proposed a completely optical method for the measurement of the photoinduced electric response of bacteriorodopsin in oriented purple membrane films (OPMF), which can be called the electrooptical pump-probe method [8]. A femtosecond light pulse initiates the bR photocycle and the charge separation. This leads to the appearance of a transient photovoltage over the OPMF. After the excitation, in the presence of a transient electric field, the refractive index of OPMF and the polarization of probing pulse will be changed due to the electrooptical (Pockels) effect in OPMF. The variation of the polarization depends on the transient electric field, and can be measured after the analyzer. Using this method one can obtain the photovoltage time dependence with high temporal (subpicosend) resolution without using an electronic device, e.g. oscilloscope. Technical application of this method in electrooptical modulators (switches), which use photoinduced or external electric field, can also be considered.

However, electrooptical effect can be tested only in the IR region, where the OPMF is transparent, because of a number of interfering polarization-dependent effects, like circular (CD) and linear (LD) dichroism, in the spectral region of the bR absorption. Furthermore, photoselection of bR molecules with polarized light influences the results considerable. In the case of an applied external electric field the electrochromic (Stark) effect will occur in the region of the absorption band. The influence of an external electric field on the absorption spectrum was investigated for all-trans retinal and the protonated or unprotonated Schiff base in solution [9], all-trans retinal in unoriented films [10], and for unoriented dried [11-12] and PVA [13] films of PM. This effect is, however, expected to be much stronger in oriented systems. Moreover, in the case of a strong effect it can be considered for possible practial applications of bR films as optical modulators. In this publication we consider the electrochromic Stark effect of OPMF.

## MATERIALS AND METHODS

The oriented purple membrane films were prepared according to ref. [14]. They were electrically deposited on a glass substrate covered with $SnO_2$ as transparent electrode. The second glass/$SnO_2$ electrode was tightly connected to the OPMF surface. This capacitor construction gives good contact with OPMF without creating a short circuit as measured in the photovoltage experiment using a Keithley 610 CR electrometer. A 10 V photoelectric signal was obtained under illumination with a 5mW He-Ne laser corresponding to the typical photoelectric response of OPMF [15]. The external 25 V (peak-to-peak) voltage was applied at 800 Hz to the $SnO_2$ electrodes by a Sweep Function Voltage Generator. The reference signal from the generator was applied to the reference input of a PARC 5206 lock-in amplifier.

The sample was placed into the sample compartment of a SPEX spectrofluorimeter. The 450 W xenon lamp and excitation monochromator of the spectrofluorimeter were used for the OPMF optical probing. When an external electric field was not applied to OPMF, the Xe lamp light was chopped at 800 Hz. The light intensity transmitted through the sample (I) was measured at this frequency with a photodiode connected to the signal input of the lock-in amplifier. The incident light was not chopped when an electric field modulated at 800 Hz was applied to the film. A part of the transmitted light, which was modulated at 800 Hz due to the Stark effect (ΔI) was measured at the same frequency. The wavelength dependence $\Delta I(\lambda)/I\ (\lambda)$ was then calculated. Electrically induced optical density changes $\Delta A\ (\lambda)$ assuming $\Delta I << 1$ can be calculated according to:

$$\Delta A(\lambda) = \lg(1 + \Delta I/I) = -\Delta I/2.3I \qquad (1)$$

The optical absorption spectra were measured with a Beckman DU-70 spectrophotometer. The maximum absorption of the film at 560 nm was 1.9. The thickness of the OPMF used was calculated according the procedure described in [8] and was estimated to 7.2 ± 0.5 µm. This means that the amplitude of the applied electric field was $3.47 \times 10^4$ V/cm. All measurements were made at room temperature and at a relative humidity of 45 %.

## RESULTS AND DISCUSSION

The absorption spectrum of OPMF is shown in Fig. 1 (solid line). The film was dark adapted and the maximum is located at 562 ± 1 nm. For dried purple membrane films complete dark to light adaptation occurs only for relative humidities above 70 % [16]. Furthermore, the light-adapted state for the films does not occur at humidities below 50 % [16]. The presented absorption spectrum is the difference between experimentally measured OPMF spectrum and the spectrum of the $SnO_2$ covered substrate. We did not measure the absorption spectrum at wavelengths shorter than 440 nm because of the strong absorption. The spectrum of dark adapted PM in suspension is shown by the dotted line in Fig. 1. Its absorption maximum is located at 560 nm, typical for dark-adapted samples.

Figure 2 (solid line) shows the relative transmittance changes of OPMF, ΔI/I, obtained at 800 Hz in the presence of an applied electric field modulated by the same frequency. The electrochromic spectrum shows bipolar signal with positive peak at 512 nm, negative peak at 608 nm, and a zero crossing point at 567 nm. A small negative band at 403 nm is also noted. The value of relative transmittance at 608 nm is $-1.46 \times 10^3$. The signal is linear with respect to the applied electric field and changes polarity as the polarity of the applied field is changed. The measurements were made at normal incidence of the depolarized probing beam. When the angle of incidence was 45°, the peaks

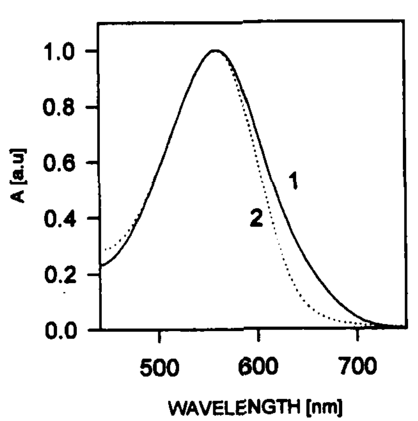

Fig. 1.

Absorption spectrum of a bacteriorhodopsin - containing oriented purple membrane film (1) obtained after subtracting the $SnO_2$ covered substrate spectrum, and the absorption spectrum of purple membranes in suspension (2).

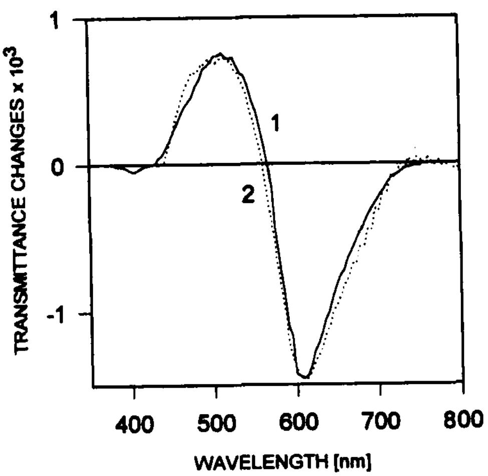

Fig. 2.

Relative transmittance changes induced in OPMF by the external electric field (1) and the first derivate of the OPMF absorption spectrum $dA/d\lambda$ multiplied by $\lambda^2$ (2).

positions and the zero crossing point did not change. However, the ratio of the 608 nm peak amplitude to the amplitude of the 512 nm peak was 2.35 insteaded of 1.92 as for normal incidence.

The theory of electrochromic effects in molecular systems was developed by Labhart [17] and Liptay [18], and especially in the case of solid films by Reich and Schmidt [19]. In solid films the molecules are not oriented by the applied external electric field. The applied field (F) changes the energy of molecular states due to the Stark effect. If the molecular dipole moments in the ground and excited electronic states are $\mu_g$ and $\mu_e$, respectively, the corresponding energy changes are

$$E_g = E_g^o - \mu_g F - 1/2\, \alpha_g F^2$$

$$E_e = E_e^o - \mu_e F - 1/2\, \alpha_e F^2 \qquad (2)$$

where $\alpha_g$ and $\alpha_e$ are the ground and excited state polarizabilities, respectively. Shift of the absorption band will then be

$$\Delta\nu = - (\Delta\mu/h)\, F \cos\theta - 1/2\, (\Delta\alpha/h)\, F^2 \qquad (3)$$

where $\Delta\mu = \mu_e - \mu_g$, $\Delta\alpha = \alpha_g$ and $\theta$ is the angle between $\Delta\mu$ and F. For oriented systems the main term is:

$$\Delta\nu = -(\Delta\mu/h)\, F \cos\theta \tag{4}$$

The relevance of this shift to the experimentally measured optical density changes $\Delta A$ ($\lambda$) is considered in [19]. Taking into account that at small signal amplitudes $\Delta A$ ($\lambda$) is proportional to the induced relative transmittance changes, $\Delta I/I$ and the transforming formula given in [19], we can find for oriented systems:

$$\Delta I/I = 2.3\, (\Delta\mu/hc)\, F \cos\theta\, \lambda^2\, dA/d\lambda \tag{5}$$

where $dA/d\lambda$ is the first derivate of the absorbance of the Stark-shifted transition. Figure 2 (dotted line) shows the first derivate $dA/d\lambda$ of the OPMF absorption spectrum multiplied by $\lambda^2$. This $\lambda^2\, dA/d\lambda$ term is normalized at the minimum of the spectrum. One can observe an agreement between the two curves presented in Fig. 2 in accordance with (5). They are different mainly at the zero crossing. The zero crossing is shifted from 562 nm for the $\lambda^2\, dA/d\lambda$ term (position of the OPMF absorption spectrum maximum) to 567 nm for the electrochromic signal. This means, however, that the absorption band, which is different from the absorption band of OPMF, is shifted due to the Stark effect and has the absorption maximum at 567 nm. Absorption spectra of PM in suspension and OPMF have similar absorption maxima as shown in Fig. 1. However, absorption spectrum of OPMF is broader, mainly in the long wavelength region. We can suggest that the long wavelength absorption is in OPMF is due to a transition that is shifted when the electric field is applied. Because this transition is more sensitive to the applied electric field than that at shorter wavelengths, it should have a higher $\Delta\mu$ value or a smaller angle $\theta$ between $\Delta\mu$ and the applied field F.

Bacteriorhodopsin molecules are organized as trimers in PM , and an excitonic interaction between the bR molecules in trimers was proposed [20, 21]. Due to excitonic interaction molecular degenerate transitions are split into a doubly degenerate excitonic transition polarized in-plane and a non-degenerate transition polarized out of plane. Recently several arguments against [22, 23] and in favor [24] at the excitonic model were published. The Stark-effect of OPMF should be very sensitive to the excitonic interaction because only the out-of-plane excitonic state should contribute to the electrically induced spectral shift. One can assume that the absorption band having a maximum at 567 nm is due to an excitonic transition which is, red shifted relative to the molecular transition and polarized perpendicular to the OPMF plane. However, in case of the excitonic interaction, the absorption spectrum of OPMF should only be due to absorption of the excitonic doubly degenerate state polarized in the film plane. This state should be blue shifted in our case compared to the molecular transition. Therefore, OPMF absorption spectrum should be red shifted in comparison with absorption spectrum of PM in suspension and have smaller absorption in red part of the spectrum. This in contradiction, however, to the absorption spectra shown in Fig. 1. Therefore, the OPMF absorption and electrically induced spectra together speaks against excitonic interaction in bR trimers. Moreover, the Stark-effect sensitive out-of-plane transition should be blue shifted as predicted [16, 21].

The absorption band with the maximum at 567 nm can be attributed to the all-trans retinal containing bR molecules of the dark-adapted film. This means, however, that these molecules contribute mainly to the electrically induced spectral shift. The value of $\Delta\mu$ is 12.7 D [13] or 13.5 D [25] for the all-trans retinal containing bR molecules. Assuming that the Stark effect in OPMF is mainly due to all-trans molecules this should indicate that the permanent dipole moment changes are several times smaller for 13-cis molecules than for all-trans molecules.

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