Model of Photovoltage Response of Bacteriorhodopsin in PVA Films

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ABSTRACT

Bacteriorhodopsin (BR) is a light-transducing protein contained in a purple membrane of microorganism *Halobacterium salinarium*. Natural function of BR is to act as a light-driven proton pump in energy balancing mechanism of the bacterium. Numerous technical applications of BR that use proton pumping have been proposed [11]. A thin film of oriented BR enclosed between two electrodes generates a temporal photovoltage response when illuminated [13]. The intensity of photoresponse depends on intensity and spectral power distribution of illuminating light. This work describes a model of wavelength dependency of photoresponse of elements containing a thin film of wild form of BR and its reconstituents and compares the model to experimentally measured values.

Keywords: Absorption, Bacteriorhodopsin, Retinal, Smart Sensors

1 INTRODUCTION

The visual system of living species has been a common source of inspiration in machine vision research. So-called smart sensors are analog VLSI devices that attempt to mimic neurological signal processing such as those in retina [10]. Although resolution and signal processing capacities of the present devices are limited by current VLSI technology, the silicon retinas currently under development may one day serve in a prosthetic device for the blind [7].

Advanced materials technology offers a potential for new and improved smart sensors. Biotechnology can provide materials especially suitable for implementing natural vision functions. One protein, bacteriorhodopsin (BR), responds to light with a differential sensitivity common in motion detection and edge enhancement [5]. Such capabilities are found in natural sensors, for example in human eye.

In our research we use BR as an active material for building an artificial retina. We have constructed a photosensor in which wild-type BR and its two synthetic modifications were combined in a single matrix [13]. Each of the three BR types have shown different opto-electric response with respect to wavelength, which makes possible to add color recognition capability to BR based photosensors [3]. This work describes a model of wavelength dependency of photoresponse of elements containing a thin film of wild-type BR and its 4-keto and 3,4-dehydro modifications and compares the model to experimentally measured values.

2 BACTERIORHODOPSIN BASED SENSOR

The purple membrane of microorganism *Halobacterium salinarium* contains the bacteriorhodopsin (BR), a light-transducing protein related to human visual protein rhodopsin. As with all rhodopsins, BR is composed of a protein part and functional retinal chromophore, a derivative of vitamin A. Natural function of BR is to act as a light-driven proton pump [1, 4] in energy balancing mechanism of bacteria moving protons from the inside to the outside of the cell in order to create a proton gradient across the cell membrane under anaerobic conditions. The proton transport through the cell membrane is coupled with photochemical conversions occurring during photocycle of BR.

BR retains its photocycle even when isolated from the purple membrane and incorporated into an artificial membrane [6] or thin polymeric film [2, 12]. A thin film of oriented BR enclosed between two electrodes generates a temporal photovoltage when illuminated (Figure 1). When the light is turned on, a transient photovoltage is generated across the film due to a charge displacement within BR molecules. The photovoltage appears as a fast rising spike which reaches saturation within approximately a millisecond following illumination and returns exponentially towards zero. The spike amplitude is proportional to intensity and spectral distribution of the light pulse.

Beside naturally occurring form, BR can also be engineered artificially. Artificial modification of BR offers unique possibility to adapt its optical and opto-electric properties to the demands of a specific application. For our purpose, we modified properties of wild-type BR by *in vitro* reconstituting bleached bacteria membrane with synthetic retinal analogues, obtaining so 4-keto BR and 3,4-dehydro BR variants.

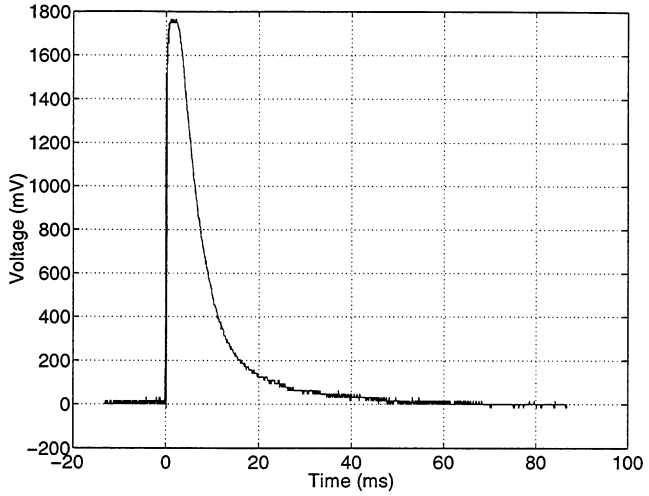

Figure 1: Photoelectrical signal from the wild-type BR element.

2.1 Model of the photovoltaic response

The mechanism of photoresponse of BR is a quantum process — after the retinal part of a BR molecule absorbs a photon, the molecule starts photocycle during which a charge shift occurs. The charge shift can be registered as a photoresponse. Assuming that quantum efficiency of the retinal is constant across the visible region of spectrum, the strength of the photoresponse should be proportional at given wavelength λ to a fraction of quanta absorbed

$$R_\lambda = k * \alpha_\lambda * \frac{\lambda P_\lambda}{hc} \tag{1}$$

where α_λ is the absorptance of the retinal, P_λ is the power of incident light, h is Planck constant, c is velocity of light, and k is a suitable constant. Absorption rate of the retinal is a function of wavelength, so the photoresponse depends also on a spectrum of illuminating light. The model response of the element is calculated by integrating (1) over λ

$$R = \frac{k}{hc} * \int \lambda P(\lambda)\, \alpha(\lambda)\, \mathrm{d}\lambda \tag{2}$$

where $\alpha(\lambda)$ is the absorptance function of the retinal, and $P(\lambda)$ is the spectral power distribution of incident light. Absorbance function is often provided instead of absorptance function. Absorptance $\alpha(\lambda)$ than can be directly expressed in terms of absorbance $A(\lambda)$ by

$$\alpha(\lambda) = 1 - 10^{A(\lambda)} \tag{3}$$

The most intricate step in modeling photoresponse of BR and its variants is to obtain a good estimate of either absorbance or absorptance functions of their retinals. Though the absorbance functions of BR elements can be easily measured e.g. with spectrophotometer, the measured functions are not absorbance functions of the retinals — the elements contain also other light absorbing matter in addition to the retinals, as protein, glass, gold, PVA, ITO, and some impurities. The measured absorbance functions have to be corrected for the nonretinal absorption. This is not straightforward, since the absorbances of some nonretinal matter cannot be measured independently. For example, the absorbance function of BR containing retinal differs from the absorbance function that could be derived by simple superposition of absorbance functions of bleached BR, that is BR without retinal, and retinal alone.

One possibility to obtain the absorptance function of a retinal is based on a structural and functional similarity between BR and rhodopsins in human photoreceptors. In this method, an absorptance template function for rhodopsins is used in place of the absorptance function of the retinal [8].

The absorptance template is parameterized by the wavelength of maximum absorption λ_{max}. The λ_{max} can be directly found from spectrophotometric measurements. As the measurements of absorption are made at fixed wavelength intervals, interpolation have be used. However, the absorption curve near the maximum is relatively flat and interpolation may provide rather imprecise value of the λ_{max}, especially in the case of noisy data. The wavelength of maximum absorption can be derived more accurately from the wavelength at half of the peak absorption $\lambda_{0.5}$ [9]. Both wavelengths are simply related through a multiplicative constant which can be obtained directly from the used template. The effect of noise is so greatly reduced, since absorption changes most rapidly with wavelength near the $\lambda_{0.5}$. The $\lambda_{0.5}$ is obtained directly by interpolating from spectrophotometric measurements. MacNichol [9] also proposes a measure of goodness of the fit as a product of slope tangent s to the measured absorptance at the $\lambda_{0.5}$ times $\lambda_{0.5}$: $Q = s * \lambda_{0.5}$. If plotted on a relative wavelength scale, the spectral absorptance function of retinal based visual pigments was found invariant to position of the λ_{max}. Therefore Q is a constant. The template we used provided $Q = 8.78$ [8].

Figure 2 contains plot of measured absorbance functions of the three variants of BR and the fitted templates.

2.2 Measurements

Elements containing polyvinylalcohol (PVA) films with wild-type BR, 3,4-dehydro BR, and 4-keto BR, have been prepared as described in [13]. Absorption spectra of all three elements were measured using standard spectrophotometer. The measurements were done in 1 nm steps from 189 to 900 nm. Figure 2 contains plot of measured absorbance functions of the three vari-

Computational Nanoscience 2001, (www.cr.org), ISBN 0-9708275-3-9.

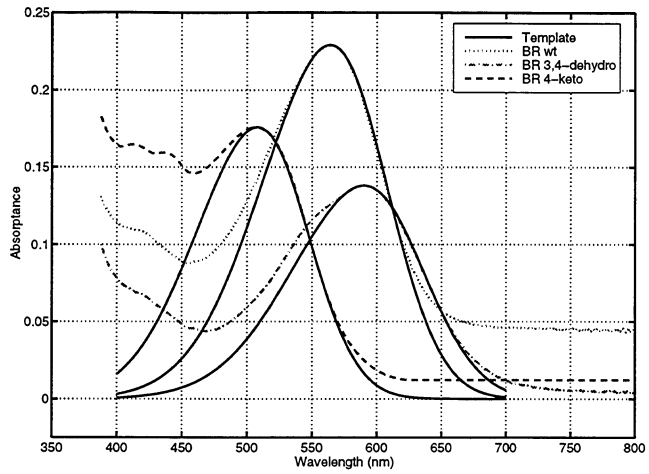

Figure 2: Absorption spectra of the three variants of BR and the fitted templates.

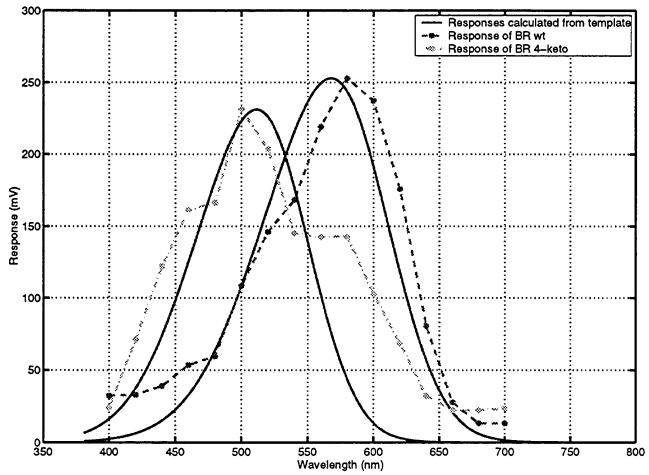

Figure 3: Compensated responses for BR wild type and BR 4-keto compared to responses calculated from template.

ants of BR and the templates fitted as described previously. The measured absorbance maxima λ_{max} for the three elements were 564 nm for wild-type BR, 590 nm for 3,4-dehydro BR, and 508 nm for 4-keto BR, respectively. The maxima λ_{max} derived from $\lambda_{0.5}$ using template method differed by less than 0.02 nm from the measured values. The value of Q was 7.44 for wild-type BR, 8.34 for 3,4-dehydro BR, and 8.81 for 4-keto BR, respectively.

In order to measure wavelength dependency of element response, the elements have been connected to a standard digital oscilloscope. A pulsed Oriel series Q xenon flash lamp was used as a source of short light pulses. The wavelength dependence of photoresponse was measured using a set of Oriel narrow band interference filters placed between the lamp and the elements. The filters were every 20 nm from 400 to 700 nm, the half width of the filters was about 10 nm. The wavelength dependence for two elements was measured — element containing wild-type BR and element containing 4-keto BR. The measured responses were compensated for transmittance of the flashlamp and for transmittance of narrow-band filters. The maximum compensated response for element with wild-type BR was at 580 nm, and for element with 4-keto BR was at 500 nm, respectively. The compensated photoelectrical responses are compared to responses calculated from templates in Figure 3.

3 CONCLUSIONS

Despite the response model is based on absorbance functions of retinals indirectly derived using templates, the modelled response functions are in good agreement with experimentally measured values. The peaks of responses differ by 16 nm for wild-type BR and 8 nm for 4-keto BR, respectively, which is less than the step between single interference filters. The results provide support both for the template method for obtaining approximations of retinal absorbance and the model of the photovoltage response.

ACKNOWLEDGEMENT

The research project has been supported by TEKES and the Academy of Finland. We wish to thank prof. Dieter Oesterhelt for the bacteria, and Ph.D Pertti Silfsten for fruitfull discussions.

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