Towards Color Sensitivity of Protein Based Artificial Retina

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ABSTRACT

Bacteriorhodopsin (BR) has been studied as a material for molecular electronics applications. Thin film elements based on BR and its variants in polyvinylalcohol (PVA) have been produced to determine the photoelectrical properties of the material for the development of an optoelectronic sensor. The measurement setup and results of basic measurements including the photoelectrical signal, intensity and wavelength dependencies of the elements are described. The results show that it is feasible to develop an artificial retina based on BR.

Keywords: Artificial retina, bacteriorhodopsin, color sensitivity.

1 INTRODUCTION

The feature size in electronic circuits has decreased so much that the laws of quantum mechanics and the limitations of fabrication technology are starting to prevent higher levels of integration. It can be seen from [1] that many problems are associated with the future production of semiconductors for which no known solutions are available at the moment. In order to continue the miniaturization of circuit elements down to the molecular scale, researchers are investigating several alternatives to the transistor for ultra-dense circuitry [2]. These alternatives include the use of new electronic, biochemical, mechanical or quantum devices. It is also possible to combine several technologies to create hybrid devices which perform better than a device based on one technology alone [1]. This is the idea behind the use of a light-sensitive biomolecules for optical information processing.

Bacteriorhodopsin (BR) has been studied as a potential material for a color sensitive artificial retina. Bacteriorhodopsin (BR) is the light-driven proton pump found in the membrane protein of *Halobacterium salinarium* [3]. It is a photosynthetic protein used by the bacteria to produce energy by a light-induced proton gradient across the cell membrane [4]. When BR is used as an oriented film between two electrodes immobilized by a polyvinylalcohol (PVA) matrix, it can produce a photoelectrical signal. The signal is due to the charge separation in the element. This film produces a stable photoelctrical signal. We have reported earlier its intensity and wavelength dependency [5]. These properties enables the use of BR-PVA elements in optoelectronic applications.

There has been quite a lot of interest to modify some characteristics of BR in search of better performing alternatives for applications [6]. Most applications of BR are dealing with optical information processing, especially holography and memory subsystems in which they utilize BR as a recording media [4]. This is why the usual attribute to vary is the set of time constants related to the state changes in the photocycle [7]. Another characteristic is the absorption spectrum connected to each state of the photocycle. The possibilities to control the properties of BR include the following according to [8]. First, modulation of the external proton availability affects the proton-dependent state changes in the photocycle. This possibility is related to the time constants of the photocycle. Second, the retinal chromophore of BR can be replaced by an analogue, e.g. 3, 4-dehydro and 4-keto. This approach changes the molecular structure of the retinal modifying the photocycle and the absorption spectra totally. Third, the amino acids like Asp85 and Asp96 acting as proton donors or acceptors can be modified to change the functionality of BR. This method is very flexible and allows the production of newly generated BR variants by conventional biotechnological methods.

The goal of our research is to use BR as a biomaterial for an advanced optoelectronic sensor to achieve color sensitivity in an artificial retina. To achieve this goal we have produced two variants of wild-type BR, 3, 4-dehydro and 4-keto using the second method, replacement of the retinal chromophore [9]. It has been shown that it is possible to alter the spectral properties of BR using this method. The method was selected to achieve large enough changes in the absorption spectra since color sensitivity of the device is dependent on this characteristic [10].

To understand the photoelectrical properties of wild-type BR and BR variants better, photosensitive elements based on BR-PVA films were produced. To be able to produce a prototype of a color sensitive device, different properties of BR types concerning the signals from the sensors and the intensity and wavelength de-

pendencies of their photoelectrical response were considered. This article describes these properties for the development of a color sensitive artificial retina.

2 MEASUREMENTS

In our previous work we have reported basic characteristics of BR films with different wavelength properties [11]. In this study, we have prepared a set of new BR elements, and used signal conditioning electronics to achieve a signal clean from noise. We have also used a pulsed Oriel series Q flashlamp with a xenon bulb which has a very short pulse (1.6 μs) when compared to the camera flash used previously. The energy of the pulse can also be set from 26 to 160 mJ. The concentration of the wild-type BR after isolation was 13.6 mg/ml. 300 μl of BR was mixed with 750 μl of PVA acting as the immobilizing matrix to form a dried film between two electrodes. The concentration of the 4-keto BR was 6.8 mg/ml, and 200 μl of it was mixed with 500 μl of PVA. The concentration of the 3,4-dehydro BR was 10.0 mg/ml, and 250 μl of it was mixed with 500 μl of PVA. The elements were connected to voltage followers for impedance matching and installed into aluminum cases to reduce electromagnetic interference from the environment. The signal was filtered using a signal conditioning stage before it was registered with a standard digital oscilloscope. The measurements were performed using the measurement equipment described in [5].

First, the photoelectrical signals from the elements were registered using a standard digital oscilloscope. The energy setting of the light source was 160 mJ, and the frequency of light pulses was 1.0 Hz. No saturation of BR was noticed even when the interval between the light pulses was decreased. The distance between the light source and the element was 400 mm, and the alignment of the equipment and focusing was performed so that maximum signal could be attained. The signals from the BR elements are shown in Figures 1, 2 and 3. The amplified photovoltage signals show that the elements are operating as expected from reports by others [12], [13], [14]. The 4-keto bacteriorhodopsin having the opposite direction of charge separation when compared to the other two BR types has been reported to possess interesting photochromic properties as well [15].

The intensity dependency of the elements was determined using the same light source and a set of neutral density filters between the light source and the element. The peak-to-peak voltage of a series of 10 measurements was registered using the oscilloscope for a constant distance between the light source and the element (400 mm) and a constant frequency of light pulses (1.0 Hz). The energy setting of the light source was varied from 30 to 150 mJ in 30 mJ steps. The result for different bias intensities including the average (solid line) and the minimum/maximum amplitude (dotted lines) for each

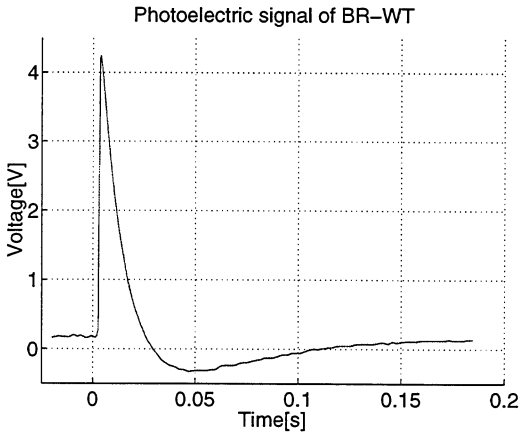

Figure 1: Photoelectrical signal from the wild-type bacteriorhodopsin element.

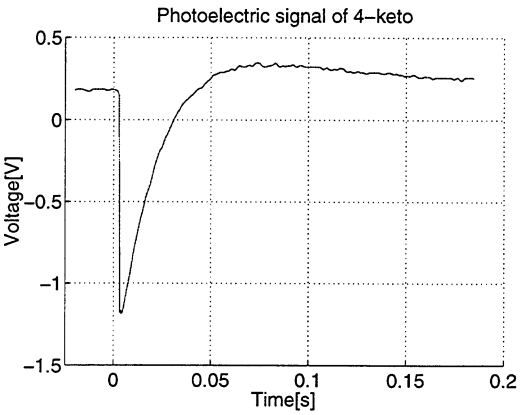

Figure 2: Photoelectrical signal from the 4-keto bacteriorhodopsin element.

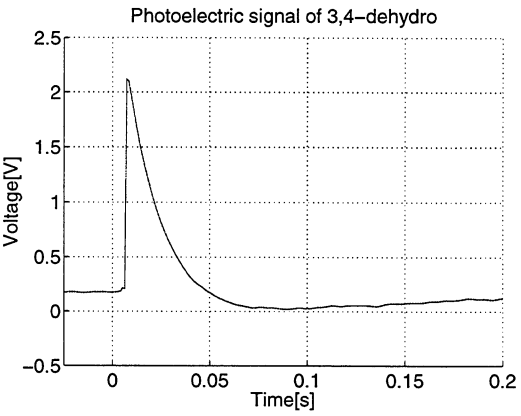

Figure 3: Photoelectrical signal from the 3,4-dehydro bacteriorhodopsin element.

BR element is shown in Figures 4, 5 and 6. The intensity dependency can be seen close to linear for the

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observed intensity range and the variance of the amplitude to be small thus allowing the sensing of different intensity levels.

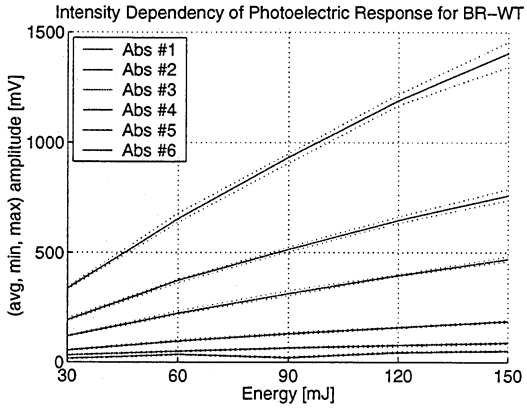

Figure 4: Intensity dependency of the wild-type bacteriorhodopsin element.

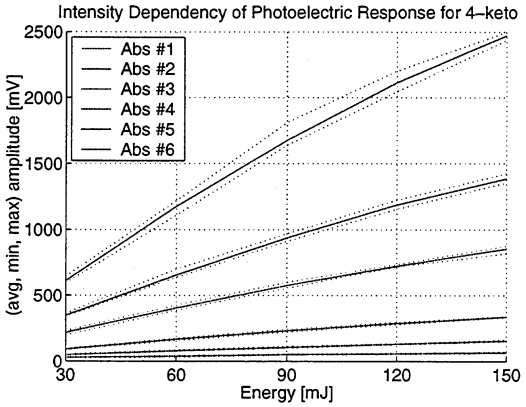

Figure 5: Intensity dependency of the 4-keto bacteriorhodopsin element.

Last, the light from the flashlamp was filtered by a set of interference filters covering the visible part of the electromagnetic spectrum. This was performed to register the wavelength dependency of the elements. The peak-to-peak voltage for a constant distance between the light source and the element (400 mm), a constant frequency of light pulses (1.0 Hz), and a constant energy setting (160 mJ) was registered with the oscilloscope. The compensated photoelectrical responses including the average (solid line) and the minimum/maximum amplitude (dotted lines) of 10 consequtive measurements for each element are shown in Figures 7, 8 and 9. The non-ideal transmittance spectrum of the light source has been compensated by measuring the power of light through the interference filters using an optical power meter, and estimating the transmittance of the interfer-

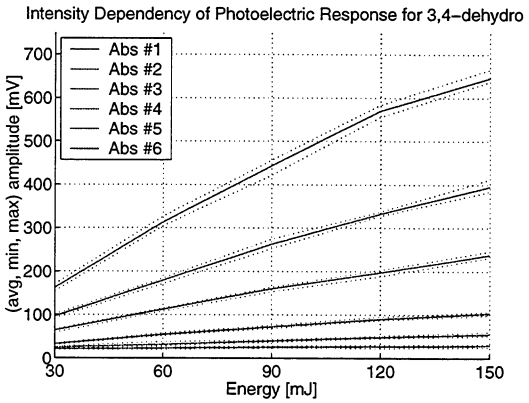

Figure 6: Intensity dependency of the 3,4-dehydro bacteriorhodopsin element.

ence filters using the parameters from the manufacturer and assuming the transmittance to be gaussian. The results containing multiple peaks show that a more accurate compensation method is required.

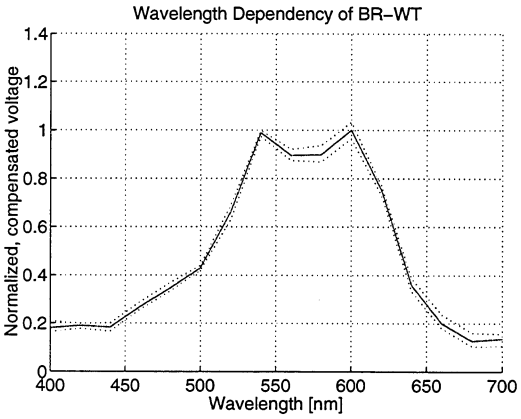

Figure 7: Wavelength dependency of the wild-type bacteriorhodopsin element.

3 Conclusion

Wild-type BR and two BR variants, 4-keto and 3,4-dehydro, were studied by registering the signals obtained from the BR elements and determing the intensity and wavelength dependency of the elements. The results verify the suitability of the material for the development of a color sensitive artificial retina. More work is required in order to clarify the effects of the measurement setup to the results.

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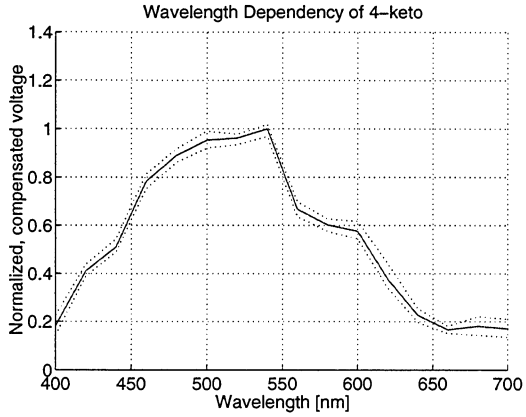

Figure 8: Wavelength dependency of the 4-keto bacteriorhodopsin element.

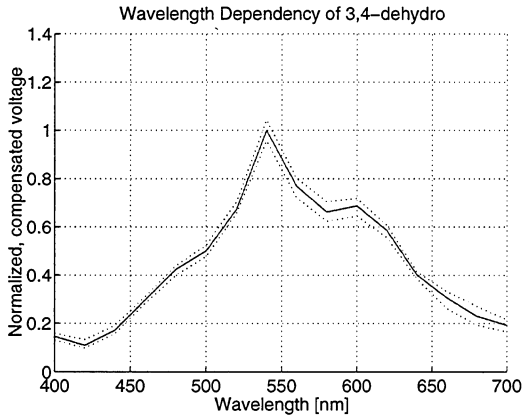

Figure 9: Wavelength dependency of the 3,4-dehydro bacteriorhodopsin element.

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