Potential applications of bacteriorhodopsin mutants

P. Saeedi,1 J. Mohammadian Moosaabadi,2,*, S. Sina Sebtahmadi,3 J. Fallah Mehrabadi,4 M. Behmanesh5 and S. Mekhilef3

1Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran; 2Department of Biochemistry; Faculty of Biosciences and Biotechnology; Malekashar University of Technology; Tehran, Iran; 3Faculty of Electrical Engineering; University of Malaya; Kuala Lumpur, Malaysia; 4Department of Genetic Engineering; Faculty of Biosciences and Biotechnology; Malekashar University of Technology; Tehran, Iran; 5Faculty of Science; Tarbiat Modares University; Tehran, Iran

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Bacteriorhodopsin (BR), a model system in biotechnology, is a G-protein dependent trans membrane protein which serves as a light driven proton pump in the cell membrane of Halobacterium salinarum. Due to the linkage of retinal to the protein, it seems colored and has numbers of versatile properties. As in vitro culture of the Halobacteria is very difficult, and isolation is time consuming and usually inefficient, production of genetically modified constructs of the protein is essential. There are three important characteristics based on protein catalytic cycle and molecular functions of photo-electric, photochromic and proton transporting, which makes this protein as a strategic molecule with potential applications in biotechnology. Such applications include protein films, used in artificial retinal implants, light modulators, three-dimensional optical memories, color photochromic sensors, photochromic and electrochromic papers and ink, biological camouflage and photo detectors for biodefense and non-defense purposes.

Introduction

Bacteriorhodopsin (BR), a seven α-helical protein taking in a chromophore molecule (retinal), is a light-dependent proton pump. Light photons activate the pump to make ATP by creating a proton gradient across the membrane.1 BR mutagenesis improves the optical and electrical properties of the protein to produce unique photoelectric, photochromic and electrochromic materials, has an important role in development of materials and tools based on BR. Mutant proteins, with different wavelength characteristics form a foundation for color sensitive sensors.

In industrial progress, having a prolonged energy supply is crucial. The incorporation of BR into electronic circuitry; leads to applications such as artificial retina, photochromic data storage, holographic memories, light batteries and information processing. Meanwhile, bacteriorhodopsin acts as a sensitive, wavelength specific photoactive proton pump and as a potential solar conversion, data storage or photo switching component when utilized in electrodes.2

Artificial Retina

Obtaining more advanced and less surgically invasive retinal implants is extremely desirable in the repair of vision of blind people with photoreceptor disorder suffering from retinal perturbation or similar diseases.3

BR is an identical protein to Rhodopsin of the retina, converting sunlight into chemical or electric energy during a photo cycle.4 It is capable of preserving the photo cycle even if secluded from the purple membrane and integrated into an artificial membrane, or a thin polymer-based film (Fig. 1).6

A “retina chip” or “silicon retina” is an electronic stimulator that activates the retinal ganglion cells electrically through a visual input repairing different degrees of vision. Electronic stimulators are not suitable for clinical application because of toxicity of foreign objects in the body. Consequently, there still remains a requirement for proper treatment/cure of this condition in patients.7

Multilayer BR films contain two-dimensional planar endogenous or exogenous lipid membrane surrounding mutant or wild type BR between thin polymers layers as in Figure 1; join to a transparent tin oxide electrode on a glass substrate. An electrolyte gel is injected in between the membrane and gold-coated glass.8 The sensor made from only wild type BR, lacks all wavelengths, so that only black and white images can be perceived, but not colored. By modifying the BR genetically, the different wavelength responses can be produced.4

Multilayer BR-based photocells respond to immediate light intensity changes and detect moving images. Therefore, relative films could provide the foundation for motion detectors or artificial vision apparatus.8,9

Light Batteries

Bacteriorhodopsin, a bionano component, is a potential candidate for integrating within nano-structures. It acts as a light sensor which can effectively be used as a solar collector to collect energy from (sun) light. This energy could either be consumed directly or stored in batteries similar to Figure 2.

*Correspondence to: J. Mohammadian Moosaabadi; Email: mohama_j@yahoo.com
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Fig. 1

Bacteriorhodopsin (BR) micrographs grown in vitro. A: red cell membrane; B: purple membrane.
equipment and clothing results in effective light diffraction and concealment.11

Biosensors. BR is a photoactive protein. So it can be employed as a photo sensor and information processor for optical computing in defensive technologies. It may also help to maintain civilian protection and reduce damage by bio-weapons that use unusual pathogen countermeasures and qualified biological systems in defense against bio-warfare and bioterrorism.12

Light detection by BR sensors can be used in both dried states and in suspension.13-15 Dry BR sensors contain a mixture of PM fragments and polyvinyl alcohol spread on a conductive glass. This conductive glass and a thin layer of gold function as the electrodes of the BR sensor. In contrast to dry sensors, BR in suspension sensors acts as a light-driven proton pump and its photocycle has more intermediates compared with the dry BR sensor.

Memories

The photocycle of BR has intermediates (states) with individual absorption spectra which can be optically identified.16 The intermediates utilized for short-term memories are the ground state (BR) and the M state complex. The lifetime of the M state differs from about 1 sec (for the wild-type bR) to a few minutes (for certain mutants of BR).17 For long-term memories, the ground state (BR) and the intermediates of branched photocycle, P or Q state should be consider.18 After deprotonation the branched photocycle initiates at the O intermediate. Upon exposure to red light, transition from O to P state occurs. Transition from P to Q state happens thermally. The Q intermediate can be isolated by a relatively large energy gap. By the exposure of protein to blue light, the Q intermediate will come back to the ground state.16 The intermediates are shown in a graph form in Figure 3.

Data are written via a branching reaction. For writing and reading, a thin layer of protein within a memory medium (page) should be selected and activated. Data are written by a beam of laser light on the page that shifts the intermediates and photoactivates the O state.19 After two milliseconds, the O state converts to P. Then the P state thermally changes to the Q state after several minutes. The writing process lasts about ~10 ms coincident to the completion time of photocycle.

Data are read by using differential absorption. The only two intermediates, K and the long-lived O intermediate absorb wavelength light of 680 nm effectively. By proper timing control of light beam, the K state will be eliminated and simply the O state will absorb light. The reading sequence initiates just as writing process by the same activating approach. Later than 2 ms, the data on the active page are read by the CCD detector at a low constant power.19

The blue light can convert both P and Q states back to BR. Using coherent light with wavelength near 410 nm abolishes both P and Q states, then pages are erased.

The exhibition of large refraction changes upon photo activation is outstanding holographic properties of the BR. The BR size is very small which is useful in fabricating thin holographic

Biodefense

Camouflage. Bacteriorhodopsin could be the basis for microwave-absorbing paints, due to its strong affinity for microwave absorption (3–40 GHz). Equipment covered with such biomaterials would be undetectable by infrared detectors.

This biomaterial is useful in military clothing and concealment, and is an excellent example of bioinspired application in biodefense. BR prototypes incorporate into camouflaged

BR acts as an optical switch and photocurrent generator in bioelectronics. Upon illumination to a single PM sheet of unidirectionally oriented BR molecules, a photovoltage is induced which may be employed either as an indicator or control element for diverse applications. The voltage is generated only during the light intensity change. The photovoltage can be simply measured by setting in the PM layer between two transparent electrodes.10

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mediums.\textsuperscript{20} The BR and its other genetically engineered versions are capable of two photon absorption and can sustain very hot temperatures and intense light. As well, based on their highly quantum efficient photocycle, they are valuable for data storage medium and holographic memories.\textsuperscript{19,21-23}

The BR, M and Q states are prepared for the data storage medium. The blue-shifted M and Q states are used in real time storage systems\textsuperscript{23} and long-lived holographic memories.\textsuperscript{19,21-23}

Mostly, the properties of BR can be regulated by the development of gene engineering which made it possible to obtain BR analogs with the individual amino acid replaced by others. Each intermediate can be constructed genetically as well and various profit-making technological devices could be developed based on the formation of these photosynthetic proteins. BR and its mutants are considered proteins of interest, as they really are potent.\textsuperscript{26}

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