Fabrication and photoresponse of novel carboxymethylcellulose (CMC) based bacteriorhodopsin (bR) sensor

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Abstract

A single-pixel dry-type reconstituted bacteriorhodopsin (bR) sensor (Au/CMC + bR/ITO) incorporating carboxymethylcellulose (CMC) as the artificial membrane is fabricated according to a protocol developed to investigate signal acquisition from the proposed biosensor. Droplets of reconstituted purple membrane (PM) containing bR applied onto a gel-like solution of CMC placed on gold electrodes (anode) are allowed to dry under an electric field, utilising the PM fragment’s net negative charge and electric dipole moment. The resulting bR matrix thin film with high molecular orientation is finally sandwiched together with a semi-transparent indium titanium oxide (ITO) counter electrode and proper electrical connections made to form a single pixel bR-CMC photosensor. Efficient and reproducible photoresponse observed upon illumination clearly exhibits the potential of the proposed biosensor for future photosensing works.

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1. Introduction

Bacterial-rhodopsin or bacteriorhodopsin (bR) is a family of the retinal-containing proteins found in extremely halophilic bacteria known as Halobacterium halobium thriving in salt marshes [1]. The only protein component in the purple membrane (PM) of the bacteria and contains a binary mixture of two proteins [2], one containing 13-cis retinal (dark-adapted bR) and the other all-trans retinal (light-adapted bR) at ambient temperature and low-light conditions. It is the simplest known light-driven ion pump performing photosynthesis that provides light-dependent ion (proton, H\(^+\))
transport and sensory functions for these organisms. This is achieved by converting light energy into an electrochemical gradient by means of pumping out protons from the cytoplasmic region into the extracellular region of the biological membrane.

bR of the halobacterial cell membrane consist of a 2-dimensional crystalline cell membrane embedded into a lipid bilayer (3:1 to protein:lipid) and grown by the bacterium when the concentration of oxygen becomes too low to sustain the generation of adenosine triphosphate (ATP) via oxidative phosphorylation, which is also how ATP is formed in cellular respiration. ATP production in the absence of oxygen is achieved by using bR as a proton pump by participating in a photocycle process [1,3] and [4], which produces H⁺ for every photon of light absorbed. The process in turn changes the bR’s shape (kinked shapes) and creates many intermediate forms while moving the protons produced across the membrane. This trans-membrane movement from the cytoplasmic side to the extracellular side creates an H⁺ gradient. The resulting energy of the H⁺ gradient is then harnessed by using ATPase to form ATP from inorganic phosphate and ADP in the same manner it occurs in cellular respiration.

Many bR-based photosensors as proposed by various research groups [5–15] and [16] worldwide were configured in a multilayered thin film sandwiched between two conducting electrodes. Crucial steps usually involve the incorporation of reconstituted bR into suitable mediums [5–15] acting as “artificial membranes. The medium to be chosen should be transparent enough to allow light penetration to reach the embedded bR in the “artificial membrane”. It should also possess cation-selective capabilities that enable proton transport through the medium. Most research groups concentrate into separate formation of bR thin film layers and “artificial membrane” medium [8,11,12,15] before sandwiching the bR-matrix together to enable forced incorporation of highly orientated bR into the active medium. In the work proposed however, an unconventional approach is used by allowing the bR to directly fuse into the medium in the form of a thick and viscous electrolyte gel that restricts free movements and rotations of the bR. By allowing the rotation limited bR-matrix to dry naturally under an electric field, bR with high molecular orientation embedded in an almost uniform multilayered matrix is formed.

2. Experiment

2.1. Materials

PM (molecular weight without retinal of 26784 kDa) of variant type V1 containing lyophilized powder of bR molecules from Halobacterium salinarium were obtained from MIB, Munich Innovative Biomaterials, Germany. Product information stated absorption maximum of bR in deionized water after light adaptation at 566 nm, while absorbance ratio (referring to the ratio of maximum optical densities, ODs at 280 nm and 570 nm), OD₂₈₀ / OD₅₇₀ at 2.3. For the purpose of the study, bR was suspended in pure deionized water to form a bR solution with a concentration of 0.2 mg/ml. The gel-like “artificial membrane” CMC-salt viscous gel electrolyte, bought in the form of powder from Sigma chemicals (product number 419273-100G), was prepared by slowly dissolving 6% CMC in 1 M of KCl (pH 7.4) prepared earlier in purified deionized water. Homogeneity of the gel electrolyte and the bR solution was achieved by means of using an agitator (Model 34524), supplied by Snijders Scientific, Holland. Gold coating (resistivity of 11.5 Ω/cm) formed on standard glass slides (2.5 cm × 7.5 cm) used in the work was achieved by using a BIO-RAD SEM sputtering system. The counter electrode used, the semi-transparent ITO conducting electrode (2.5 cm × 7.7 cm) had a surface resistivity of about 75.0 Ω/cm. Single pixel light-receptive area of 1.3 cm × 0.9 cm on the gold sputtered electrode was prepared by simply using a sharp metal point to define the dimensions of the light receiving area and thoroughly wiping of the rest of the sputtered gold on the slide with a filter paper. Electrical connections for the pixel from the edge to the underside of the pixel bearing glass slide were made using silver conductive paint (resistivity of 1.20 Ω/cm) obtained from RS Components, UK, while copper wires were attached to the individual electrical connections using the silver conductive paint. Sensor sealing was accomplished using a silicon based adhesive sealant (Selley Silicone Sealant).

2.2. Device optimizations and fabrication

bR-sensors fabricated by many researchers in their previous publications [8–10,12,15] seems to centre on the semi-dry type version, which basically involves the junction type bR-matrix sensor (metal /
bR / medium (electrolyte gel) / metal). Incorporating bR layers deposited as single or multilayered thin films employed through various deposition techniques and additions of charge enhancing ionophores [17] and [18] increases the production of efficient photosresponse from the fabricated photosensor. Much more basic configurations were employed in this work, yet still allowing a high level of photosensitivity to be achieved.

A glass slide prepared and washed with methanol and deionized water is sputtered with gold using a Scanning Electron Microscope (SEM) coating unit. Using the sharp metal point as mentioned earlier, a single pixel configuration is prepared on the gold coated glass slide (working electrode), which acts as the gold electrode. Electrical connection is made by using silver conductive paint on the edge of the gold pixel (the side of the glass slide) to the underside of the gold electrode. A copper wire is then attached by using the silver conductive paint to the electrical connection made on the underside of the pixel bearing glass slide. Using a 1 ml syringe, a drop of the gel-like CMC solution (about 0.1 ml) is applied onto the gold pixel. The use of CMC as the active medium was mainly due to ready availability and the intrinsic cation exchange capability possessed by the negatively charged carboxymethyl groups in CMC, enabling proton transport through the matrix. A drop of bR solution, about 0.1 ml applied from a 1 ml syringe is later deposited directly onto the CMC droplet. The OD measurement of the resulting light adapted bR-CMC mixture placed in a cuvette showed a value of 0.345 at 566 nm.

To induce high molecular orientation of bR as it rotates and fuses into the CMC material, the gold pixel bearing the bR-matrix is placed on an aluminium base and kept under a high electric field of 50 V/cm to utilise the bR’s net negative charge and electric dipole moment. Silver paint connecting the edge of the gold pixel to the underside of the glass slide bearing the pixel enables electrical connection to the aluminium base, which is connected to the positive polarity of the dc supply. Puu et al. [19], in his paper has shown that this method of bR matrix film formation will retain their molecular orientation even after the removal of the electric field and the drying of the electrode while retaining the natural intrinsic properties of bR. Such thin films incorporated into the dry-type photosensor design enables a high efficiency in generating photoelectric current as shown by Nicolini et al. [20], since almost all proton pathways are orientated in the same direction.

The whole setup is kept in dark for about 48 h to allow the pixel bearing electrode to dry naturally under the electric field and normal room temperature. An ITO slide washed with methanol and deionized water is dried and used as a counter electrode by sandwiching it with the prepared bR-matrix on the gold pixel. To allow good adhesion, the working electrode is wetted using a drop of deionized water from a 1 ml syringe before sandwiching. If the weight of the ITO slide is placed immediately on top of the “gel-like” bR matrix
before allowing complete drying, the viscous matrix will spread all over and flow out of the gold pixel. By keeping the fabricated bR-sensor overnight in dark conditions to dry naturally under the same electric field and room temperature, the electric dipole moment of bR will be oriented in the same direction. After complete drying, the edge of the sensor is sealed using the silicon based adhesive sealant to reduce the effects of humidity fluctuations. Finally the entire sensor is placed permanently by using adhesive tapes onto a solid polystyrene base to give physical support and kept in dry and dark conditions overnight [21] before any signal acquisition works are carried out (see Fig. 1).

2.3. Signal acquisition

Signal acquisition from the biosensor was carried out using a 500 W halogen projector lamp as the light source. To reduce excessive heat build-up, a distance of 0.5 m was maintained between the protein-based biosensor and the light source, allowing about 1.3 mW/cm² of light intensity to fall onto the pixel of the sensor.

3. Results and discussions

When the light source was switched on, an increase or “rise” in open voltage generation was observed. As a result of using a continuous source of illumination, a steady photovoltage generation or open voltage is observed. The peak values registered characterized by the bR concentration and light intensity used in the experiment shows a steady state photovoltage, which represents the proportional open voltage measurement of the net charge (proton) transport. When the light source was removed, a sudden decrease or “fall” in photovoltage generation was observed indicating sudden loss of proton gradient.

The sensors’ switching profiles on days 1, 2, 3 and 4 registered different initial (at $t = 0$ s) or starting open voltage values suggesting incomplete proton discharging of the sensor or exposure to background light before signal acquisition was obtained. It was evident that the initial values registered do not alter the switching profiles of the sensor but inversely influences the time it takes to reach the peak photovoltage (see Fig. 2).

The basic underlying mechanism that occurs, involves creation of the build-up of positive charges or protons through direct response towards absorption of light photons through the ITO semi-transparent electrode. This being as a consequence of the photocycle process triggered in the bR incorporated into the CMC matrix, creating a proton gradient across the “artificial membrane”. As in nature, the single pixel gold electrode represents the cytoplasmic side from where the proton or charge transfer takes place towards the extracellular side of the “artificial membrane”, which is represented by the ITO slide. The ITO slide collecting the charges or the electrical current produced will then be registered as photoresponse or an open voltage value.

![Fig. 2. Switching profiles of the bR sensor as obtained on days 1, 2, 3 and 4, exhibiting similar stable profiles. Peak values for days 1, 2, 3 and 4, self-stabilising at about 45.5 mV, 47.6 mV, 47.5 mV and 46.7 mV respectively were achieved after 170–270 s. On day 1, an initial open voltage value of 24 mV takes 170 s to reach peak value, day 2 with 22.9 mV takes 180 s, day 3 with 10 mV takes 270 s and day 4 registers 16.9 mV and 260 s.](image-url)
As discussed earlier, there are many methods for immobilizing bR into the “artificial membrane”. Most of these works involve either the semi-dry sandwich-type voltaic cells with metal-bR matrix-metal [11,12,14] or liquid junction cells comprising a bR-loaded membrane separating two aqueous solutions [8,22]. But the work presented in this paper involves a truly robust and easy to fabricate completely dry sandwich-type photosensor with repeatable and stable switching profiles.

4. Conclusions and future works

The work presented serves as a crucial preliminary investigation into the possibility of fabricating a multi-pixel CMC-based bR sensor to be integrated into an optical data processing system. Observation of photoresponse results clearly exhibits the switching profile of the proposed biosensor and by further perfecting the bR-matrix deposition method a faster rise-time could be achieved allowing fabrication of a more sensitive and practical photosensing device. Future works will also include important characterization works on the proposed bR sensor so as to better understand the light sensing mechanisms involved.

The basic bR-based photosensor design architecture presented and fabricated in this work demonstrates good photoresponse as shown by the switching profiles obtained while keeping many aspects of the fabrication process as simple as possible.

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