Original Article

Assay of Bacteriorhodopsin Activity and Structure on Polycarbonate Surface by Spin Coating Method and Photochemical Activity Analysis

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Abstract

Introduction: Protein-based memory is a novel technology that employs proteins ability to participate in electronic processes. Bacteriorhodopsin (BR) is a membranous proton pump that it's applications in bio-molecular electronic devices has been widely studied. The results of this research show that BR bounded to modified polycarbonate surface has higher activity for spin coating method.

Materials and Methods: In an in-vitro study, BR-containing polymer matrix of polyvinyl alcohol and gelatin with different w/v ratios was prepared. Spectroscopic and enzyme activity analysis was performed and the optimized concentration for BR-containing films was determined to be 3.2 mg/ml. BR-polymer was then immobilized on the polycarbonate surface with spin coating method and AFM microscopy was used to characterize BR-coated polycarbonate.

Results: Based on the obtained results we conclude that polymer concentrations below 1% significantly reduced BR activity levels. A280/A570 of 3.64 for 3.2 mg/ml BR solution and 4.97 for BR in 1% polymer confirmed the quality prepared film. AFM study of BR-coated polycarbonate surface revealed the overall thickness of 25nm, indicating that we were able to prepare a surface with suitable thickness for nano electronic devices

Conclusion: The results of this research show that BR bounded to modified polycarbonate surface has higher activity for spin coating method.

Keywords: Protein Memories, Baceriorhodopsin, Spin Coating, Polycarbonate Surface

1. Introduction

Protein memories are novel technologies that use the intrinsic ability of proteins and macromolecules to participate in electronic processes. Some of the advantages of protein memories include: low cost, providing reliability of magnetic hard discs, high optical storage capacity and high speed signal processing. Considerable interest has been shown toward the potential use of light-transducing proteins as active components in molecular electronic memories [1]. Bacteriorhodopsin (BR) is a membranous protein extracted from Halobacterium Salina [2]. BR is a light-driven proton pump and a photochromic retinal protein with photosynthesis ability. BR serves as a light harvesting system for generating ATP under aerobic conditions [3 , 4]. BR'S chromophore (all trans-retinal) is

attached to Lys-216 as a Schiff base. Following light absorbance, bR undergoes a sequence of photochemical intermediates (I, J, K, L, M, N and O) involving electrogenic protonation/de-protonation that results in a proton being trans located across the membrane [5].

Because of its photochromic properties, long-term photochemical and thermal resistance due to its bi-dimensional crystalline structure, high forward and reverse quantum yields, wavelength-independent quantum yields, its ability to form thin films with good holographic efficiencies and excellent optical properties, bR is a great candidate for electronic applications such as data storage or optical devices and BR films have been widely studied in optical data storage and process [1 , 6-9].

Matrices made from natural polymers such as gelatin and synthetic polymers such as polyvinyl alcohol have been used for BR film preparation. These matrix materials yield mechanically stable films with good optical quality [10 ,11].

The purpose of this study (in vitro) was to use BR and optimization of various protein concentrations, which was performed by determining biological activity. For this reason, different concentrations of BR and polymer film were prepared and studied for biological activity and structure. Also, BR-coated polycarbonate surface was prepared by spin coating and BR-coated polycarbonate surface was used in the fabrication of protein disks.

2. Materials and Methods

2.1. Materials

Wild-type and S9 strain BR, polyvinyl alcohol (PVA) and gelatin (GE) were purchased from Sigma Aldrich, triethanolamine (TEA) from Fluka and Polycarbonate was provided by CD producer companies. Moreover, the test conditions were determined according to in-vitro standards.

2.2. Spectroscopic Analysis of BR Structure

UV visible spectroscopic analysis was performed through Thermo Unicam UV-300. BR solution containing 3.2 mg/ml BR in 350 µl distilled water was prepared. Absorption of the sample suspension was recorded at 280 nm and 570 nm. The test was carried out in room temperature. A280/A570 ratio was used to estimate protein film quality [12 , 13]. A280 and A570 indicate the absorptions of the aromatic amino acid residues in the BR and the absorption of the protonated Schiff base retinal, respectively. The retinal chromophore is central to the proton pumping activity of the protein.

2.3. Investigation of Photocycle and Biological Activity of BR

To study BR activity, changes of pH following light exposure ware studied according to Kouyama et al (1987) [14]. The initial pH was adjusted to 7. Light exposure was provided by a 200W lamp which was placed in a 30 cm distance from the samples and changes in pH were monitored for 30 minutes.

2.4. BR-Containing Polymer Film Preparation

BR-containing polymer film in the matrix of GE and PVA was prepared according to Korposh et al (2005) [15]. Briefly, different concentrations of BR suspension were prepared (1.5, 3.2, 4 and 6mg/ml), sonicated for 20 minutes and placed on shaker for additional 6 hours. Polymer solution was then prepared by adding GE and PVA to double distilled water (0.01%, 0.1% and 1% w/v). The suspension was mixed at room temperature for 20 minutes and again mixed thoroughly for additional 40 minutes at 60º C.

BR suspension was added to polymer solution and mixed for 20 minutes. Finally, TEA was added to the polymer matrix. The molar ration of TEA: BR was 250:1. The matrix suspension was mixed for 30

minutes and then placed in a vacuum desiccator.

2.5. Polymer Immobilization on Polycarbonate by Spin Coating

For polycarbonate surface preparation, a CD was soaked in 65% citric acid for an hour and then in methanol for 20 minutes. AFM microscopy was used to confirm that polycarbonate surface was ready for BR immobilization. BR-containing film was immobilized on the polycarbonate surface by spin coating. Different concentrations of BR and polymer films were tested. The optimized polymer matrix contained 1% w/v polymer and 3.2 mg/ml BR. Polycarbonate surface was covered with polymer film and placed in a spin coater. When coating was complete, the surface was left at room temperature for 24 hours to dry.

2.6. Morphological Analysis of CD Surface

BR-coated polycarbonate surface was further studied with AFM according to S.O.Korposh et al (2005) [15].

2.7. Statistical Analysis

The t-test and one-way ANOVA was performed for statistical analysis of data with SPSS software (version 19) and the P value <0.05 was considered significant.

3. Results

Photochemical Activity of Wild-Type BR and BR Films

Changes in pH of different concentrations of BR solution (1.5, 3.2, 4 and 6 mg/ml) in 1% polymer film were analyzed. The optimum concentration of BR solution with maximum activity was 3.2 mg/ml. BR activity was also analyzed in 0.1% and 0.01% polymer films (figure 1). Based on the obtained results, 3.2 mg/ml BR in 1% polymer film showed the highest activity.

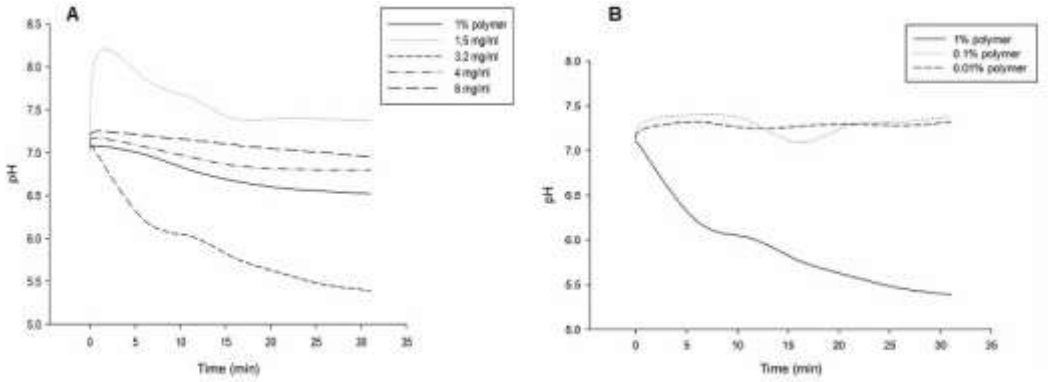

Figure 1. PH changes of BR solution following light exposure. (A) Different BR concentrations in 1% polymer (B) 3.2mg/ml BR in polymers with different w/v rate

Effect of BR Concentration and PVA-GE Ratio of Maximum Absorbance

UV-Vis absorption spectra of different concentrations of BR (1.5, 3.2, 4 and 6 mg/ml) in 1% polymer film was investigated (figure 2). As it can be seen from figure 2, the optimum concentration of polymer-bound BR is 3.2 mg/ml.

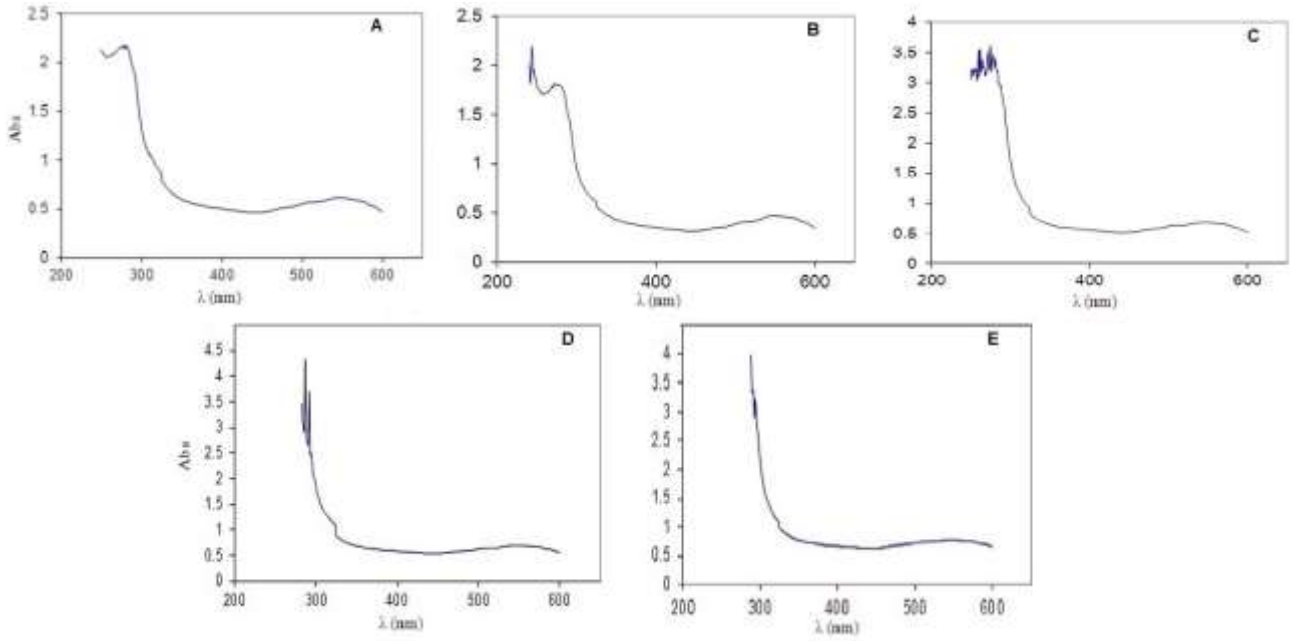

Figure 2. Absorption spectra of films based on BR in 1% polymer. (A) 3.2 mg/ml BR solution without polymer (B) 1.5 mg/ml BR with 1% polymer (C) 3.2 mg/ml BR with 1% polymer (D) 4 mg/ml BR with 1% polymer (E) 6 mg/ml BR with 1% polymer

Absorbance of 3.2 mg/ml BR was then studied in 0.1% and 0.1 percentage polymers as well (figure 3). Based on the obtained results, 1% Polymer film containing 3.2 mg/ml BR was chosen for further studies.

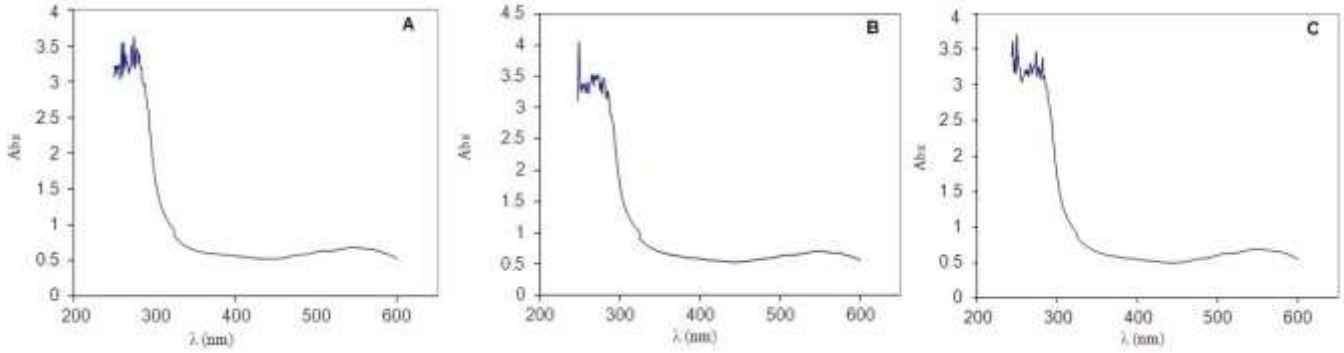

Figure 3. UV-Vis absorption spectra of BR-containing polymers (A) 1% polymer (B) 0.1% polymer and (C) 0.01% polymer.

Morphological Study of bR-Coated Polycarbonate Surface by AFM

Figure 4 represents the morphology of polycarbonate surface before and after BR-polymer immobilization. Dark lines in figure 4.A represent empty grooves on CD surface before BR coating. Upon BR-containing polymer coating, the depth of the grooves is reduced and 25 nm thicknesses are observed (figure 4.B).

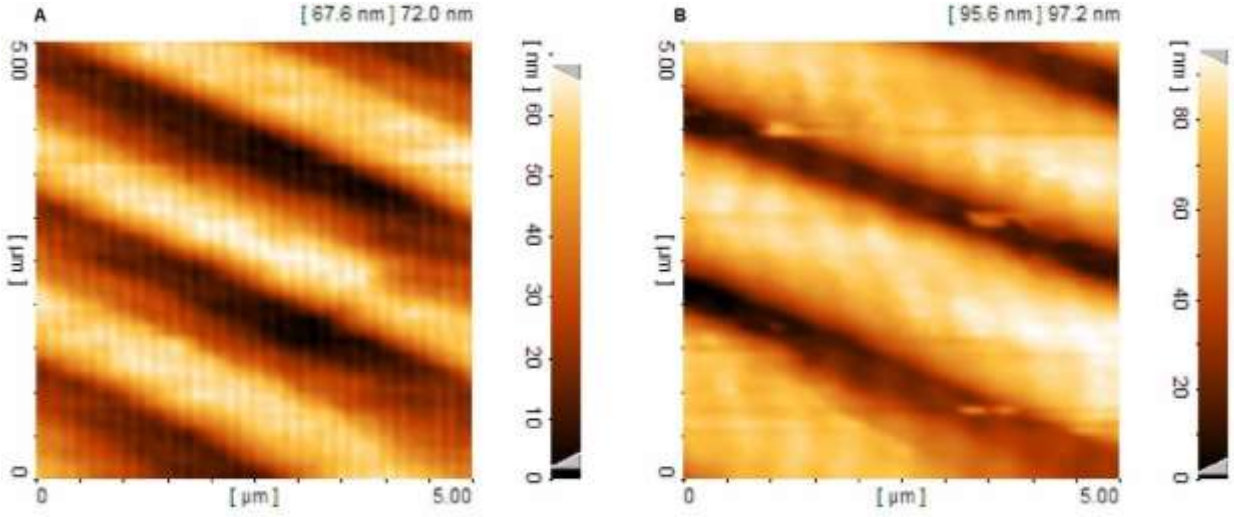

Figure 4. AFM images of polycarbonate surface. (A) Untreated polycarbonate surface (B) polycarbonate surface following BR-containing polymer coating. Dark lines represent grooves on CD surface.

4. Discussion

In the present study, BR biological activity and optical properties were assessed in PVA-GE polymer matrix for optical data storage in electronic device applications. Biological activity analysis of different concentrations of BR in 1% polymer matrix showed that 3.2 mg/ml was the optimum concentration with greater activity. Lower BR concentration showed reduced activity presumably due to unbindingness from the film. Loss of activity in higher concentrations due to the presence of too much BR molecules indicates that only a certain amount of BR can bind to the polymeric film. The optimum concentration was further analyzed for its activity in 0.1% and 0.01% polymer matrices. Polymer bound-BR significantly lost its activity in lower PVA-GE concentrations which could be attributed to the lower mechanical stability provided by the polymer.

BR has an absorption maxima at 568 nm. Presence of aromatic amino acids results in another absorption peak at 280 nm [16]. Thus, A_{280} and A_{570} indicate the absorptions of the aromatic amino acid residues and the absorption of the protonated Schiff base retinal, respectively. BR is considered as the most important contamination of BR with absorption peaks at 497, 533, 475 and 392 nm [17-19]. Presence of additional bands in absorption spectra could be related to Bacterioruberin contamination. Obtained maximum absorbance at 570 nm and 280 nm in 3.2 mg/ml BR and 1% polymer film indicates excellent optical quality and low light scattering, also proving that polymer-bound BR retained its optical properties and conformation and is not denatured. Prepared films with higher BR concentrations showed decreases sensitivity and lower optical quality and high light scattering. As the concentration of PVA-GE decreased, wavelength of maximum absorbance (λ_{max}) was lowered, indicating decreased film optical transparency. Taken together, these results suggest that BR binding to GEL-PVA polymer matrix does not affect maximum absorption wavelength or photocycle and that biological activity of BR is preserved.

Since GE is a water-soluble material, it preserves the native structure of bR. Electron microscopy studies have indicated that GE-containing polymer films with homogeneous surfaces could be synthesized [20 , 21]. PVA is also water soluble which upon dehydration yields a resistant thin film. PVA and GE are widely used in polymer films for biosensors and optical memories since they provide high optical quality and minimum light diffraction. To determine BR-film quality, A_{280}/A_{570} ratio

was used. The ratio for 3.2 mg/ml BR was 3.64 and for bR in 1% polymer was 4.97, indicating good film qualities. Following aluminum and silicon removal from the CD surface, empty CD grooves with the depth of 67 nm were visualizes by AFM. AFM study of bR-coated polycarbonate surface confirmed the prepared surface quality with overall thickness of 25 nm. Since the diameter of a single bR molecule is less than 10nm [22], results show that we were able to prepare a surface with at least three layers of bR molecule which is a suitable thickness for nano electronic devices. This study reports on the use of BR embedded in GE-PVA polymer for bioelectronic storage devices. BR films with GE and PVA solutions of 1% (w/v) with good optical quality were prepared. In conclusion, prepared polymer matrix was suitable for immobilization of BR protein and could be used in optical storage devices. In order to improve the accuracy of microscopic analysis, one way is to take advantage of computational imaging [23-24]. There have also been some sorts of projects which try to restore the 3D surface model of microscopic object to allow further interpretation of 3D micro surfaces rather than only 2D images, providing better visualization and qualitative information for the specimens [25-27].

5. Conclusion

The results of our research on different concentrations showed that 3.2 mg/ml concentration BR was optimal for spin coating, and the BR application is confirmed to be used in polymer film making. As a part of our plan, we may take 3D microscopy vision was taken into account and micro surface attributes was examined in a more reliable manner.

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Conflict of interest

The authors declare no conflict of interest.

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