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Writing ability of bacteriorhodopsin by beaming laser light

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ABSTRACT

Bacteriorhodopsin (bR) is a transmembrane protein which can exist in a variety form of chemical states. By choosing two states, O state for binary zero and Q state as binary one, it is possible to use this protein as a memory device. In order to increase O state life time and follow that Q state the new matrix was design to make a bR film. Thus, different ratios of Gelatin-polyvinyl alcohol matrices weight/volume were provided in order to find the optimal condition to light absorption. In addition, triethanolamine (TEA) was also used as photosensitizing chemical additive to film forming suspension. It found that the bR film matrix of 0.0005% (w/v) is the best ratio for bR light absorption. Furthermore, TEA changed both absorption and the lifetime of M intermediate. After wards, bR film was chemically immobilized on glassy surface and then illuminated by laser beam in different times. Radiation of bR films in various intervals after chemical immobilization demonstrated that bR ground state; M, P, and Q intermediates of bR photocycle are existed.

Keywords: Protein memory, Bacteriorhodopsin (bR), Photocycle, Laser beam.

INTRODUCTION

Bacteriorhodopsin (bR) is a transmembrane protein which consists of a single polypeptide chain of 248 amino acids forming seven transmembrane α -helices. It has a similar structure and same activity to the rhodopsin protein which founds in the eyes [1, 2]. The bR has a chromophoric group contains a retinal molecule that linked via a protonated Schiff base to lysine 216 [3]. This molecule use solar energy as a supply to transport protons through the Halobacterium salinarium cell membrane and causing a potential difference required for driving the synthesis of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) [2, 4, 5, 6, 7]. Absorption of a photon by bR trigger a photocycle that involve a series of intermediates identified as K, L, M, N and O states [8, 9, 10, 11]. The bR exclusive property as a light sensitive receptor makes it a good candidate to create an artificial photoreceptor [2, 12, 13, 14, 15, 16]. In the last few years, scientists are looking at protein-based memory to compete with the speed of electronic memory, the reliability of magnetic hard-disks, and the capacities of optical/magnetic storage. The data writing technique proposed by Dr. Birge involves the use of a three-dimensional data storage system [17]. In this case, a cube of bR in a polymer gel is surrounded by two arrays of laser beams [18, 19, 20, 21] placed at 90 degree angles from each other. One array which called "paging" beams is set to green and activates the photocycle of the protein in any selected square plane, or page, within the cube. After a few milliseconds, the number of O states

intermediate of bR in reaches around maximum. The other array is set to red color, a beam which switching molecules from O to P state and following relaxes to the highly stable Q state quickly. In this process O state, the first state of excited state, assign to a binary value 0 and P-, Q- states are assigned as a binary value 1. In the present study, we have tries to use the unique characteristic of bR [22, 23] as an analogous to the binary switching system which is used in existing semiconductor and magnetic memories. In this respect, the new bR film was constructed and the photocycle activity of its in film was investigated before and after laser beam radiation.

MATERIALS AND METHODS

2.1 Materials

Bacteriorhodopsin (bR), polyvinyl alcohol (PVA), HCl and gelatin (GE) were provided from Sigma. Triethanolamine (TEA) was provided from Fluka. Sulphoric acid (H₂SO₄), GlycidOxy PropyltrimethoxySilane (GOPS), 1,1'-carbonyldiimidazole and acetonitrile were provided from Merk. The Glassy surface was provided from Sinagen.

2.2 preparation of bR film

Bacteriorhodopsin film in polymer matrix was prepared followed by standard protocol as described in [24]. 1 mg of bR was soaked in 1ml tri-distilled water for 20 min. In addition, 0.4 M of triethanolamine (TEA) solution was added to the bR suspension to give a TEA: bR with 250:1 molar ratios (which give maximal photosensitivity). Then 0.00025 gr of PVA and GE powders were solved in 100ml tri-distilled water for 20 minutes to prepare PVA and GE solutions. The resulting solution was heated and stirred at 60 °C for 40 min. Finally, the appropriate ratio of bR - TEA and GE-PVA solutions were mixed and stirred for 20–30 minutes to make a film-forming solution [24].

2.3 Chemical Immobilization

The chemical immobilization of bR film in GE-PVA matrix was done on a glassy surface. First the glassy surface was soaked for 10 min in 20 mM HCl and washed out with distilled water. Next, the glassy surface was soaked 10 min in sulphoric acid solution for 10 min and once more it was washed with distilled water again. After that the glassy surface was embedded into 10% (v/v) GOPS solution and mixed by 1:1 ratio with distilled water at 90°C for 3 hours. Lastly the glassy surface activated by soaking 30 min in 1,1'-carbonyldiimidazole mixed by 100 mg/ml acetonitrile solution and dried at 4°C for 12 hours. At the end the film based on bacteriorhodopsin in Gelatin-polyvinyl alcohol matrix was dropped on treated glassy surface and dried at 4°C.

2.4 photon induction and assay of bR activation

After chemical immobilization of film based on bR in GE-PVA matrix on glassy surface, two orthogonal beams, green laser (vertical) and red laser (horizontal) have been used to consider absorbance spectrum of bR.

UV/Vis spectroscopy was used as a quantitative determination of bR film. Furthermore, a solution of 3M of KCl and 80 mM of MgCl₂ adjusted to pH 7.1 and the bR film was embedded into the solution. A projector lamp (200 W) was used as a light source to induce the activity of bR proton pump. The changing of pH was measured by pH meter [25].

RESULTS AND DISCUSSION

The matrices based on polymer such as GE and PVA are normally used to construct the bR film. These types of the matrices mechanically make the stable films with good optical quality [24]. In this study, the films were provided based on bR in GE-PVA matrix with various weight/volume ratios (w/v). The results show that increasing of bR concentration in bR film or decreasing of w/v ratio of GE-PVA matrix in bR film increase the light absorption. Among different GE-PVA w/v ratios, the bR film matrix of 0.0005% (w/v) shows highest absorption to light (Fig. 1).

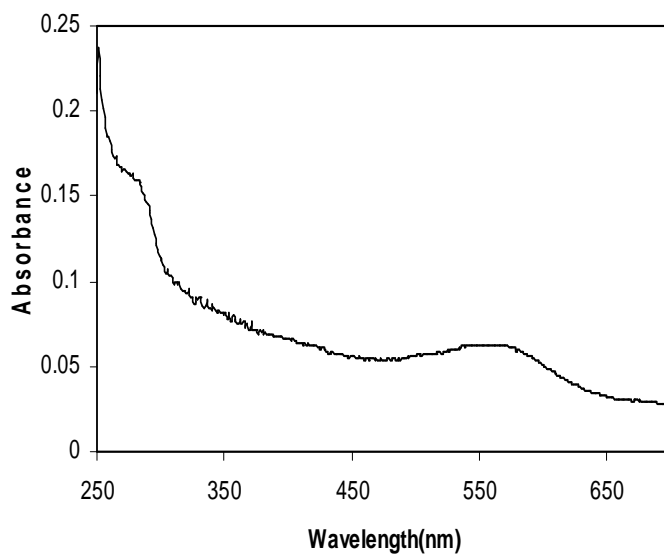


Fig.1. Absorption spectrum of bR film in GE-PVA matrix at 25°C.

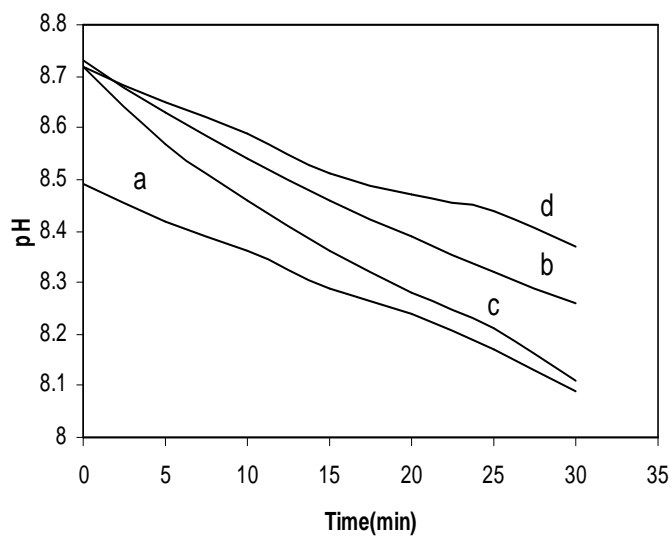


Fig.2. Activity of proton pump of bR film 0.0005%, immediately (a), 18 hours (b), 7 days (c), and 30 days (d) after chemical immobilization.

Hence, this ratio was designated as an optimal condition for the rest of study. The previous studies reported that the amounts of bR intermediates, mainly O state, are highly pH sensitive [2].

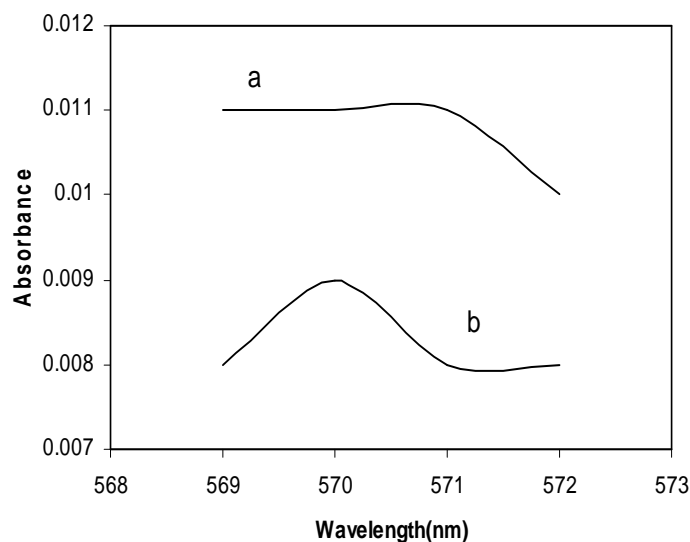


Fig.3. Comparison of bR state in bR film 0.0005%, 7 days after chemical immobilization; a, after laser; b, before laser.

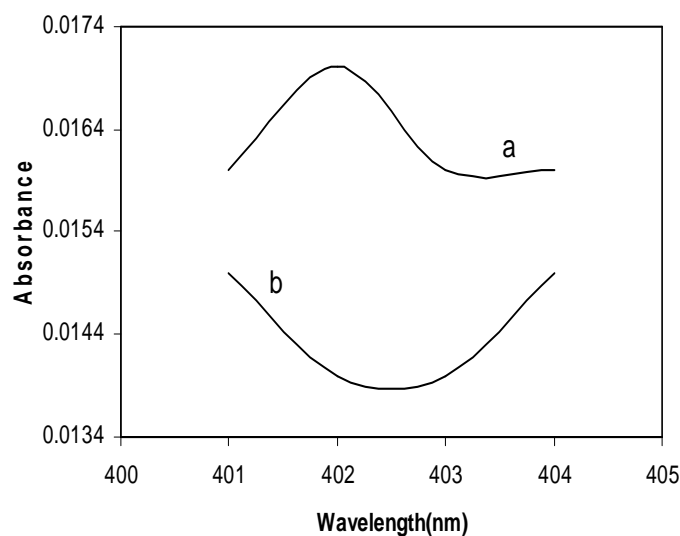


Fig.4. Comparison of M state in bR film 0.0005%, 7 days after chemical immobilization; a, after laser; b, before laser.

In addition, it has been shown that the in pH= 6 earlier to polymerization enhanced O state [2]. For that reason, the buffer phosphate with pH values ranging from 4.48 to 9.06 was selected as a solvent of the bR film. The results show that bR film in 0.0005% (w/v) GE-PVA matrix of buffer phosphate with pH=6.04 as a solvent has the highest absorption to light (data not shown).

Follow that, the activity of the light-driven proton pump has been extensively investigated by pH meter to measure the activity of bR in the film in GE-PVA matrix of 0.0005% (w/v).

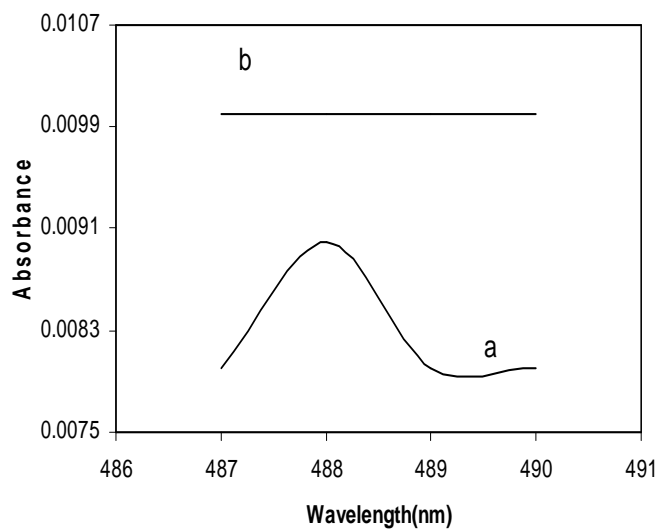


Fig. 5. Compare of P state in bR film 0.0005%, 18 hours after chemical immobilization; a, after laser; b, before laser.

It confirmed that bR in film has been active 18 hours, 7 days and 30 days after chemical immobilization on glassy surface (Fig. 2).

Although pH decreasing exposed bR protein to different conditions, however bR is able to maintain its structure and activity. Consequently, the bR films were illuminated by two orthogonal beams immediately, 18 hours and 7 days after chemical immobilization for 30 minutes.

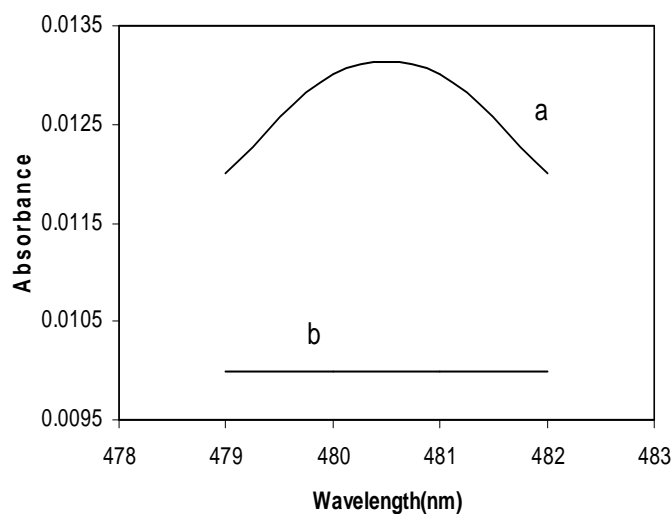


Fig. 6. Compare of P state in bR film 0.0005%, 7 days after chemical immobilization; a, after laser; b, before laser.

Figure 3 indicates the bR activation in bR film 0.0005%, 7 days after chemical immobilization. Figure 3 displays a peak at 569-571 nm which indicates bR state before radiation.

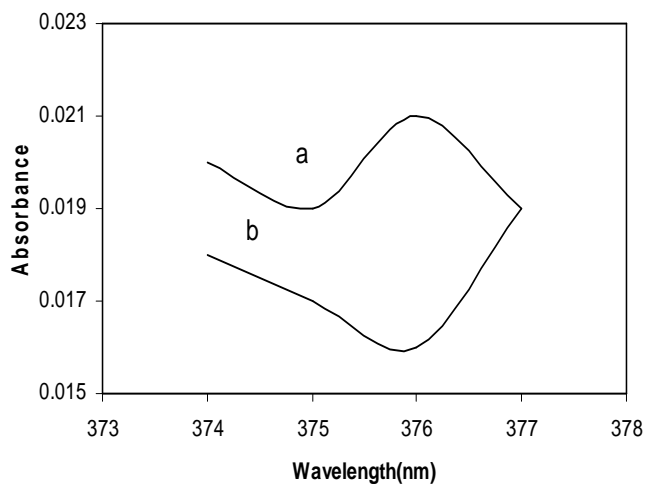


Fig.7. Comparison of Q states in bR film 0.0005%, 7 days after chemical immobilization; a, after laser; b, before laser.

Although there is no absorption peak after laser beam. There is a peak at 401-404 nm (Fig. 4) which represents native M states, respectively. As it shows this peak appears after laser beam while there is no absorption peak before radiation.

Figures 5 and 6 show the P states 18 hours and 7 days after chemical immobilization. Although in both case there is no absorption peak before laser beam, however the absorption peaks appear after radiation. Figure 7 displays an absorption peak at 375-377 nm via illumination after laser beam. This peak is corresponding to Q state of the bR photocycle. Altogether, the comparison of absorbance spectrums, before and after lasers, demonstrated that in optimized pH and GE-PVE matrix w/v ratio, the bR photocycle intermediates namely bR, M, P, and Q are existed and they are activated after laser irradiation.

CONCLUSION

One of the special characteristics of bacteriorhodopsin is its ability to enter to into branched photocycle represented as P and Q states. Illuminating the bR protein in O intermediate with red light will drive protein into the branched photocycle. Increasing the life time of O intermediate will enhanced the photochemical conversion into the P and Q states [2, 26]. Thus, the optimal condition to embed bR membrane protein and the photocycle activity of bR membrane has been investigated. The TEA in combination with halogen containing organic compounds was added to GE-PVA matrix as photosensitizing chemical agent. Among different GE-PVA w/v concentration matrices, bR film in GE-PVA matrix of 0.0005% (w/v) has the highest absorption to light. TEA dramatically changed both absorption and the lifetime of the M and follows that O intermediate. As a result, developing of O state enhanced photochemical conversion into the P state and so increasing the writing ability of bR film.

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