Surface modification by using of immobilized electrostatic self-assembly of bacteriorhodopsin as protein memory

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ABSTRACT

Bacteriorhodopsin (BR) is the light harvesting and photoactive proton pump found in the membrane of a salt marsh bacteria. This protein has significant potential to use in optical computing and memory devices due to unique intrinsic physical properties of photo and bioelectric. All these features make BR one of the most promising protein candidates in protein memories. Protein memory is a kind of optical memory with a large storage capacity and high speed processing features. BR protein was used with the polymer film in order to create better stability. In order to investigate immobilization of electrostatic self-assembly of BR on glass and polycarbonate as protein memories was used. Polycarbonate is a layer of compact disc (CD) structure which considered dye immobilized on its surface and have reading and writing abilities of information via 0,1 bites. In this study, surfaces of polycarbonate modified by the mixture of 5% sulfuric acid and 20% acetic acid; furthermore, by using of PEI as cationic resin the surface of polycarbonate was charged and BR immobilized on it electrostatically. The modified surfaces were characterized by AFM technique**.** Also, light activity for reading data is retained. This is an appropriate method for optimal stability and activity assay of the protein and also is suitable for preparation of protein memories.

Keywords: Bacteriorhodopsin; Protein memories; Electrostatic self-assembly; UV-visible spectrometry techniques; FTIR-ATR spectroscopy; AFM technique

INTRODUCTION

There are significant reduction in the size of future tools and increase in their speed, benefits manufacture and design of molecular devices .Molecular computers could be a size 2-3 times smaller and faster than today's computers. Due to the size and speed, molecular electronics plays important role in the development of computer tools [1, 2]. *Halobacteriun salinarum* is a kind of halobacteria, which exist in environments with high concentrations of salt, such as salt lakes and saline waters [3-5]. Halophiles aerobic organisms use light instead of electron respiratory chain in the low pressure of oxygen [6]. These organisms are capable of producing energy through alternative route to maintain their survival. The concentration of dissolved oxygen in the pond of salt water (saturation salt) is five times less than ordinary water, so in this condition, low concentrations of oxygen and light, the present system does not use Chlorophyll revival path; instead retinal (energy conversion) and bacteriorhodopsin (BR) protein are used as proton pump [5, 7, 8]. BR is chromophores retinal protein. This protein was discovered in 1970 by Dr. Estoeckinus and Dr.Osterhelt. During the past four decades, many studies have been conducted in this field [9, 10]. *Halobacteriun salinarum* growth, stops under anaerobic stress conditions, and this start synthesis of purple membrane (PM). BR is 75% of the PM and 25% the rest consists of mixture of connecting lipids [2]. BR requires the presence of lipid to maintain its conformation ,so optical memory applications uses of thin BR films [11, 12]. Matrix of polyvinyl alcohol and gelatin: polyvinyl alcohol (PVA) is a water-soluble polymer. As soon dehydration, thin film will stable with this method, since containing

hydroxyl functional groups [13, 14]. (BR) immobilized through self assemble electrostatic methods that can be employed in the preparation of the protein memory. Nature of BR is anionic; therefore, electrostatic method can be used [15]. In this method, layers of material are linked together with opposite charges. Immobilization of these surfaces was carried out with immersion in aqueous solutions with opposite charge [16]. CD is a kind of plastic polycarbonate which made of 4 layers with 1.2 millimeter thickness and 15-20g weight [17]. In this respect, development of chemical components on the disks surface improves the immobilization of active biological molecules on it [18-21]. Polycarbonates are a kind of compact polymers that are highly resistance to the physical damage and temperature. These properties make it one of the best components to immobilize the thermophilic enzymes [10, 22, 23]. The present study attempts to immobilize the BR on glass and polycarbonate surface by modification of those functional groups[17, 24].

MATERIALS AND METHODS

Wild type and S9 strain Bacteriorhodopsin (BR), potassium chloride, magnesium chloride, potassium hydroxide 2%, gelatin, polyvinyl alcohol (PVA), polyethylene Aimin (PEI) were purchased from Sigma. Triethanolamine (TEA) was purchased from Fluka. Polycarbonate was provided from CD producer companies. Glass substrate, sulfuric acid and acetic acid were purchased from Merck.

Methods

The study of BR structure and its photocycle was performed by UV-visible spectrometry (Unicam UV 300 model) techniques. To measure the BR activity, pH changes were determined. As a result, in order to define the PM activity, pH changes we measured by baloni pH 720 precision pH meter. In pH = 7.2 Kcl 3M and 80M solutions were prepared. Then lamp with 200W power at a 30 cm distance from the sample was used. Finally pH variations were measured for 30 minutes.

Preparation of polymeric matrix

The preparation of BR film by GE-PVA solution in distilled water was followed by standard protocol as described by korposh and his colleagues [6]. GE-PVA solution (1%wt) was prepared. After cooling the solution different concentration of bacteriorhodopsin suspension was added to the solution, then 0.4 M Triethanolamine solution with 250: 1 ratio was added to improve BR light sensitivity.

Electrostatically immobilized BR on glass and polycarbonate

The immobilization technique chosen is based upon an electrostatic layer-by-layer self-assembly technique [25]. In the first step polycarbonate surfaces was sulfonated by the mixture of 5% sulfuric acid and 20% acetic acid. This process was done at 80° C for an hour. Next, glass or Polycarbonate components charged via 25 minutes submersion in to a 2% KOH solutions, washed and dried. Afterward, the components were soaked in PEI for 5 minutes at room temperature, washed and dried. Finally, each substrate component was submerged in PM solution for 10 minutes in an ultrasonic bath, washed and dried [25]. To provide protein memory CD, polycarbonate should be ridge. So, it was decided to carry out reverse engineering.

Figure 1. Removing the CD layers to achieve the polycarbonate layer

Layer removing protocol

CDs were soaked in nitric acid solution 60% and distilled water for an hour and fifteen minutes, and then were rinsed. After that, they were immersed for 20 minutes in methanol. Finally, samples were washed and dried.

RESULTS

The study of (BR) structure and its photocycle was done by UV-visible spectrometry (figure2). Film containing a suspension of 1.2 mg/ml BR shows pH levels less than others. 0.5 pH units decrease a film containing a suspension of 3.2 mg/ml BR towards decrease to 0.24 and 0.31 units, respectively, for film containing suspensions 1.2 mg/ml and 2.2 mg/ml BR, have the highest biological activity(figure 3) .BR suspension was used to immobilize BR on glass and polycarbonate surface. pH changes, indicate activated proton pump BR protein after immobilization (Figures 4,5) .

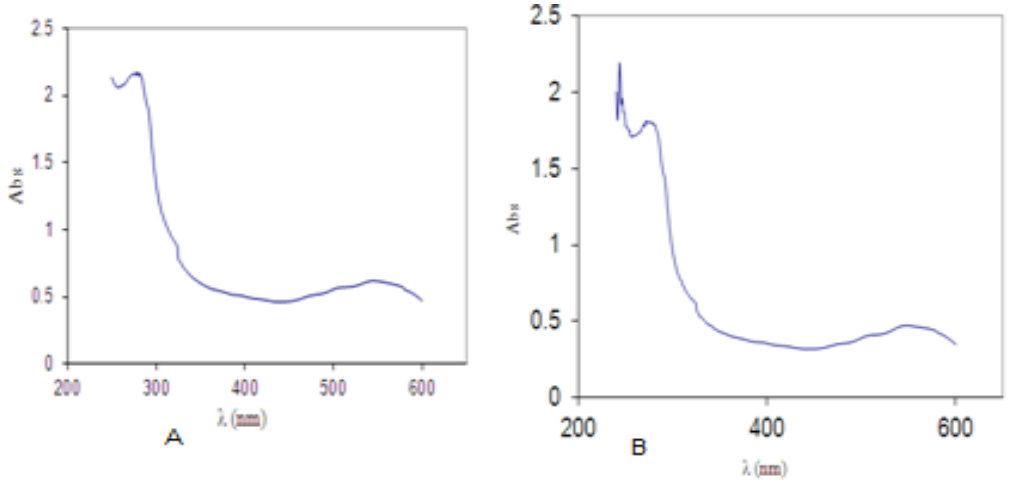

Figure 2. A) UV-visible spectrum in a wavelength 650-200 nm for (BR) suspension 3.2 (mg/ml)
B) UV-visible spectrum in a wavelength 650-200 nm for (BR) 3.2(mg/ml) in a polymeric film 1%

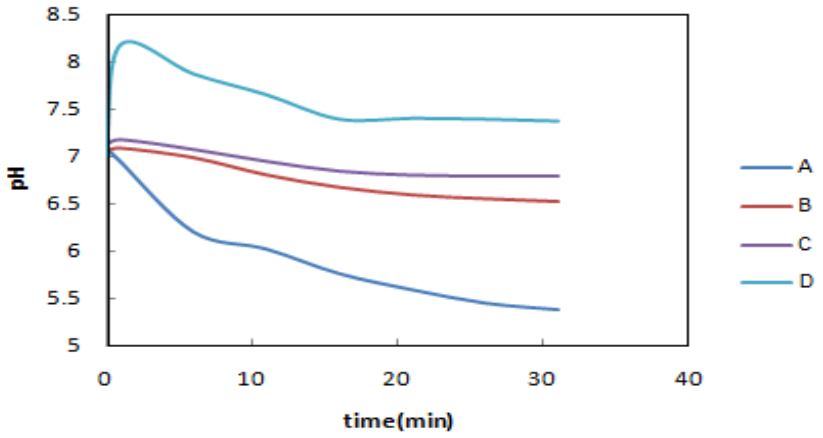

Figure 3. A) Activity of BR 1.2(mg/ml) in a polymeric film 1% B) Activity of BR 2.2(mg/ml) in a polymeric film 1% C) Activity of BR 3.2(mg/ml) in a polymeric film 1% D) Activity of BR suspension 3.2 (mg/ml)

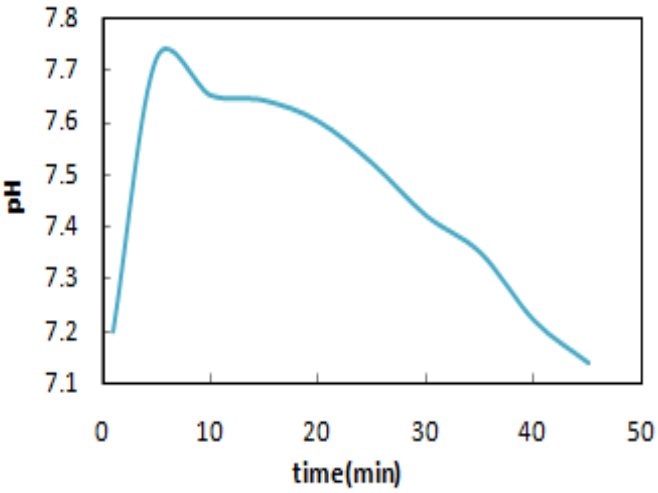

Figure 4. Activity of self assemble electrostatic immobilized BR 3.2(mg/ml) With PEI on the glass surface.

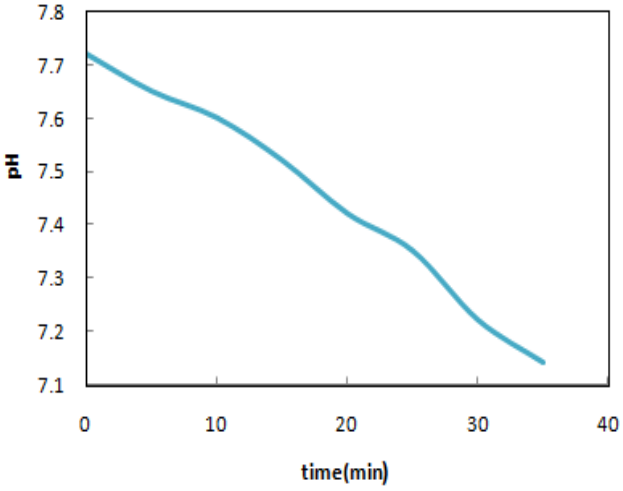

Figure 5. Activity of self assemble electrostatic immobilized BR 3.2(mg/ml) With PEI on the polycarbonate surface.

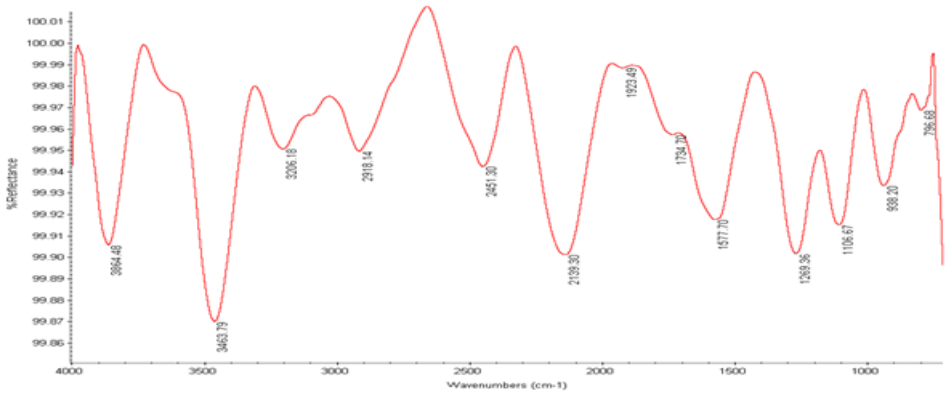

Figure 6. The FTIR-ATR spectrum of the polycarbonate surface before immobilization of BR.

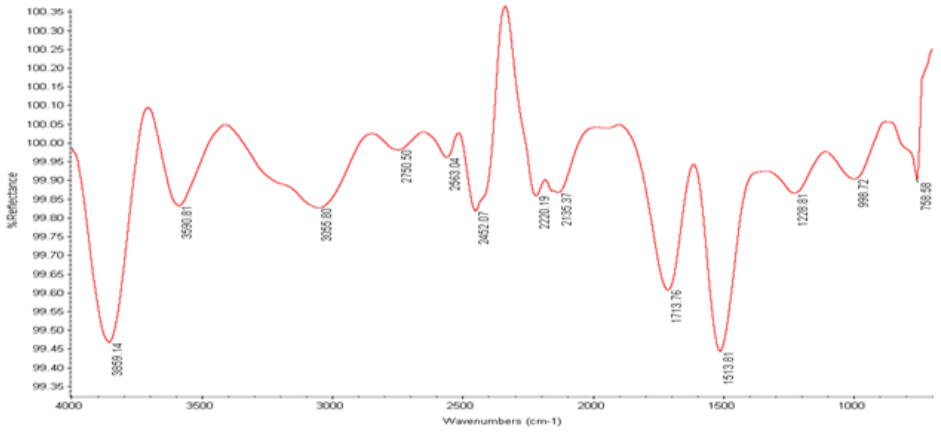

Figure 7. The FTIR-ATR spectrum of modified polycarbonate surface after immobilization of BR.

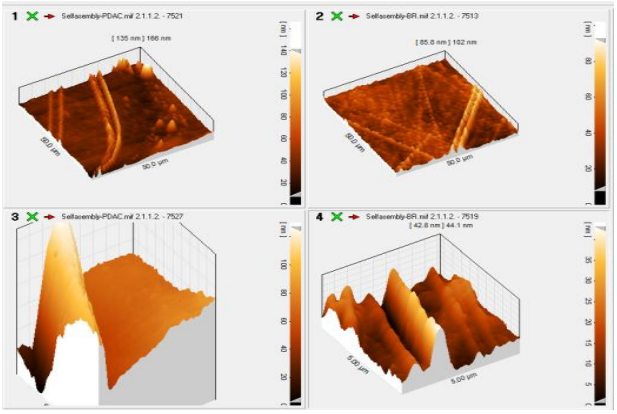

Figure 8. Morphological studies of polycarbonate surface by AFM. Panel shows polycarbonate surface after electrostatically immobilization of BR.

PEI made the polycarbonate surface positively charged and the BR has a negative charge on it. Thus, BR interacted to the PEI modified surface. The peaks on 3595 and 1517 in figure 7 showed that the BR was bind to the modified polycarbonate surface electrostatically.

To identify the functional groups formed on polycarbonate surface, Thermo Nicolet Nexus 870 with SMART MULTI-BOUNCE HATR was for FTIR-ATR. The morphology of polycarbonate surface changed after immobilization of BR proteins was studied by AFM technique (See figure 8).

DISCUSSION

Bacteriorhodopsin is applied as a model for data storage application via electrostatic interactions onto biological compact disc. By the use of self-assembly electrostatic strategies of these protein molecules onto polymer films, memory storage purposes are tangible. For this reason, polymer films (Polyvinyl alcohol matrix-gelatin) were provided; and then, optimization of different concentrations of the protein was done by determining the biological activity [17, 26]. By utilizing spectroscopy methods, functional characteristic techniques, and microscopic evaluations, the absorption of BR proteins to polymer and glass was studied. In fact, spectral techniques such as UV-visible spectrometry and FTIR-ATR spectroscopy enable reorganization of protein assembly features and candidate surface attachment. In addition, AFM study provides morphological alteration of the chosen surface that can be an approval for earlier evaluations. UV-visible spectrum provides information related to protein structure [27] as it is depicted in figure 2. This result shows that similar spectrum pattern of these two samples can be obtained via this method. This indicates that the BR protein is successfully loaded on film material as the concentration of protein is similar to suspension proteins. Another evaluation is pH metery, which is a useful method for protein activity assessment. Here by the use of this technique related outcome has been achieved [28]. Protein activity determination was carried out by pH metery of different concentrations of samples. As it is shown in figure3, protein as suspension shows a typical activity including maintenance of pH in the range of biological environment, while this concentration in the presence of film has no similar activity. For more resolution, the concentrations of protein in the presence of film decreased (2.2(mg/ml) and 1.2(mg/m)) .

The finding indicates that protein ability decreases due to unbinding on the film. It can be concluded that the certain percentage of protein can bound to the film. However, this part is a significant value to consider. It can be seen from figure 4 and 5 that pH alteration range is different among BR proteins loaded on glass sample and BR protein loaded on polycarbonate surface sample, but both of these findings approve the proton pump activity as the pH amount alters. That is, the confirmation of biological activity for BR protein on film surface. Techniques such as FTIR-ATR spectroscopy provide information related to surface characterization [29]. Results from figure 6 and 7 depict that by the use of this technique, it is confirmed that the surface of polycarbonate chemical is modified after immobilization of BR. In addition to this, AFM is another applied technique for imaging protein patterns and measuring dimension of proteins [30].

The findings specify the accuracy of previous conclusions by the high- resolution method. In a way that, protein binding on the surface can be approved by visualizing methods as it is illustrated in figure 8. After applying the designated proteins on the film, noticeable

changes, can be observed. These changes are due to electrostatically generated arrayed protein. Consequently, all these findings proved that this immobilization can be used to prepare protein memories instead of chemical dyes in the polycarbonate layer of CD, which is attained by different methods. In fact, designed protein storage model is approved by these techniques since presenting the promising stability and activity aspects.

CONCLUSION

In this work, we studied the activity of BR in variety of concentrations. The results show that the best spectrum was measured 3.2 mg/ml for concentration of polymer films which contains BR. Afterwards, the activity of BR on glass and polycarbonate surfaces was measured. The results show that BR immobilized on polycarbonate surface and it retained biological activity to read data.

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