Discussing the stabilizing bacteriorhodopsin through spin coating method in protein nano-memory

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ABSTRACT

Protein memories are novel technologies which use the hidden abilities of bacteria proteins and biological molecules in order to the electronic processes. Four decades before baceriorhdopsin (BR) protein was extracted from the membrane of *Halobacerinum Salina* rum found in high salt water depositions after its original discovery in 1967. In this study, for immobilizing the protein a film has been provided from the different weight/volume use of polyvinyl alcohol and gelatin polymers. Then a suspension of protein bacteriorhodopsin with the 3/2 $mg.ml^{-1}$ has been used that according to the spectroscopy and determining their activity, optimizing concentration of a film containing bacteriodospian has been obtained. Polymeric film containing bacteriorhodopsin protein has been injected on the glass and by use of spin coater, after obtaining optimizing protocol become stabilized. After that the biological activity of the bacteriorhodopsin protein has been studied. Several biological molecules are being considered based on their usage in computers sciences, as though the bacterial protein – BR has generated much interest among scientists. Functional immobilization of membrane proteins requires consideration of their physiological needs, often dictated by the quality and components of the natural hydrophobic environment surrounding this class of proteins.

Keywords: Protein memories; baceriorhodopsin; spin coating; glass surface.

INTRODUCTION

Protein memories are novel technologies which used the hidden abilities of bacteria proteins and biological molecules in order to the electronic processes [1, 2, 3]. In recent years attentions has been directed toward the storage tools of more rapid and with better viability. Recently, researchers pay attention to the memories based on protein which is the ameliorative of electrical memory speed, insurance of magnetic hard discs and the capacity of optical magnetic storage [4]. Early researches protein-based memories yielded some serious problems with using protein for practical computer application. The most serious problems are the instability and unreliable nature of proteins which are subjected to thermal and photochemical degradation of them, making rooted premature or higher-temperature use impossible [5].

Optical applications such as light modulators and optical storage based on organic photochromic materials have been under investigation since 1970. One of the major problems of conventional organic photochchromic materials has been unwanted side reactions that have caused fatigue in the read craze cycles [6]. Robust immobilization techniques that preserve the activity of biomolecules have many potential applications. For example, microarrays, micro beads, and biosensor chips play increasingly important roles in characterizing the function and interaction of biomolecules, purification schemes, and point of care diagnostics [7].

Although appropriate for many applications, these schemes can, at time, be limited by the reduction in bimolecular activity due to protein denaturation, unintended reactions at or near the active domain, or random orientation at the solid-liquid inter face that reduces access to molecular

recognition sites [8]. Four decades ago baceriorhdopsin (BR) protein was extracted from the membrane of *Halobacerinm Salina* rum found in high salt water depositions after its original discovery in 1967 [9, 10]. The BR contains the chomophore retinal and this protein is known to be one of the simplest biological energy convertors [11]. Bacteriorhodopsin protein which is in the group of photo chrome proteins has been under scrutinize in last 3decades.This membranous protein is produced by *Halobacteria* and is a key protein with photosynthesis ability. What makes this protein different from photochromic organic and nonorganic materials is its bi-dimensional crystalline structure which shows persistence at the thermal and chemical degradations[12]. The BR is found in the cell membrane of bacteria. In these organisms, it serves as a light – harvesting system for generating adenosine triphosphate (ATP)- the fuel of most living organism, under aerobic conditions [6]. Matrices made from natural polymers such as gelatin [13] and synthetic polymers such as PVA [14] have already been used for BR film preparation. These matrix materials allow mechanically stable films with good optical quality to be manufactured [15] PVA is a water-soluble polymer, which forms a thin stable film-upon dehydration. The main functional group is hydroxyl. Gelatin is derived from skin or bones, the principle component is the collagen, which in gelatin normally contains 300-4000 amino acids [16].

MATERIALS AND METHODS

Materials used in this study include: Potassium chloride (Germany brand), Magnesium chloride(Germany brand), Chloridric acid (Sigma Aldrich),Potassium hydroxide (Germany brand), Three etalon amino (Fluka), Polyvinyl alcohol (Sigma Aldrich) and Bacteriodohopsin protein (Sigma Aldrich). For spectrometer analysis of bacteriorhodopsin UNICUM UV-300 machine has been used ,which the method is as follow:

1- A mixture of 10ml bacteriorhodopsin with the concentration of 3/2-mg.ml1 with 350 microl double distilled water were prepared and uniformed.

2- First spectrophotometer machine was calibrated by two distillated water.

3- The rate of the absorb of the suspension was recorded.

4- Absorb range of the sample in the 570mm and 280mm wave length were recorded the maximum absorption in the basic state were related to Aromatic Amino acids.

5- Test was done in the room temperature and for the best result, it repeated 2 or 3 times.

Investigation of bacteriorhodopsin biological activity

To prove the bacteriorhodopsin activity, pH of light changes were studied. Because the bacteriorhodopsin is a protein pomp which depends on the light and under the condition of radiation of light it pumps protein and lead to the changes of the pH. Also the concentration of the magnesium ion is effective in the rate of protein pump. In this study for proving the activity of BR and examining the concentration of the magnesium in the rate of protein pumping ,the method of Dr. Ikegami and Dr. Kouyama which has done in 1933 has been used which is as follow:

1-For examining the activity two 50ml flask (one as a control and two as samples) were chased.

2-Control erlen contains a solution of 3M KCL and 80mM MgCl2

3-One of them contains BR 3/2 mg.ml-1 and the other one constitutes 3M KCL and 80mM MgCl2 and BR 3/2mg.ml-1:The important note is that the pH alterationhappens only in some minutes so BR increased during the test,

4-The initial pH of each flask was adjusted to 7.1 using KOH and HCL,

5-Erlens' door was closed by a plastic cap

6-A study lamp installed 30 cm far from the relents

7-Electrod in pH meter put in the erlen. Then so fast 50ml of bacteriorhodopsin suspension has added to it and the door was closed with a cap

8-In the time of 30minutes, solution pH examined by a machine.

9-Test was done in the room temperature.

Providing polymer films containing bacteriorhodopsin

In this study, we used the polymer film containing the bacteriorhodopsin in the matrix of polyvinyl alcohol and gelatin and Korposh method. Gelatin

as a matrix with amino-agent groups has advantages:
1-Various applications and its usage for the processing technology
2-Is water soluble and can prevent of changes in the nature of bacteriorhodopsin.
3-Images from electron microscopy show that the homogeneous films can be made.
PVA is also a water soluble material with Hydroxyl agent group which shows a resistant thin film after dehydration. So, these matrixes produce a resistant film with high light quality in which photo cycle of bacteriorhodopsin is done completely to provide polymer films containing bacteriorhodopsin :

The first phase: preparing a suspension contains BR

1-Suspension 3/2mg.ml1- bacteriohodopsin with two times distilled water.
2-The resulted suspension has spinout for 20 minutes.
3-It was put on the shaker for 6 hours.

The second phase: Providing gelatin and polyvinyl alcohol

1- 0.1gram gelatin and 0.1 gram polyvenylalcohol with 19.8 ml two times distillated water was put in the room temperature for 20 minutes.
2-The final mixture was put on the shaker annotator device for 40 minutes with the temperature of 60 degree to produce a 1% weight mixture of the film.

The third phase: Adding bacteriorhodopsin suspension

1- 0.056 ml of bacteriorhodopsin suspension is mixed with 3/2mh.ml1-with 0.083 ml of gelatin polymeric and polyvinyl alcohol.
2-the final volume of the film becomes 0.167ml by using two times distillated water.
3-Erlen containing this mixture was put on the magnetic agitator for 20 minutes.

The fourth phase: Adding three ethanol amino

1-Three ethanol amino increases the light senility of the bacteriorhodopsin films. As a result the liquid solution of the three ethanol amino of 0.4 molar was added to the mixture that the ratio of TEA: BR became 250:1,
2-The mixture was put in the magnetic agitator for 20-30 minutes then it was put in the dedicator in the vacuum condition,
With the same way, films with different concentrationswere provided in order to optimize the concentrations of the films. Soluble stabilizer film formed on the glass substrate by spin Address. To optimize the stability of the films containing different concentrations, bacteriorhodopsin device spin coater was tested at different periods. At this stage it was decided to either Svspansvyn protein and protein film on a glass substrate stabilized. The last result used 1% film of volume and weight containing bacteriorhodopsin [sin with concentration of 3/2mg.ml1- also after multiple layering, obtain the speed profile of spin coating.

Methodology:

1-providing bacteriorhodopsin with the concentration of 3/2mg.ml-1,
2-providing 1% film,
3-contaminating a glass surface with 1%film containing bacteriorhodopsin and put the surface in the spin coater. Settle the programmed on the optimizing profile,
4-Putting the glass in the room temperature for 24 hours to become dry,
5-In the last phase for determining the rate of the bacteriorhodopsin protein activity which is stabilized on the glass bacteriorhodopsin suspension as a determinant was used.

RESULTS

Determining biological activity of bacteriorhodopsin protein with the concentration of 3/2mh.ml-1 in polymer films with the ratio of different volume/weight. First the bacteriorhodopsin suspension was inspected to determine its activity with the measuring of the pH changes.
For examining polymer films, we proved polymer films with the concentrations of 0.1%, 0.01% and 1% which contains bacteriorhodopsin
3/2mh.ml-1 that after determining biological activity the results sketched in the bellow diagram.

Somas a bacteriorhodopsin is a photonic pump based on light and the movement of proton across linen leads to pH change. The observed pH changing in this study proves photonic pump activity of bacteriorhodopsin in different conditions.

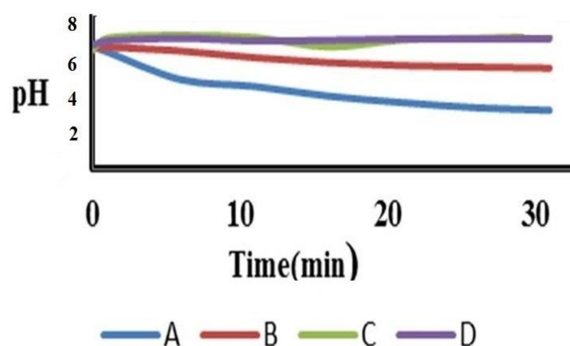

Figure 1: BR is a light dependent proton pomp, which can transfer the proton out of the cells due to light excitation and cause to pH reduction there. This characteristics is used in order to prove the proton pomp activity of BR . **A:** Investigation of biological activity of BR protein in 3/2mg.ml-1 bacteriorhodopsin suspension. **B:** Investigation of biological activity of BR protein for polymeric film containing 1% BR suspension.**C:** Investigation of biological activity of BR protein for polymeric film containing 0.1% BR suspension. **D:** Investigation of biological activity of BR protein for polymeric film containing 0.01% BR suspension.

Results obtained from analysis of absorption range of polymer films based on bacteriorhodopsin

In this phase, first spectroscopy of bacteriorhodopsin suspension was measured. Then,3/2mg.ml-1 concentration bacteriorhodopsin became stabilized in polymer films 0.1 , 0.01 and 1% and was used. These results were shown by UV-visible in the bellow diagram (Fig 2).

In this study the results from analysis of polymer film with different optimizing concentrations from bacteriorhodopsin showed that polymer film 1%contains bacteriorhodopsin with 3/2mg.ml-1 concentration had the highest absorption in 280mm to 570mm and the highest light transparency.

The ratio of A570/A280 for bacteriorhodopsin suspension was almost 3/64 and for 1% film containing 3/2mg.ml-1 this amount became 4/97 which shows the low ratio of light distribution of polymer film and made an appropriate route for stabilizing bacteriorhodopsin and making films based on bacteriorhodopsin.

Results showed that the stabilizing bacteriorhodopsin in gelatin and polyvinyl alcohol matrix did not change the wave length absorption and the light cycle of bacteriorhodopsin was done completely. So light activity of bacteriorhodopsin was kept in the gelatin and polyvinyl alcohol matrix.

Examining results of fixing with the spin coating method

For fixing in this way films with different concentrations were used and for obtaining the optimize speed protocol, try and error of different speeds were done with spin coater. At last, polymer film 1% containing bacteriorhodopsin with concentration of 3/2mg.ml-1 stabilized on the glass which was expanded uniformly. The obtained optimized speed for spin coater and the biological activity diagram of bacteriorhodopsin protein which was fixed on the glass is shown on figures 3 and 4.

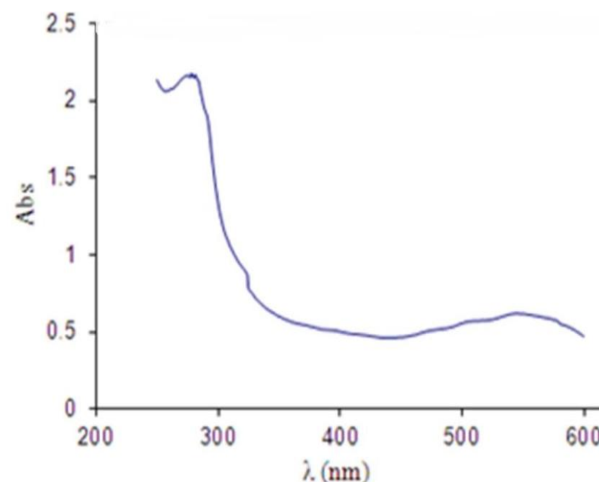

Figure 2: Optimized profile obtained for layer making, Stabilization of BR in polymeric gelatin matrix and polyvinyl alchol didn't alter the wave length maximum absorption and the light cycle of BR was completely done. Hence, this indicated that the light activity of BR was conserved through gelatin and polyvinyl alchol matrix.

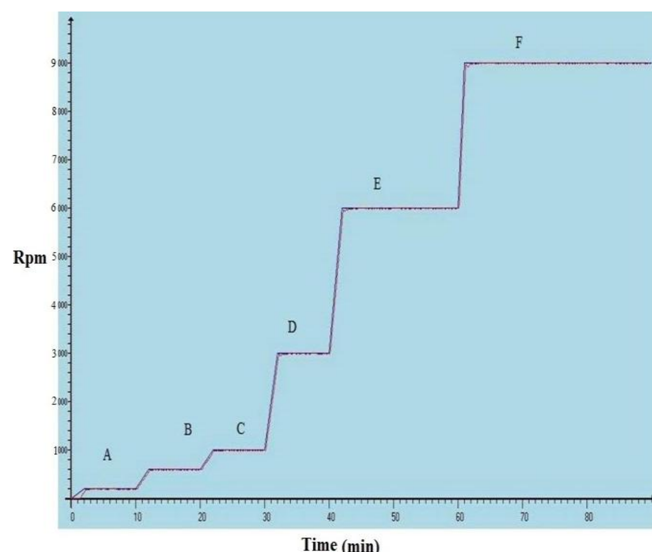

Figure 3 . Optimized profiles for spin coating method, the biological activity diagram of bacteriorhodopsin protein which was fixed on the glass, The investigation showed that the biological activity of proteins pump and pH optimum of bacteriorhodopsin will change.

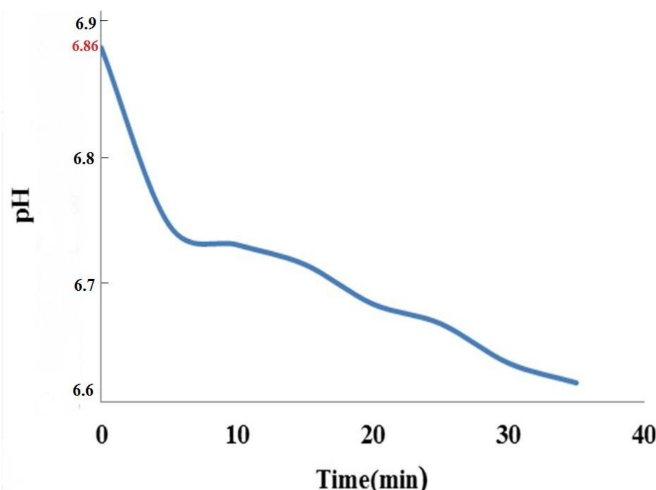

Figure 4. Biological activity of bacteriorhodopsin protein for polymer film 1% : containing suspension 3/2mg.ml-1 stabilized bacteriorhodopsin on a glass from spin coating method.

The biological activity of protein and pH investigations showed the optimum activity of photonic pump of bacteriorhodopsin. Also spin coating on the glass because of providing films with nanometer thickness provides a suitable method for using Nano tools.

DISCUSSION

Several biological molecules are being considered for use in computer sciences, but the bacterial protein – BR has generated much interest among scientists. Bacteriorhodopsin, a light harvesting bacterial protein, is the basic unit of protein memory and is the key protein in *Halobacteria* photosynthesis. It functions like a light- driven photo pump [17]. Orientation of the BR membrane is crucial for one-directional proton pumping and thus generation of maximum electrical potential [18]. To prove the bacteriorhodopsin activity, the changes related to pH of the light has been studied. Because the bacteriorhodopsin is a protein bomb which depends on the light and under the condition of radiation of light it pumps protein and lead to the changes of the pH. Also the concentration of the magnesium ion is effective in the rate of protein pump. In this study for proving the activity of BR and examining the concentration of the magnesium in the rate of protein pumping ,the method of Dr. Ikegami and Dr. Kouyama which was done in 1933 used (19). Bacteriorhodopsin comprises a light absorbing component known as chromophore, that absorbs light energy and initiates a series of complex internal structural changes to alter the protein‘s optical and electrical characteristics. This phenomenon is known as photo cycle [17].

The overall result includes the transfer of protons from the cytoplasmic side of cell membrane to the extracellular environment. It contributes to build up of the proton gradient across the membrane resulting in electrochemical potential and thus local pH changes [20]. The photochromic cycle (read-erase cycles of more than 10^8 reports) of the protein memory is quite uniqne: the ground state has abroad absorption band centered at 568 nm and a metastable state centered at 412 nm with a thermal relaxation time of 10 ms. The metastable state offers two potential advantages. First, it can be stimulated either by electrical field or photons to decay into the ground state in 200 ms. Second, the thermal relaxation time of this state may be extended by five orders of magnitude by suitable chemical treatment of the protein memory [6]. In most of the technological application, it is

however impractical to use the naked molecule: the polypeptide chain is folded nine times across the lipid bilayer membrane [21].

Bacteriorhodopsinhas a characteristic absoptance peak at 570 nm, which is attributed to the retinal chromophore covalently, bound to the apo protein of bacteriorhodopsin molecule through a protonated Schiff base linkage [15]. While bacteriorhodopsin can be used in any number of schemes to store memory [5, 6]. Soluble proteins have been readily immobilized through cross-linking aldehydes or this's with protein amines or carboxyl groups for applications such as micro arraying by printing [22, 23, 24, 25]. Functional immobilization of membrane proteins requires consideration of their physiological needs, often dictated by the quality and components of the natural hydrophobic environment surrounding this class of proteins [26]. In most of the technological applications, it is however impractical to use the naked molecule, the polypeptide chain is folded nine times across the lipid bilayer membrane [5]. Apart from optical memories, another promising application is as a chemical sensor [27, 28]. Molecules such as NH_3 or CH_2 engender significant changes in the optical spectra of the ground state and the photocycle intermediates [15, 16]. In PVA, water is strongly act ached via numerous hydrogen bonds to the hydroxyl groups of the polyol, and consequently the bacteriorhodopsin chromophore will be less exposed to water [28]. Furthermore, this process also increases the actinic sensitivity of the material by several orders of magnitude [6].

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