Microbiorhodopsin a powerful candidate for a photosensitizer of the TiO2-based pollutant degradation system

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Abstract. Bacteriorhodopsin (BR) is a light driven proton pump firstly found in the membrane of Halobacterium salinarum. Because of its high quantum efficiency, high stability under harsh conditions and many interesting photochemical properties, BR was shown to be able to enhance the performance of TiO₂ materials in TiO₂-based solar cell, photocatalytic water splitting and photocatalytic degradation of pollutants. However, high cost of the preparation of the BR restricts its industrial application. Although numerous attempts have been made, E. coli-expression system of the BR, which is most economic and fast way to prepare proteins, has not been developed yet. In the present work, we successfully expressed BR homolog, Archaelrhodopsin (AR) found in a Halorubrum species by E. coli-expression system. The E. coli expressed AR retained its photoactivity and showed similar photoreaction properties as that of BR. The E. coli expressed AR might be an excellent candidate for a photosensitizer of the TiO₂-based solar energy conversion system.

Keywords: Bacteriorhodopsin, Archaelrhodopsin, TiO₂ nanoparticles, Photocatalytic degradation.

1. Introduction
Solar energy is the primary energy source of our planet. The sun provides energy for all living creatures on the earth through the process of photosynthesis. Today solar energy also becomes one of the most important renewable energy sources for human development. Titanium dioxide (TiO₂) is a widely utilized semiconductor for transforming the solar energy. The TiO₂-based system has been utilized in solar cell, photocatalytic hydrogen production and photocatalytic degradation of organic pollutants[1]. The Schematic TiO₂ sensitization to visible light with dye. However, wide bandgap (3.2 eV) of TiO₂ requires UV light irradiation for excitation, that limits the application of solar light TiO₂-based system since UV light (λ< 387 nm) in solar light is less than 5 %[2]. To improve the performance of TiO₂-based system, surface of TiO₂ materials can be modified by the organic dyes that show absorbance in the visible region. Many chemically synthesized organic dyes were proven to be able to enhance the performance of TiO₂-based system. However, there are common limitations in these organic dyes: (1) most of the chemically synthesized organic dyes are prone to photo-
degradation; (2) most of the chemically synthesized organic dyes contain aromatic groups and degradation of these dyes would bring persistent and toxic aromatic compounds in the environment. Thus, it is important to find a stable and environmentally friendly materials for the application in the dye-sensitized TiO2 system.

Bacteriorhodopsin (BR) is a photoactive protein found in the membrane of Halobacterium salinarum[3]. The protein shows wide absorption band in the visible light region and extremely stable under sunlight irradiation[4]. As it is made of peptide and a retinal cofactor, degradation of BR does not produce any pollutant. BR was shown to be able to enhance the performance of TiO2 materials in TiO2-based solar cell[5], photocatalytic water splitting[2] and photocatalytic degradation of pollutants[6]. The limitation of BR for an industrial application is its price. Although numerous attempts have been made, E. coli-expression system of the protein, which is most economic and fast way to prepare proteins, has not been developed yet. Present methods of preparation of BR are time consuming and inefficient, that makes the cost of BR very high. According to Sigma-Aldrich (China), price of BR is 7,686.90 Chinese Yuan per microgram that makes the application of BR in industrial scale impossible. In the present work we expressed a BR homolog, HeAR found in a Halorubrum species isolated from Ejinoor salt lake of China[7]. The protein showed similar photoreaction properties as that of BR and most importantly the E. coli-expression of the protein was achieved. The protein might be an excellent candidate for a photosensitizer of the TiO2-based system.

2. Materials and Methods

2.1 Isolation of the purple membrane from Halobacterium salinarum

Bacteriorhodopsin proteins make a characteristic membrane domain in the cell membrane of Halobacterium salinarum, which is called the purple membrane composed of BR and lipids. The purple membrane was prepared by a sucrose density step gradient according to the reported procedure [8]. The collected purple membrane was suspended in 50 mM Tris-HCl buffer, pH 7.0 at room temperature.

2.2 Expression and purification of HeAR from E. coli cells

HeAR with a histidine tag at the C-terminus was expressed in E. coli BL21 (DE3). The expression plasmid was constructed using pET-21c (+) vector (Novagen, Madison, USA). The E. coli with the expression vector was cultured with 1mM IPTG and 10 μM all-trans retinal for 4 hr. The total membranes of the E.coli solubilized in 1% n-dodecyl-β-D-maltoside (DDM) were purified by a Ni-NTA agarose (Qiagen, Tokyo, Japan) column and gel filtration column chromatography. Absorption spectra were measured by using MPS 2000 recording spectro-photometer (Shimadzu, Kyoto, Japan).

2.3 Photocatalytic degradation of phenol with the purple membrane

TiO2 nanoparticle (particle size: 21 nm; surface area: 50 m²/g) was purchased from Dalian Heptachromia SolarTech Co., Ltd, China. The photo-degradation experiment, suspension of TiO2 nanoparticles and dye solutions were transferred into 50 mL flask and magnetically stirred for 30 min in the dark to modify the surface of TiO2 with dye. Subsequently, phenol solution was added to a final concentration of 20 mg/L and the mixture was stirred for 5 min in the dark before light irradiation. A mercury lamp (500 W) was applied as a light source. The concentrations of phenol were measured with a time interval of 30 min by 4-aminoantipyrine spectrophotometric method.

3. Results and Discussion

3.1 Isolation of the purple membrane fragment

In the case of Halobacterium salinarum, the purple membrane containing BR and the red membrane containing many other different membrane proteins and a characteristic carotenoid, bacterioruberin, can be separated by sucrose density step gradient centrifugation. As shown in Figure 1, the red
membrane and the purple membrane of *Halobacterium salinarum* were well separated.

![Image](image.png)

**Figure 1.** Separation of membrane fraction from *Halobacterium salinarum* by a sucros step density gradient.

### 3.2 Expression of HeAR in the E. coli cells

The expression and purified HeAR were shown in Figure 2. The total proteins of the non-induced (column T₀ in Figure 2-a) and induced whole cells (column T⁺ in Figure 2-a) were separated by SDS-PAGE. A conspicuous band was observed at the molecular mass of about 31 kDa only in the column T⁺. The molecular size of purified HeAR after the Ni-chelating chromatography (column E in Figure 2-a) was similar to that of the conspicuous band. The absorption spectrum of the purified HeAR was shown in Figure 2-b (red line). The absorption maxima of the retinylidene chromophore were identified at 550 nm for HeAR, or at 558 nm for BR.

![Image](image.png)

**Figure 2.** The expression of histidine tagged HeAR. Fig 2-a, The expression of histidine tagged HeAR analyzed by SDS-PAGE. M, molecular weight marker; T₀, non-induced cells; T⁺, induced cells; E, purified HeAR. Fig 2-b, Comparison of the color of the *E. coli* cells and the purified HeAR. A, HeAR plasmid free *E. coli* cells. B, *E. coli* cells containing HeAR plasmid. C, the purified HeAR.

### 3.3 Photo-degradation of phenol with purple membrane

During the dark incubation and the following light irradiation we monitored the change in phenol concentration of TiO₂ suspensions with different amount of BR. The decrease in the phenol concentration did not exceed 5 % of the initial concentration in the dark for 2 hours (data not shown). Light irradiation of the system containing TiO₂ (0.5 g/L) and phenol (20 mg/L) gradually decreased the concentration of phenol. After 90 min of irradiation, 3.56 % of phenol had been degraded. It should be noted that the mercury lamp has an irradiation in the UV range, that may be absorbed by
TiO$_2$ nanoparticles and caused photocatalyzed degradation of phenol. We measured the photocatalytic degradation by addition of different concentration of BR or alizarin red as a photo sensitizer. In the case of alizarin red, the optimum concentration was 2.1×10$^{-5}$ mol/L. At this concentration, 18.0 % of phenol was degraded after 90 min of light irradiation as shown in table 1. BR showed a similar sensitizing effect as alizarin red, the optimum concentration was 4.94×10$^{-5}$ mol/L. At this concentration, 14.2 % of phenol was degraded after 90 min of light irradiation. These results indicate that BR has a sensitizing efficiency similar to that of alizarin red. We measured the photocatalytic degradation by addition of different concentration of HeAR as a photo sensitizer. The optimum concentration was 0.4 μM, at this concentration, 28.0 % of phenol was degraded after 90 min of light irradiation and 37.0 % of phenol was degraded after 150 min of light irradiation as shown in Figure 3. The optimum concentration was 0.8 μM, at this concentration, 20.0 % of phenol was degraded after 90 min of light irradiation and 25.0 % of phenol was degraded after 150 min of light irradiation as shown in Figure 3. The results showed that the efficiency of 0.4 μM HeAR was higher than that of 0.8 μM HeAR. These results indicate that HeAR and BR has a sensitizing efficiency similar to that of alizarin red.

Table 1. Photo-degradation of phenol catalyzed by TiO$_2$ with alizarin red or BR as a sensitizer.

| Sensitizer   | Concentration (mol/L×10$^{-5}$) | Irradiation time (min) |
|--------------|---------------------------------|------------------------|
| (-)          | -                               | 0.69 1.48 3.56         |
| alizarin red | 2.1                             | 4.15 15.6 18.0         |
|              | 4.2                             | 6.50 11.3 15.1         |
|              | 8.4                             | 5.38 9.62 11.5         |
|              | 16.8                            | 4.49 8.65 10.0         |
| BR           | 2.05                            | 2.26 4.73 5.01         |
|              | 3.95                            | 7.38 11.2 14.1         |
|              | 4.94                            | 9.94 13.2 14.2         |
|              | 5.93                            | 5.76 11.3 12.8         |

Figure 3. Photo-degradation of phenol (20 mg/L) catalyzed by TiO$_2$ (0.5g/L) with different concentration of HeAR (0.4 μM, 0.8 μM). The degradation rate (%) was calculated as: Ct/C0×100. C0, concentration of phenol before light irradiation; Ct, concentration of phenol after light irradiation of t min.

4. Conclusions
We showed that the purple membrane isolated from the total membrane fraction of *Halobacterium*
*salinarum* represented a good performance as a sensitizer of TiO$_2$-based phenol photocatalyzing system as alizarin red as reported previously. We successively expressed HeAR in *E. coli* and showed that the *E. coli* expressed HeAR retained its photoactivity. The performance of HeAR as photosensitizer of TiO$_2$-system will be tested and able to modified by gene-engineering technologies.

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