Evaluation Techniques of Bacteriorhodopsin Thin Films

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Bacteriorhodopsin (bR) exhibits a light-driven proton pump function, and photo-sensor devices having the bR thin film show a transient photo-current signal under the “on” or “off” of photo-irradiation. Therefore, it is important to understand the orientation of bR in the thin film for fabricating such devices and elucidating the transient photo-current signal characteristics. In the present paper, we show that by second harmonic generation interference technique, the bR thin films prepared by a dip coating technique have a chiral and polar orientation with C_∞ symmetry. Furthermore, we discuss the absolute orientation of bRs in bR thin films.

1 Introduction

Bacteriorhodopsin (bR) is found in the purple membrane of halobacterium salinarum and is a protein that has a covalently bound protonated Schiff base retinal chromophore. bR releases protons (H^+) into the extracellular side of the cell with light absorption and takes up protons on the cytoplasmic side of the cell. This process accompanies the photoisomerization reaction of retinal chromophore in bR, and bR experiences a photochemical cycle, passing through a series of intermediate states. Thus bR has a light-driven proton pump function. Additionally, bR is tolerant to a number of environmental elements, including pH and temperature. Optical sensors that utilize differential photoelectric response related to the aforementioned light-driven proton pump function have been developed[1]. Furthermore, applications in a number of other photoelectric devices that utilize the characteristics of bR have been proposed[2][3]. In recent years, we have also conducted research into the development of biomimetic optical sensors, which utilize those properties. It is important to reveal the symmetry of bR thin film and the orientation of bR in bR thin film to assess the performance of such devices and to reveal the origin of transient photo-current signal characteristics. In this research, we used the second-harmonic generation (SHG) interferometry method to examine the symmetry of bR thin film and the absolute orientation of bR in bR thin film. We prepared the bR thin films by a dip-coating technique.

2 Preparation and evaluation of bR thin films

2.1 Preparation of bR thin films

In order to fabricate various devices using bR, bR thin films have to be prepared on a substrate. There are a number of ways to prepare bR thin films, including the Langmuir-Blodgett (LB) technique, the electro-phoretic deposition technique, the electrostatic layer-by-layer self-assembly technique, a technique that uses antigen-antibody recognition and the spin-casting technique, all of which have both advantages and disadvantages[4]. For this research, the dip-coating technique, which will be discussed below, was used to prepare bR thin films. We prepared a purified purple membrane solution, in which purple membrane fragments are included in a 10 mM Tris-HCl buffer with pH 7.2. The concentration of bR in the solution was 4 mg protein/ml. A pre-cleaned glass substrate with a thickness of 1 mm was dipped into the purple membrane solution in a cuvette and withdrawn at a speed of 500 μm/s. Usually, bR thin films are deposited on both sides of the glass plate when using the dip-coating technique. The thickness of the deposited bR thin films was approximately 50 nm. For the investigation of the symmetry of bR thin films using the SHG interferometry method, the glass plate with the deposited bR thin films on both sides was used as is, as a sample glass plate. On the other hand, for the investigation of the absolute orientation of bR in bR thin films, the bR thin film of one side was wiped and then used as a sample glass plate.

2.2 Evaluation of macroscopic symmetry of bR thin films using the SHG Interferometry Method

Figure 1 shows the optical setup of the SHG interferometry method used to investigate the symmetry of the bR thin films deposited on both sides of the glass plate using the dip-coating technique. A nanosecond pulse laser at a wavelength of 1,064 nm was used as the fundamental light and the second harmonic generation (SHG) with a 532 nm wavelength was detected.

Measurements were carried out with fundamental light and SHG polarization-state combinations of p-p, s-p, p-s and s-s while its incident angle was changed. p and s represent p-polarization and s-polarization respectively. Of the combinations p-p, s-p, p-s and s-s, SHG in p-s was observed in addition to p-p and s-p, in which combinations SHG is usually observed in symmetry of $C_{\infty v}$. SHG was not observed in the s-s combination. Figure 2 shows the results of the experiment on the incident angle dependence of SHG in the p-p and p-s combinations. The SHG from the bR thin films deposited on both sides of the glass plate interfere with each other. An SHG interference fringe was observed due to the change in optical path length caused by the rotation of the glass plate and the frequency dispersion of the refractive index of the glass plate at the fundamental light and SHG frequencies. The p-p combination is related to the achiral components of the nonlinear optical susceptibility of SHG while the p-s combination is related to the chiral components. The almost opposite phase between the interference patterns of the p-p and p-s originates from the difference for the coordinate transformation between the achiral components and chiral components. These experiments have revealed that the macroscopic symmetry of bR thin film is chiral polar C_∞ symmetry[5]. It was found that nonlinear optical susceptibility components are χ_{333} = 0.35 pm/V, $\chi_{311} = \chi_{322}$ = 0.2 pm/V, $\chi_{113} = \chi_{131} = \chi_{223} = \chi_{232}$ = 0.2 pm/V, $\chi_{213} = \chi_{231} = -\chi_{123} = -\chi_{132}$ = 0.036 pm/V. Here z (3) is the surface normal direction and the ∞ rotation axis in C_∞ symmetry, and x(1) and y(2) are in plane. The determined values are in agreement with previously reported results using the bR films prepared by using other techniques[6]. Furthermore, in the course of the experiment on the circular dichroism in SHG, an intriguing phenomenon was observed when interference of SHG caused by two thin films with chiral polar C_∞ symmetry properties was investigated[5].

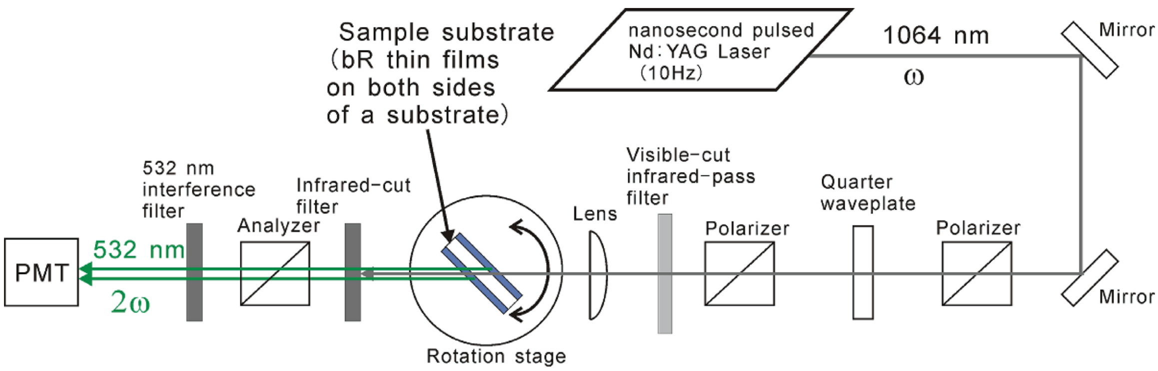

Fig. 1 Optical setup of SHG interferometry method

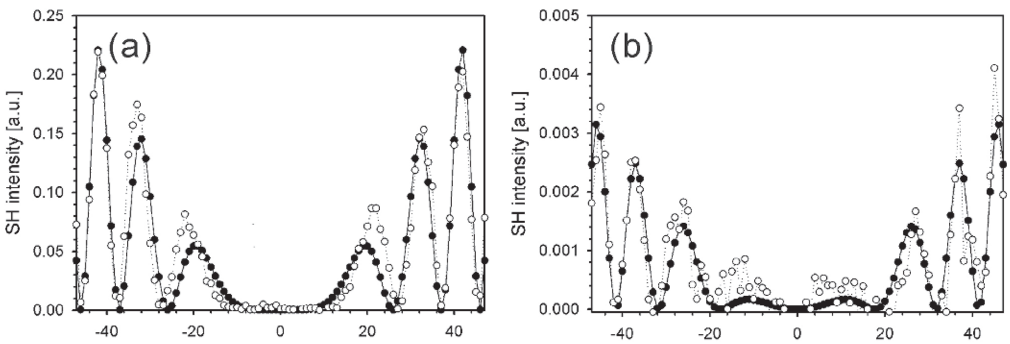

Fig. 2 Incident angle dependence of SHG in the p-p (a) and p-s (b) combinations

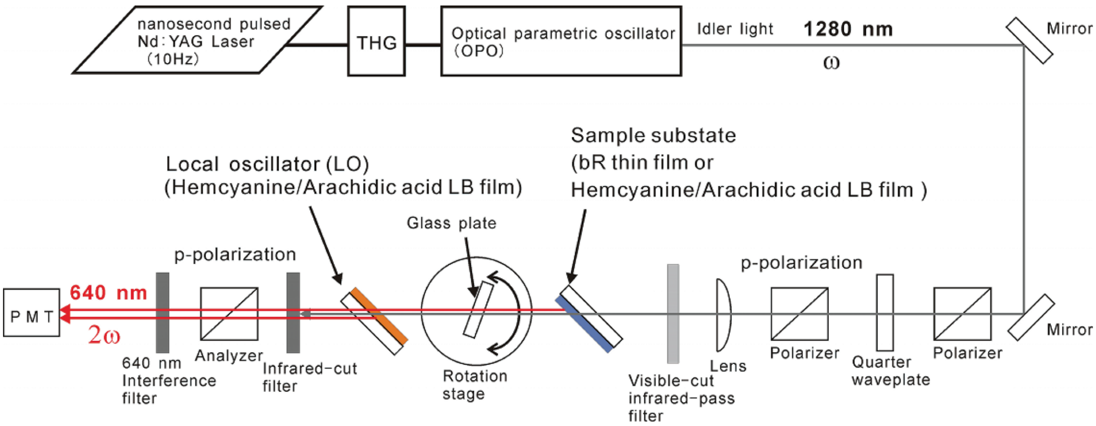

Fig. 3 Optical setup of SHG interferometry method to investigate the absolute orientation

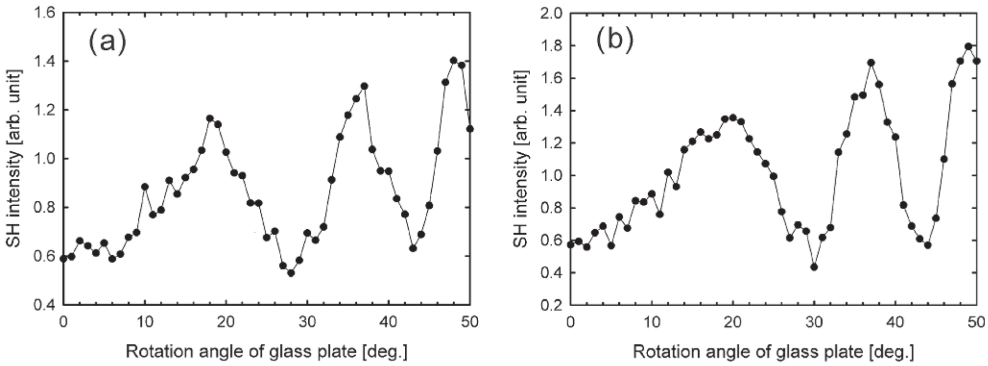

Fig. 4 (a) SHG interference fringe between the bR thin film and the local oscillator and, (b) SHG interference fringe between the mixed monolayer LB film of hemicyanine dye and arachidic acid, and the local oscillator

2.3 Evaluation of the absolute orientation of bR in bR thin film using the SHG interferometry method

Figure 3 shows the optical setup to study the absolute orientation of bR in bR thin film using the SHG interferometry method. The absolute orientation of bR in bR thin film was investigated by comparing the SHG interference fringe between the mixed monolayer LB film of hemicyanine dye (C_{22}-Hemicyanine) and arachidic acid, which has a known absolute orientation (the direction of polar orientation)[7], and an appropriate local oscillator, with the SHG interference fringe between the bR thin film and the same local oscillator. A nanosecond pulse laser at a wavelength of 1,280 nm was used as the fundamental light and second harmonic generation (SHG) with a 640 nm wavelength was detected. The SHG interference fringe was studied by setting the polarization state of the fundamental light and SHG as p-p and rotating the glass plate placed between the sample plate and the local oscillator[8].

Figure 4(a) shows the SHG interference fringe between the bR thin film and the local oscillator. Figure 4(b) shows the SHG interference fringe between the mixed monolayer LB film of hemicyanine dye and arachidic acid, and the local oscillator. The retinal chromophore in bR is responsible for second-order nonlinear optical response. Therefore the absolute orientation of bR in the bR thin film is determined by evaluating using the absolute orientation of the retinal chromophore as the marker. The SHG fringe in Figs. 4(a) and 4(b) follow an almost identical pattern,

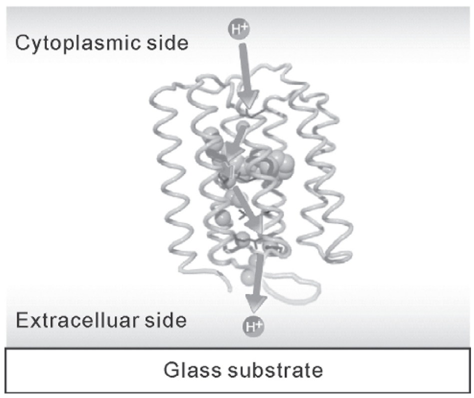

Fig. 5 The absolute orientation of bR in bR thin film

revealing that bR in the bR thin film prepared on the glass plate using the dip-coating technique has a tendency to orient the cytoplasmic side towards the air and the extracellular side towards the glass plate, as shown in Fig. 5[8].

3 Conclusion

It has been revealed that the bR thin film prepared by the dip-coating technique has a chiral polar C_∞ symmetry and the bR in the bR thin film has a tendency to orient the cytoplasmic side towards the air and the extracellular side towards the glass plate, by means of the SHG interferometry method. We expect that the knowledge of the absolute orientation provides us with insight for interpreting the transient photocurrent signal of the photo-sensor devices using the bR films and improving the performance of the devices.

The experiment has shown that the bR exhibits orientation tendencies as shown in Fig. 5. However, we do not yet know to what extent this occurs. In the future, we will develop more advanced methods of manipulating bR orientation and investigate means of improving the performance of bR photo-sensor devices and explore potential applications that can benefit from these characteristics.

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