The Fast Photovoltaic Response from Multilayer by Alternate Layer-by-layer Assembly of Polycation and Bacteriorhodopsin

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(Received October 29, 1999; CL-990923)

A thin multilayer film, formed by alternate deposition of poly(dimethyldiallylammonium chloride) (PDAC) and bacteriorhodopsin (bR) (pH=9), generated a photovoltage with defined polarities upon one flash excitation. Three time constants of 2.59 μs, 13.64 μs and 58.03 μs fitting to the kinetics of photovoltage were assigned to the decay of K, L_1 and L_2 intermediates of bR photocycle respectively. The positive photovoltage accompanying the formation of M indicated that the cytoplasmic side of PM fragments was directed toward the substrate.

Bacteriorhodopsin (bR) is a transmembrane protein regularly arranged in the purple membrane (PM) of Halobacteria and functions as a light driven pump.[1] Upon illumination, the proton's displacement distance from the initial position accompanying the photocycle of bR produces an observable photoelectrical signal in an orderly oriented sample. Several techniques such as traditional LB film,[2] electrophoretic deposition[3] and applying an external electrical field to PM suspension[4] have been used to orient the PM fragments for the study of photoelectric response.

Recently, a simple technique, alternate layer-by-layer electrostatic deposition of positively charged poly(dimethyldiallylammonium chloride) (PDAC) polyelectrolyte and PM fragments, has been used to form thin bR multilayer.[5] However, many optical and photoelectrical properties of such a film have been unclear so far. In this work, we successfully prepared a sandwiched-in $(PDAC/bR)_{14}$ multilayer film and reported a photovoltaic signal generated from it for the first time, and determined several intermediates of bR photocycle from photovoltaic signal according to the fitting time constants. Of most significance, the orientation of PM fragments has been decided from the polarities of photovoltaic signal.

The preparation of $(PDAC/bR)_{14}$ multilayer on quartz plate was done according to reference 5. In brief, a solid support (quartz plate or indium tin oxide (ITO)-quartz plate) with negatively charged surface was first immersed in 2 mg/ml PDAC solution at pH 6.8 for 5 min, and then rinsed in Mili-Q water for 2 min. After dried with nitrogen, the layered support was then immersed in 0.5 mg/ml bR suspension at pH 9 for 3 min and dried again by nitrogen. This process was repeated until $(PDAC/bR)_{14}$ multilayers was formed.

Since PDAC polyelectrolyte had no absorption from 250 nm to 750 nm, the deposition of bR on quartz substrate was monitored by a UV-250IPC spectrophotometer. For the photovoltage study, the electrochemical cell is shown in Figure 1. The circuit used was the same as before.[6] The input resistance was 100 MΩ. A frequency doubled Nd:YAG laser at a wavelength of 532 nm was used as the exciting light source. A halogen lamp was used as the monitoring light and the low intensity was used to avoid photoreaction of intermediates. To prevent short circuit, apomembrane (AM),[7] which has no retinal and can not pump protons as a result of the bleaching of PM fragments, was deposited alternatively with PDAC to form two $(PDAC/AM)_{10}$ thin films on both sides of $(PDAC/bR)_{14}$.

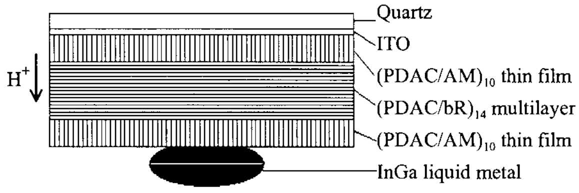

Figure 1. The electrochemical cell prepared for the study of photovoltage from PDAC/bR multilayer.

Figure 2 shows the UV-vis spectra changes upon $(PDAC/bR)_{14}$ film assembly. It can be seen that bR in the film is not denatured because its characteristic peak is similar to that in suspension. In addition, the absorbance of bR at 565 nm increases rather linearly with the deposition number. We therefore conclude that PM fragments are deposited layer-by-layer uniformly on quartz substrate.

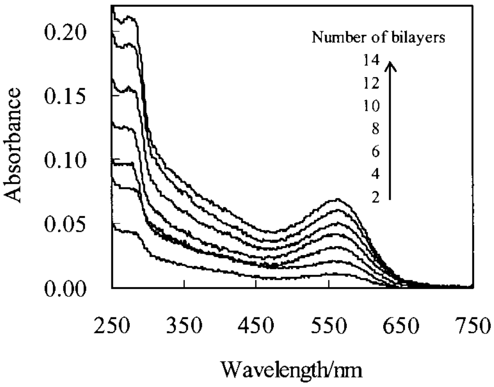

Figure 2. UV-vis absorption spectra changes of PDAC/bR multilayers on both sides of a quartz substrate.

Figure 3 shows the fast photovoltaic response from the $(PDAC/bR)_{14}$ film. After illumination, a small negative photovoltage was formed immediately and then changed its polarity within several microseconds to a large positive phase. The conversion of polarity within in the same range was also found in other oriented bR systems[6,8] and could be explained by the opposite movement of positively charged amino acid chains and protons. At about 160 μs, the maximal photovoltage was reached.

The total kinetic phase fits well to the sum of three expo-

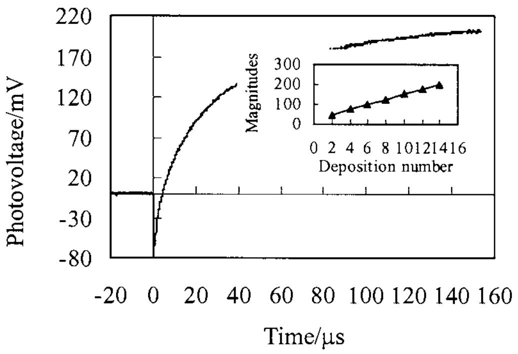

Figure 3. The photovoltatic response from (PDAC/bR)$_{14}$ multilayer on one side covered with ITO of a quartz substrate. The inset shows the linear increase of the positive maximum magnitude of photovoltages with the deposition number.

nentials as follow, $A_1e^{-t/\tau1}+ A_2e^{-t/\tau2}+ A_3e^{-t/\tau3}$ with three time constants of 2.59 µs, 13.64 µs and 58.03 µs respectively. These time constants are related with the intermediates during bR photocycle. K is the first state due to the decay of J and the decay time constant is only several microseconds.[9] Accordingly, among the constants above, τ_1 is associated with the decay of K. After K decays into L, L further decays into M in microseconds smaller than 200 µs according to one or several exponentials. Thus, it is inferred that both τ_2 and τ_3 belong to the process of L (including L_1 and L_2)→M. However, the relation between L_1 and L_2 can not be determined by present study.

In addition, the inset in Figure 3 also shows the linear increase of positive maximum magnitudes of photovoltages with the deposition number of PDAC/bR multilayers. This result is additional evidence that the assembly of PDAC/bR multilayer is formed uniformly.

Since the proton is pumped in the direction from the cytoplasmic side to extracellular side, the orientation of PM fragments can be determined by observing proton movement through the membrane in orientated PM film.[10] Namely, the orientation of PM fragments can be adjudged from the polarity of photovoltage accompanying the formation of M.[8] In present study, the photovoltage caused by the movement of protons from ITO surface to InGa liqulid metal was experimentally defined as the positive. Thus, the observed positive photovoltage caused by the formation of M indicated that the protons move in the direction from ITO to InGa liqulid metal in PDAC/bR multilayer, namely, the cytoplasmic side of PM fragments was directed toward substrate.

The reason for the generation of this orientation is that PM fragments have a large, permanent electric dipole moment perpendicular to the membrane plane in suspension and the influence of pH to this permanent dipole moment.[11] Under alkaline condition, the net negative charge is at the cytoplasmic side of the PM fragments. When positively charged PDAC is immersed in alkaline PM suspension, the cytoplasmic side of PM fragments is adsorbed by PDAC selectively.

It has been reported[12] that molecules in multilayer by alternate layer-by-layer assembly tend to orient in the direction of nitrogen flow during the drying process. In present study, frequent drying of PDAC/bR multilayer with nitrogen flow might also influence the orientation degree of PM fragments and the relative photovoltage signal. The detailed study will be done later.

In summary, the PDAC/bR multilayer, formed uniformly by the electrostatic deposition of PDAC and bR, generated a photovoltage with defined polarities. The positive photovoltage due to the formation of M indicated that the cytoplasmic side of PM fragments was directed toward the substrate under the present preparation conditions. The results in this paper are our preliminary investigation about PDAC/bR multilayer and the further study is in progress.

This work was supported by National Natural Science Foundation of China (69731010) and Academia Sinica (KJ-951-A_1-501-05).

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