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Short communication

A novel method for artificial lipid-bilayer formation

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Abstract

Many proposals have been made regarding the development of biosensors using single-channel recording with an artificial planar bilayer. The fragile nature of bilayer membranes is the major difficulty for the application of the artificial bilayer technique to the development of biosensors. We have developed an apparatus that promptly forms artificial bilayers. This technique is more efficient than other techniques for forming artificial bilayers. Bilayer membranes could be formed within 10 s requiring 1 μl of analyte solution to record single-channel currents using our apparatus. A bilayer was formed by pressing the membrane on an agarose layer with hydraulic pressure. With this novel apparatus, we have recorded single-channel currents of various types of channels such as the BK-channel, the nicotinic receptor channel and the ryanodine receptor channel. The properties of the channels determined with this novel technique agreed well with those determined with conventional techniques.

Keywords: Ion-channel; Artificial bilayer; Single-molecule imaging; Single-channel recording

1. Introduction

Single-channel recording techniques with artificial bilayer membranes have been applied to various types of channel proteins in order to observe their physiological and pharmacological properties. Furthermore, these techniques enable us to measure the properties of intracellular membranes, a task not possible with patch clamp pipettes. It is also possible to examine the effects of changes in the channel environment because in artificial bilayer experiments the experimental conditions, such as ion concentration in aqueous solution and lipid contents in the membranes, can be controlled. For example, we reconstituted the K^+ and the Ca^{2+}-channels from the membrane of the sarcoplasmic reticulum, which is an intracellular organelle, into artificial planar bilayers and characterized their electrophysiological properties(Ide et al., 1991a,b; Ohkura et al., 1995). Since it is possible to control the channel environment, we could directly examine the effects of the electric charge of the membrane on the channel conductance as well as the effect of membrane fluidity on the channel gating.

Over the past few years, a number of studies have been made on making biosensors utilizing planar bilayer membrane techniques. For example, the staphylococcal α-haemolysin channel was incorporated into artificial bilayers and used for the detection of a variety of particles from small ions (Braha et al., 2000) to large polynucleotides (Akeson et al., 1999; Howorka et al., 2001) by observing the single-channel current fluctuations. Bayley and his coworkers call this type of biosensor a "stochastic sensor" because the binding of particles to the single α-haemolysin channel, or blocking the channel current occurs stochastically (Bayley and Cremer, 2001). Recently, we have developed the method for simultaneous optical and electrical observations of single ion-channels (Ide and Yanagida, 1999; Ide et al., 2001, 2002a,b). Using this technique, we have been able to detect the interaction between channel proteins and the regulator molecules such as ligand molecules at the single molecule level. Borisenko et al. (2003) reported an

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approach for simultaneous fluorescence imaging and electrical recording of single gramicidin channels in planar bilayer membranes. These apparatuses developed for simultaneous detection of optical and electrical signals from single ion-channel ought to allow us to make electro-optical stochastic sensors.

On the other hand, the planar bilayer technique has several limitations that restrict its use although it has the potential to be a powerful tool to study channel proteins. For example, a planar bilayer formed in electrolyte solution is fragile. Additionally, it is easily broken by electrical or mechanical noises. It normally takes at least several tens of minutes to prepare the measurement. In particular, it sometimes takes hours to form artificial bilayer membranes in electrolyte solution (Alvarez, 1986 and Niles et al., 1988). Furthermore, the conventional bilayer technique requires a trained researcher to form bilayer membranes and to incorporate channels into the membranes (Hanke, 1986). A number of attempts have been made to reinforce artificial bilayer membranes. We have, for example, formed bilayers on an agarose gel that lengthens the lifetime of the membranes (Ide et al., 2002a). Schuster et al. (1999) reported a method to reinforce artificial bilayers using bacterial proteins and they recorded single α-haemolysin channel currents with the reinforced membranes (Schuster and Sleytr, 2002). Bilayer membranes formed across a very small pore seem to be durable compared to larger membranes. Hence, a number of researchers have been trying to make artificial membranes across very small apertures made by a microfabrication technique on a silicon chip (Bayley and Cremer, 2001). But unfortunately, it can be hardly said that these reinforced membranes are durable enough for biosensor use although the reinforcement lengthens the lifetime of bilayer membranes from several hours to several tens of hours. They are easily broken by large membrane voltages and by flow of electrolyte just like the conventional bilayers. In order to widen the application range of the artificial bilayer technique to biosensor use, we have developed a simple and prompt method for making artificial bilayers instead of developing a method for forming durable bilayers.

2. Materials and methods

2.1. Materials

Diphytanoylphosphatidylcholine (DPhPC) was purchased from Avanti Polar Lipids, Inc. (Alabama, USA), β-BODIPY 530/550 HPC from Molecular Probes (Oregon, USA), and asolectin, agarose type VII, alamethicin and gramicidin D from Sigma (USA). All the other chemicals were commercial products of analytical grade. The smooth muscle sarcolemmal vesicles were prepared according to the method of Slaughter et al. (1987). The sarcoplasmic reticulum vesicles that contained the ryanodine receptor channels were prepared from porcine heart according to the method of Anderson et al. (1989). The nicotinic acetylcholine receptor channels were purified from electric organ of *Torpedo californica* (Ochoa et al., 1983).

2.2. Plastic aperture

Artificial bilayer membranes were formed across a small pore pierced in a thin plastic sheet. The pore in the sheet was made as follows. A patch clamp glass pipette with an outer diameter of 1.5 mm was pulled with a pipette puller and melted with a Bunsen burner to make a fine tip which had the curvature diameter of several tens of microns. This pipette made as above was heated at its tip with a Bunsen burner and pushed onto a plastic sheet, 0.2–0.3 mm in thickness and made of polypropylene or PVC, to make a projection. Then the top of the projection was shaved with a razor blade to make a round aperture with the diameter of approximately 100 μm.

2.3. Bilayer chamber

Fig. 1 shows the assembly of the apparatus. The numbers in the Fig. 1(A) indicate: (1) a glass slide with a pore of 1–2 mm in diameter, (2) a plastic sheet with bilayers formed inside the aperture, (3) a spacer made of PDMS (polydimethylsiloxane) by a wax mold, (4) a coverslip. Fig. 1(C) shows the photograph of the spacer. The aperture was moved vertically by turning an adjust screw. The coverslip was coated with agarose to prevent adhesion of the lipid solution on the glass surface. This agrose layer was formed by painting warmed agarose solution (0.1–0.2% agarose type VII, Sigma) with a plastic rod on the glass surface and the glass was air-dried. Fig. 1(B) shows the cross sectional view of the apparatus. Ionic current across the membrane was measured with a patch clamp amplifier connected to the upper side of the membrane through an Ag–AgCl electrode.

2.4. Formation of artificial lipid-bilayers

Fig. 2 shows the procedure to make lipid-bilayer membrane from lipid solution. The bilayers were formed by "painting" a lipid solution across the aperture in a plastic sheet. In this study, negative pressure was applied to the lower side of the aperture to expand the membrane towards the glass coverslip in order to actively promote the thinning process. In contrast the conventional methods are much slower requiring a thick bulky membrane spontaneously thin into a bilayer membrane. The procedure was as follows: (a) A drop of electrolyte solution was added to the well. (b) The aperture was moved towards the cover glass to compress the silicon spacer until the aperture was <10 μm from the coverslip. (c) After removal of excess electrolyte, ∼1 μl of lipid solution (20 mg/ml DPhPC) was added to the aperture on the opposite end from the coverslip. Then electrolyte was added onto the lipid layer. (d) The aperture was moved by turning an adjust screw gradually away from the coverslip. The thick membrane was enlarged in order to contact with the agarose layer.