Single Ion Channel Sensitivity in Suspended Bilayers on Micromachined Supports

Yaling Cheng, Richard J. Bushby, Stephen D. Evans,* Peter F. Knowles, Robert E. Miles, and Simon D. Ogier

Centre for Self-Organising Molecular Systems, University of Leeds, Leeds LS2 9JT, United Kingdom

Received October 10, 2000. In Final Form: December 7, 2000

This paper describes the formation of bilayer lipid membranes on a micromachined support, consisting of a 128 μm (diameter) aperture on a gold/SU8 surface suspended over a small aqueous reservoir (∼25 nL). The bilayers are highly impermeable to ion transport with specific resistance values up to 10^7 Ω cm^2. Single-channel activities of gramicidin and alamethicin were observed in these systems. This work demonstrates that micromachined supports for bilayers are promising for the development of a biosensor with single-channel sensitivity.

Proteins that are located in the phospholipid bilayer of cell membranes are important in many biological processes including, for example, acting as receptors for the recognition of disease-causing organisms, hormones, and other signaling molecules. One class of these proteins is involved in forming transient pores, or channels, through which ions such as sodium or potassium can pass as part of the intricate signaling system which regulates cellular activity. These activities are highly specific and sensitive. For example, upon binding two acetylcholine molecules the acetylcholine receptor channel opens to permit K^+ and Na^+ transport through the cell, at a rate of ∼20 000 ions (of each type) per millisecond. To translate the high specificity and high sensitivity of these biological systems into sensor technology has been the topic of many research efforts over the last 10 years.[1−5] In general, the strategy has been to reconstitute proteins into artificial bilayer lipid membranes. To mimic biological membranes, the lipid bilayers must be fluid, robust, and, most importantly, highly impermeable to metal ions so that the functionality of the embedded proteins can be detected.

There are two main ways to produce artificial lipid membranes. The first is to attach bilayer lipid membranes onto a solid support or soft polymer cushion via self-assembly or some other sophisticated tethering technology.[6−15] Such bilayers are, in general, stable for days and are relatively easy to form; however, they do tend to be too permeable to permit the detection of single-channel activity. An alternative approach is to form a "suspended" lipid bilayer (black lipid membrane) over small apertures or filter supports.[1−3,16,17] Eray et al. demonstrated that stable bilayers can be formed on 40 μm (diameter) microfabricated polyimide apertures and that the activity of alamethicin and acetylcholine receptor could be tested over a long period of time.[2] Nikolelis et al. have used porous glass filters (0.7 μm) to support bilayers with a high resistance (10^8 Ω cm^2) and were able to detect acetylcholine, urea, and penicillin.[18]

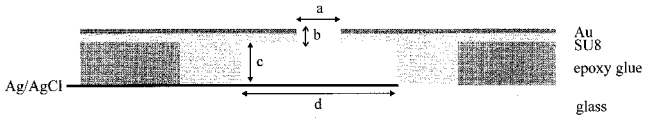

Figure 1. Schematic representation of a cross section of one element of the micromachined structure. The dimensions in μm are a = 128, b = 10, c = 100, and d = 560.

We recently introduced a new design for a micromachined support for bilayers (MSB).[19] The device consists of an ∼100 μm micromachined hole in a gold/SU8 (EPON resin SU-8, a negative photoresist developed by IBM) surface suspended above an aqueous reservoir that can hold ∼25 nL of electrolyte. A silver/silver chloride electrode at the bottom of the reservoir enables current flow through the bilayer to be measured (Figure 1). Bilayers with resistance values up to 10^6 Ω cm^2 were formed. This communication presents an advance on

* To whom correspondence should be addressed. E-mail: s.d.evans@leeds.ac.uk.

(1) Sugao, N.; Sugawara, M.; Minami, H.; Uto, M.; Umezawa, Y. *Anal. Chem*. **1993**, *65*, 363−369.
(2) Eray, M.; Dogan, N. S.; Liu, L.; Koch, A. R.; Moffett, D. F.; Silber, M.; Van Wie, B. J. *Biosens. Bioelectron*. **1994**, *9*, 343−351.
(3) Nikolelis, D. P.; Siontorou, C. G.; Krull, U. J.; Katrivanos, P. L. *Anal. Chem*. **1996**, *68*, 1735−1741.
(4) Tien, H. T.; Ottova, A. L. *Colloids Surf., A* **1999**, *149*, 217−233.
(5) Cornell, B. A.; Braach-Maksvytis, V. L. B.; King, L. G.; Osman, P. D. J.; Raguse, B.; Wieczorek, L.; Pace, R. J. *Nature* **1997**, *387*, 580−583.
(6) Sackmann, E. *Science* **1996**, *271*, 43−48.
(7) Groves, J. T.; Ulman, N.; Boxer, S. G. *Science* **1997**, *275*, 651−653.
(8) Duschl, C.; Liley, M.; Corradin, G.; Vogel, H. *Biophys. J*. **1994**, *67*, 1229−1237.
(9) Lingler, S.; Rubinstein, I.; Knoll, W.; Offenhäusser, W. *Langmuir* **1997**, *13*, 7085−7091.
(10) Cheng, Y.; Boden, N.; Bushby, R. J.; Clarkson, S.; Evans, S. D.; Knowles, P. F.; Marsh, A.; Miles, R. E. *Langmuir* **1998**, *14*, 839−844.
(11) Williams, L. M.; Evans, S. D.; Flynn, T. M.; Marsh, A.; Knowles, P. F.; Bushby, R. J.; Boden, N. *Langmuir* **1997**, *13*, 751−757.
(12) Jenkins, A. T. A.; Bushby, R. J.; Boden, N.; Evans, S. D.; Knowles, P. F.; Liu, Q.; Miles, R. E.; Ogier, S. D. *Langmuir* **1998**, *14*, 4675−4678.
(13) Jenkin, A. T. A.; Boden, N.; Bushby, R. J.; Evans, S. D.; Knowles, P. F.; Miles, R. E.; Ogier, S. D.; Schönherr, H.; Vancso, G. J. *J. Am. Chem. Soc*. **1999**, *121*, 5274−5280.
(14) Plant, A. *Langmuir* **1999**, *15*, 5128−5135.
(15) Raguse, B.; Braach-Maksvytis, V. L. B.; Cornell, B. A.; King, L. G.; Osman, P. D. J.; Pace, R. J.; Wieczorek, L. *Langmuir* **1998**, *14*, 648−659.
(16) Thompson, M.; Lennox, R. B.; McClelland, R. A. *Anal. Chem*. **1982**, *54*, 76−81.
(17) Andreou, V. G.; Nikolelis, D. P. *Anal. Chem*. **1998**, *70*, 2366−2371.
(18) Nikolelis, D. P.; Siontorou, C. G. *Anal. Chem*. **1995**, *67*, 936−944.
(19) Ogier, S. D.; Bushby, R. J.; Cheng, Y.; Evans, S. D.; Evans, S. W.; Jenkins, A. T. A.; Knowles, P. F.; Miles, R. E. *Langmuir* **2000**, *16*, 5696−5701.

10.1021/la001423h CCC: $20.00
Published on Web 01/27/2001

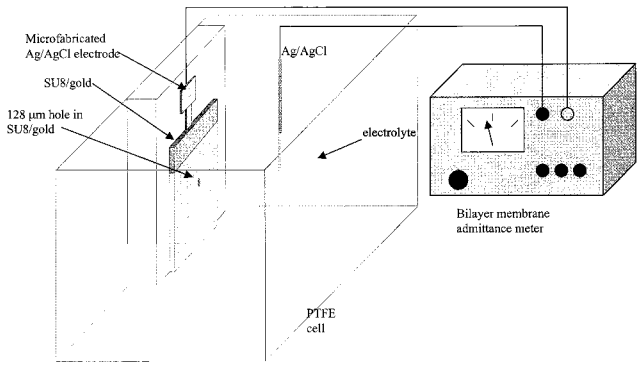

Figure 2. Arrangement for electrical measurement. A membrane admittance meter was connected via the micromachined Ag/AgCl electrode and a chloridized silver wire counter electrode. Current traces as a function of time were obtained for fixed applied voltages (between +100 and −100 mV). The device was screened in a Faraday cage to reduce background noise.

this approach in which a higher resistance and a longer lifetime of suspended bilayers were achieved. In addition, single-channel activity of the peptides gramicidin and alamethicin has been observed. This demonstrates that MSBs are a promising candidate for development into biosensors based upon ion channel conduction.

Suspended lipid bilayers were formed from egg phosphatidylethanolamine (eggPE)/decane solution at a concentration of 20 mg/mL. Gold surfaces (100 nm thick) were modified with perfluorothiol such that the septums (Au/SU8 polymer) have a hydrophobicity similar to that of the bilayer-forming solution. Membranes were painted across the aperture above one reservoir with a plastic stick.[20] Electrical measurements were made using a high input impedance (10^{13} Ω) current amplifier designed to measure conductance and capacitance of black lipid membranes and for use in patch-clamp experiments (Industrial Developments type ID 562, Bangor, Wales). The experimental setup is shown in Figure 2 in which the membrane admittance meter was connected through the micromachined Ag/AgCl electrode below the reservoir and a "chloridized" silver wire counter electrode, situated in the outer compartment.

Before the membranes were painted, the reservoir was filled with electrolyte (0.1 M NaCl). Filling of the micromachined cavity with electrolyte was confirmed by a background current of 10^{-7}−10^{-8} A at an electric potential of ∼10 mV. Once a lipid film was successfully painted over an aperture, the current fell to 10^{-12} A. Meanwhile, the capacitance of the membrane started to increase with time, from 0.1 μF cm^{-2} to reach a final specific capacitance value in the range of 0.3−0.7 μF cm^{-2}. This is consistent with typical values found for black lipid membranes.[20,21] As can be seen in Figure 3, the current response to an applied potential is linear up to 100 mV. The specific resistance values of the bilayers were typically around 10^7 Ω cm^2. In the best case, the bilayer lasted for ∼5 h, which is typical for a bilayer lipid membrane.

To demonstrate the suitability of MSBs for the development of biosensors, the ion channel peptides gramicidin D (mixture containing 80% gramicidin A, 5% gramicidin B, and 15% gramicidin C) and alamethicin were incorporated separately into the bilayers and their activities were recorded. Gramicidin was used because this peptide will only form transmembrane channels (head-to-head

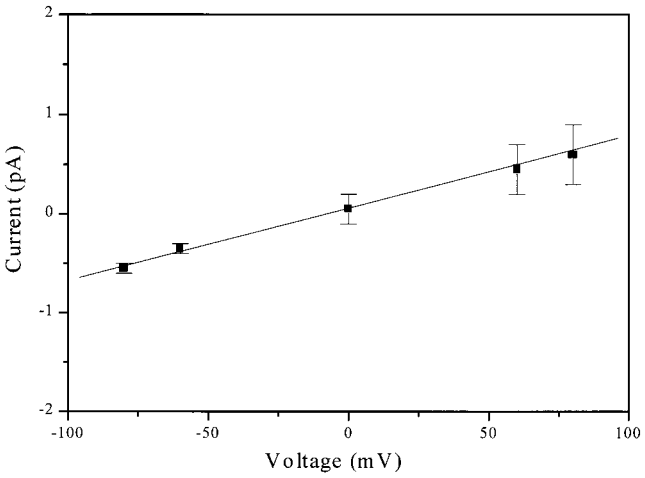

Figure 3. Typical current−voltage response for the suspended bilayers before peptide incorporation. The error bars indicate the variation between two experiments on different structures.

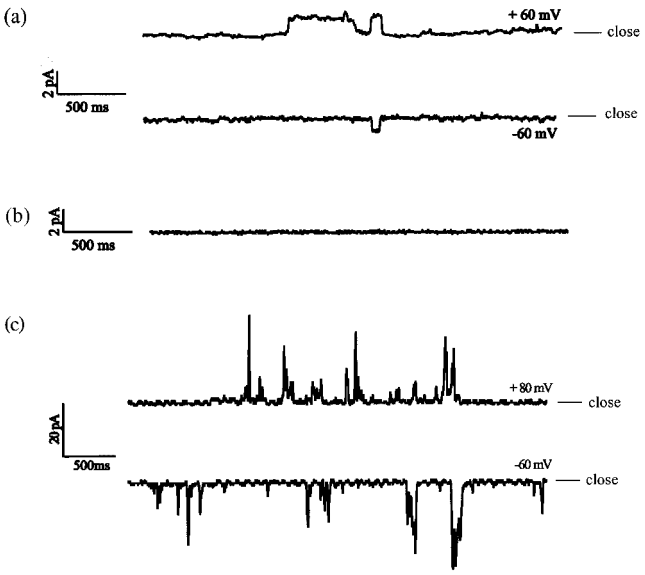

Figure 4. (a) Single-channel recordings for gramicidin D incorporated into a suspended bilayer, in a 0.1 M NaCl electrolyte. The peptide was incorporated into the suspended bilayer by vesicle fusion (1 mg/mL lipid/peptide vesicles in Millipore water with peptide-to-lipid ratio of 0.001) to the presuspended bilayer. Data are shown for positive and negative bias +60 mV (upper trace) and −60 mV (lower trace). (b) Current trace of eggPE bilayer prior to interaction with gramicidin D/eggPC vesicles. (c) Single-channel recordings for alamethicin incorporated in the suspended bilayer, in a 0.1 M NaCl electrolyte. The peptide was incorporated by adding 10 μL of alamethicin/ethanol solution (0.05 mg/mL) into the electrolyte. Data are shown for positive and negative bias +80 mV (upper trace) and −60 mV (lower trace).

β-helix dimer) in single bilayers.[22] The peptide was introduced into presuspended bilayers by the fusion of gramidicin/eggPC ($R_{P/L}$ ∼ 0.001) sonicated vesicles in Millipore water. Single-channel current traces of gramicidin are shown in Figure 4a. Opening and closing of individual channels are clearly seen. The conductance (∼30 pS) and duration (100 ms − 500 ms) of the channels are similar to that of typical gramicidin A channels.[23,24] In

(20) Tien, H. T.; Ottova, A. L. *Membrane Biophysics*, 1st ed.; Elsevier: 2000; pp 184.
(21) Benz, R.; Fröhlich, O.; Läuger, P.; Montal, M. *Biochim. Biophys. Acta* **1975**, *394*, 323−334.
(22) Goodall, M. C. *Arch. Biochem. Biophys.* **1971**, *147*, 129−135.
(23) Guihard, G.; Falk, S.; Vachon, V.; Laprad, R.; Schwartz, J.-L. *Biochemistry* **1999**, *38*, 6164−6170.
(24) Smart, O. S.; Coates, G. M. P.; Sansom, M. S. P.; Alder, G. M.; Bashford, C. L. *Faraday Discuss.* **1998**, *111*, 185−199.

contrast, no channel activity was observed before introducing the peptide into the bilayers (Figure 4b).

Under the influence of an electric field, the peptide alamethicin self-assembles into α-helical bundles to form ion pores in lipid bilayers. The number of helices in each aggregate varies from 4 to 10, which therefore gives different conductance levels, and they are not integral multiples of each other.[25,26] More importantly, alamethicin channel activity can be observed only in a bilayer.[27] Alamethicin was added to the electrolyte solution (0.1 M NaCl) from an ethanolic stock solution (0.05 mg/mL) following bilayer formation. It is believed that upon the application of a sufficient potential difference across bilayers the peptide alamethicin inserts into lipid bilayers to form channels.[26,27] When the electrical potential is between +60 and −40 mV, no notable channel activity is observed, indicating the voltage-gating nature of the alamethicin channel. Two current traces recorded at +80 and −60 mV applied potentials are shown in Figure 4c. The current fluctuations appear in bursts containing different conductance levels with more frequent appearance of lower ones, which is found by other research groups for alamethicin channels.[25,26,28] The lifetimes of these individual channels are typically in the millisecond range, and conductance values are in the range between ∼60 and ∼400 pS. These are in reasonable agreement with that reported by Bezrukov and Vodyanoy for alamethicin in the same salt solution.[29]

To conclude, this work has demonstrated that our micromachined supports are suitable for forming lipid bilayers with a high electrical resistance and allowing the observation of a single-channel conductance (as low as 30 pS). The device has exciting possibilities for the development of biosensors with high sensitivity and selectivity, based on ligand-gated ion channel receptor proteins. Furthermore, the system described can be thought of as a semisynthetic cell with both "cytoplasm" and "cytoplasmic membrane" and hence can provide a substrate for creating other types of cell-mimic systems.

Acknowledgment. This work was funded by a grant from EPSRC (Grant No. GR/M17037). Y.C. thanks Mr. L. C. Salay for help with using the membrane admittance meter.

LA001423H

(25) Hanke, W.; Boheim, G. *Biochim. Biophys. Acta* **1980**, *596*, 456−462.
(26) Sansom, M. S. P. *Q. Rev. Biophys.* **1993**, *26*, 365−421.
(27) LaTorre, R.; Alvarez, O. *Physiol. Rev.* **1981**, *61*, 77−150.
(28) Boheim, G. *J. Membr. Biol.* **1974**, *19*, 277−303.
(29) Bezrukov, S. M.; Vodyanoy, I. *Biophys. J.* **1993**, *64*, 16−25.