Ion Transport across a Bilayer Lipid Membrane in the Presence of Hydrophobic Ions

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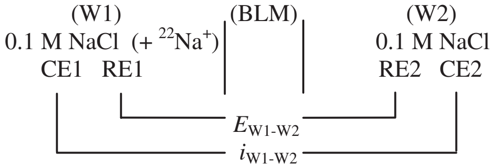
Potential-controlled electrolysis was performed between two aqueous phases containing 0.1 M NaCl across a bilayer lipid membrane (BLM) in the presence of dipicrylaminate (DPA$^-$). By use of radioisotope (^{22}Na), it has been confirmed that Na$^+$ is transported mainly and that DPA$^-$ serves as a carrier of Na$^+$ transport.

The bilayer lipid membrane (BLM) is frequently used as the simplest model of biomembranes. BLM serves as a high energy barrier for the transfer of hydrophilic ions such as K$^+$, Na$^+$, and Cl$^-$.[1,2] It has been reported that the ion transport between two aqueous phases (W1 and W2) across a BLM easily occurs in the presence of a hydrophobic ion such as dipicrylaminate and tetraphenylborate in W1 or/and W2, even when the hydrophobic ion is fairly dilute (e.g., 10^{-7} M).[3–5] It is believed that the ion-transfer current is attributed to the transfer of the hydrophobic ion between W1 and W2 across the BLM, since the transfer energy of the hydrophobic ion from the aqueous phase to the BLM is much smaller than those of other hydrophilic ions.[6–8] However, the transport of hydrophobic ion only breaks the electroneutrality within the BLM.

In contrast, the authors have reported that the magnitude of the ion-transfer current is proportional to not only the hydrophobicity of the additive ion but also that of the counter ion in aqueous phases.[9–11] It is suggested that the counter ion spontaneously distributes into the BLM with the added hydrophobic ion and that the hydrophilic counter ion is mainly transported across the BLM. However, direct evidence of the transport of hydrophilic counter ion has not been provided. Here, we first report the direct evidence of the transport of hydrophilic counter ion across the BLM in the presence of dipicrylaminate, DPA$^-$, by potential-controlled electrolysis. The authors identified that the transfer ion was mainly Na$^+$, which was distributed into the BLM as a counter ion, by detection of the radioisotope (^{22}Na).

The electrochemical cell was essentially the same as that used in our previous work.[9–11] Two aqueous compartments (W1 and W2), which were filled with 15 and 5 mL of aqueous solutions containing 0.1 M NaCl, respectively, were separated by a 0.2-mm thick tetrafluoroethylene resin sheet (a product of Du Pont-Mitsui Fluorochemical Co., Ltd.). The BLM was obtained as a black lipid membrane by brushing the *n*-decane solution of lipids on the 0.8-mm diameter aperture created on the tetrafluoroethylene resin sheet. Formation of the BLM was confirmed by a microscopic observation and a capacitance measurement. Lipids used to form the BLM were lecithin (Wako Pure Chemical Ind., Ltd., ALH5049, PC), and cholesterol (Kanto Chemical Co., Inc., No. 001G1157, Ch). The BLM-forming solution was prepared by dissolving a mixture of about 10 mg of PC and about 5 mg of Ch with *n*-decane to be 1 mL. The aqueous solution of ^{22}NaCl (10 µL, 8.94 mCi mL^{-1}) was purchased from Perkin-Elmer Life and Analytical Sciences (No. NEZ081 09061J8). The stock solution of ^{22}NaCl was prepared by dilution of the original solution with water until the total volume was 1 mL.

The electrochemical cell system employed in the present study is shown bellow:



(1)

Here, M denotes mol dm^{-3}. NaCl was used as an electrolyte in the aqueous phases. The cyclic voltammograms for the ion transfer from W1 to W2 across the BLM in the presence and absence of dipicrylaminate (DPA$^-$) were recorded by scanning the potential difference between W1 and W2, $E_{\mathrm{W1-W2}}$, and by measuring the current, $i_{\mathrm{W1-W2}}$, at 25 ± 0.5 °C. The potential scanning rate of $E_{\mathrm{W1-W2}}$ was 0.01 V s^{-1}. $E_{\mathrm{W1-W2}}$ was applied through two Ag|AgCl electrodes, RE1 and RE2, and $i_{\mathrm{W1-W2}}$ was measured by two Pt wire electrodes, CE1 and CE2. Model HA-1010mM1A (Hokuto Denko Corp.), model HB-105 (Hokuto Denko Corp.) and model F-5C (Riken Denshi Co., Ltd.) were used for the voltammetric measurements as a potentiostat, a function generator and a X–Y recorder, respectively. Then, potential-controlled electrolysis was performed in the presence of DPA$^-$ and ^{22}Na$^+$ in W1 for 95 min. After the potential-controlled electrolysis, aqueous samples of 1 mL were taken from W1 and W2 at the same time to prevent the rupture of BLM. The amounts of ^{22}Na in the samples were measured by Liquid Scintillation Analyzer (Packard, Tri-carb Model 2555TR/RB).

Figure 1 shows cyclic voltammograms for the ion transport across the BLM between W1 and W2 containing 0.1 M NaCl. In the absence of DPA$^-$ in W1 and W2, the ion-transport current was not observed in the voltammogram by scanning $E_{\mathrm{W1-W2}}$ between -0.1 and 0.1 V (curve 1). In the presence of 150 µL of DPA$^-$ solution (10^{-3} M NaDPA) in only W1, the sigmoidal shaped voltammogram with a pair of anodic and cathodic current waves symmetrical about the origin (0 V and 0 A) was recorded to be in steady state. It is inferred that the positive current is caused by the transport of Na$^+$ from W1 to W2 and/or that of DPA$^-$ from W2 to W1. Oppositely, the negative current seems to be caused by the transport of Na$^+$ from W2 to W1 and/or by that of DPA$^-$ from W1 to W2. When 0.050 V was applied as $E_{\mathrm{W1-W2}}$, about 4 nA of the current was passed through the BLM as the ion-transfer current (Faradaic component).

After the voltammetric measurement, 150 µL of the ^{22}Na$^+$ stock solution was added into only W1 around the BLM. The potential-controlled electrolysis was performed in the presence of DPA$^-$ and ^{22}Na$^+$ in W1 by applying $E_{\mathrm{W1-W2}} = 0.050$ V for 95 min. In order to prevent membrane rupture, the W1 phase was not stirred during the electrolysis. Figure 2 indicates the time course of the electric current during the potential-controlled electrolysis. The observed current was almost constant and the mean value was about 4.3 nA. The total charge during the elec-

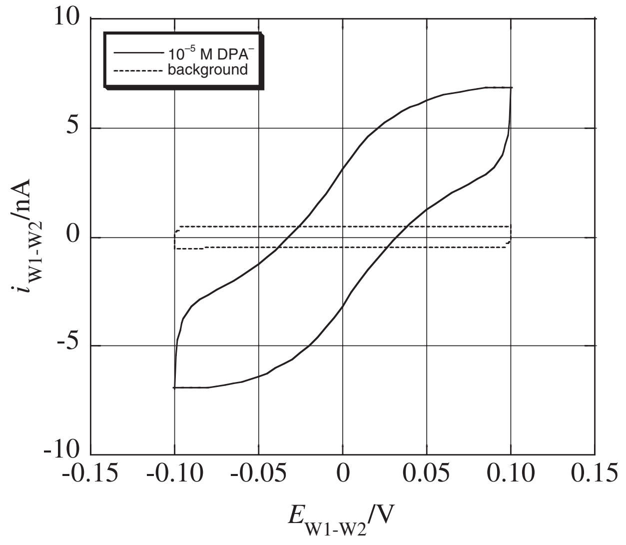


Figure 1. Cyclic voltammograms for the ion transfer across the BLM between W1 and W2 containing 0.1 M NaCl.

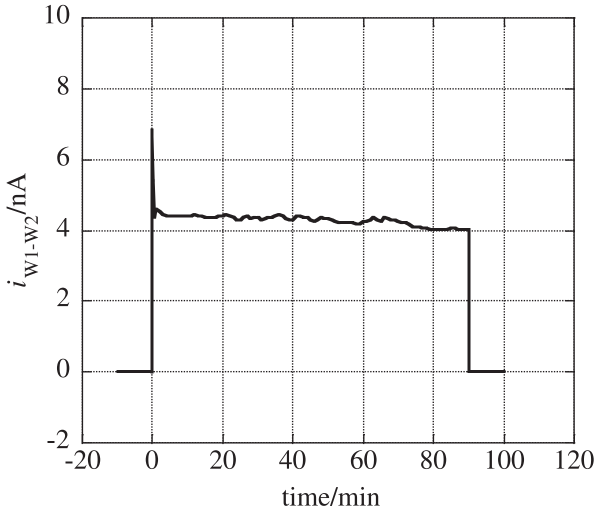


Figure 2. Time course of the ion transfer current across the BLM durining the potential-controlled electrolysis.

trolysis was 2.45×10^{-5} C, which corresponded to 2.54×10^{-10} mol. After the electrolysis, 1 mL of samples was collected slowly from W1 and W2 at the same time to prevent the rupture of BLM. Since the BLM still remained at this point, the aqueous solution in W1 did not blend with that in W2. The concentrations of ^{22}Na in the W1 and W2 phases were 89.4 µCi mL^{-1} and 7.92 nCi mL^{-1}, respectively. This result strongly supports the idea that the ion-transfer current is attributed to the transfer of the counter ion, Na^+, across the BLM between W1 and W2, and DPA^- serves as a carrier of Na^+. Note that the concentration of $^{22}Na^+$ in W2 was about 100 times higher than the estimated value (75.6 pCi mL^{-1}) assuming the homogeneous dilution of $^{22}Na^+$ in W1. This may be caused by the concentration gradient of $^{22}Na^+$ near the BLM in W2. On the other hand, the ion-transfer current was not flowed across the BLM by applying $E_{\mathrm{W1\text{-}W2}} = 0.050$ V in the absence of DPA^-. In this case, the transport of $^{22}Na^+$ was not identified either.

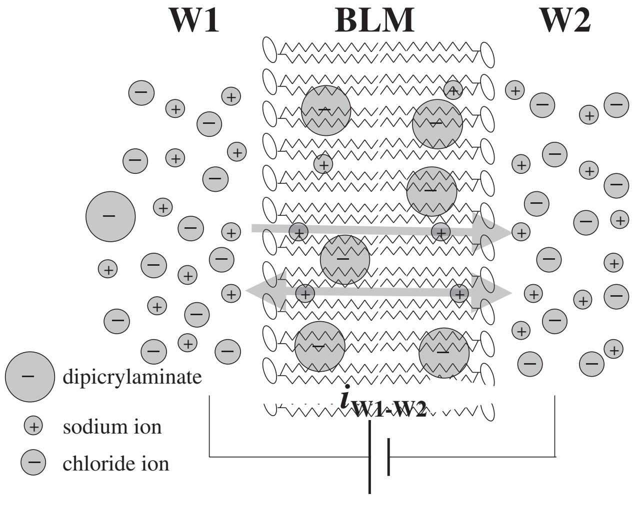


Figure 3. Ion-transport mechanism in the presence of DPA^-.

These results directly demonstrate that the hydrophilic ion (Na^+) spontaneously distributes into the BLM with the additive hydrophobic ion (DPA^-) as a counter ion and that the hydrophilic ion is mainly transported across the BLM (schematic model in Figure 3).[9–11] The electric current was derived from the transfer of Na^+ from W1 to W2 only, because DPA^- was not added into W2. If DPA^- was transported from W2 (or BLM) to W1 by the electrolysis, the depletion of DPA^- in the BLM should cause the decrease of the ion-transport current. Since the current was almost constant during the electrolysis, the present result indicates that DPA^- serves mainly as a carrier of Na^+. Although it has been reported that uncouplers such as 3,5-di(*tert*-butyl)-4-hydroxybenzylidenemalononitrile, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone, and 2,4-dinitrophenol play a similar role as a carrier of H^+,[12] it had not been previously demonstrated that hydrophobic ions may serve as carriers of the hydrophilic counter ion. The current must depend, however, on the permeabilities of both the hydrophobic ion and the counter ion, and it seems to be related to both the distribution coefficient of the ion pair and the diffusion coefficients of the hydrophobic ion and the counter ion in the BLM essentially. In the present case, the carrier transport occurred because it is likely that the diffusion coefficient of DPA^- in the BLM is much smaller than that of Na^+.

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References

1 H. T. Tien, *Bilayer Lipid Membranes*, Marcel Dekker, New York, **1974**.
2 H. T. Tien, *Prog. Surf. Sci.* **1985**, *19*, 169.
3 R. B. Genis, *Biomembranes*, Springer-Verlag, New York, **1988**.
4 E. A. Liberman, V. P. Topaly, *Biochim. Biophys. Acta* **1968**, *163*, 125.
5 O. H. LeBlanc, Jr., *Biochim. Biophys. Acta* **1969**, *193*, 350.
6 R. F. Flewelling, W. L. Hubbell, *Biophys. J.* **1986**, *49*, 541.
7 B. H. Honig, W. L. Hubbell, R. F. Flewelling, *Annu. Rev. Biophys. Biophys. Chem.* **1986**, *15*, 163.
8 C. J. Bender, *Chem. Soc. Rev.* **1988**, *17*, 317.
9 O. Shirai, Y. Yoshida, M. Matsui, K. Maeda, S. Kihara, *Bull. Chem. Soc. Jpn.* **1996**, *69*, 3151.
10 O. Shirai, Y. Yoshida, S. Kihara, *Anal. Bioanal. Chem.* **2006**, *386*, 494.
11 K. Sasakura, S. Kubota, J. Onishi, S. Ozaki, K. Kano, O. Shirai, *Electrochemistry* **2008**, *76*, 597.
12 H. Terada, *Biochim. Biophys. Acta* **1981**, *639*, 225.