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Ion transport across a bilayer lipid membrane facilitated by gramicidin A – Effect of counter anions on the cation transport

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Abstract

The facilitated ion transport from one aqueous phase (W1) to another (W2) across a bilayer lipid membrane (BLM) containing gramicidin A (GA) as an ionophore was investigated by cyclic voltammetry. Cyclic voltammograms for the ion transport across the BLM were symmetrical about the origin (0V, 0A) and the magnitude of the ion transfer current increased with an increase in the absolute value of the applied potential. The magnitude of the ion transfer current at a definite potential in the voltammograms depended on the hydrophobicity of not only cation species but also anion species, and the ion transfer current was proportional to the squared value of the GA concentration in the BLM. Taking into account the conjugated ion transfers at the W1|BLM and BLM|W2 interfaces and the distribution of ion pairs, the positive current that flowed from W1 to W2 across the BLM was attributable to both the transfer of the complex-forming cation from W1 to W2 and the transfer of the anion, which was distributed in the BLM as a counter ion, from W2 to W1. The negative current was then attributed to the opposite reaction.

Keywords: Bilayer lipid membrane; Gramicidin A; Facilitated ion transport; Hydrophobicity; Ion pair; Voltammetry

1. Introduction

The ion transport from one aqueous phase (W1) to another (W2) across a bilayer lipid membrane (BLM) has been extensively investigated with the aim of achieving a fundamental understanding of the features of ion transfers across a biomembrane [1–3]. The ion transports have been observed in the presence of ion transporters such as ion channel-forming peptides and ionophores in the BLM [1–9] or a hydrophobic ion in aqueous phase(s) [1–3,10–12]. In most of these previous studies, it was assumed that the ion transfer current was generated by transfer of the ion associated with the transporter or by transfer of the hydrophobic ion, since the energy generated by transferring associated ions or the hydrophobic ions from the aqueous phase to the BLM is much smaller than that generated by comparable transport of hydrophilic ions. However, the electroneutrality within the BLM cannot be substantiated in the general ion transfer mechanism, since the role of the counter ion is not considered.

We previously observed that the magnitude of the ion transfer current was proportional to not only the hydrophobicity of an objective ion such as tetraphenyl-boron ion, dipicrylamine ion, etc. but also that of a counter ion coexisting in aqueous phases, even if the hydrophobic ion was fairly dilute [13,14]. It was proposed that the counter ion spontaneously moves into the BLM with the hydrophobic ion and that the counter ion is usually transfers across the BLM. The transfer of the counter ion has also been observed in the case of ion transfer

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facilitated by an ionophore [4,5]. Sato et al. reported the antiport of the ion associated with the ionophore and the counter ion [15]. They considered that the ion transfer current was the sum of the cation transfer current and the anion transfer current and concluded that the anion transport and the cation transport occurred independently. Using the NMR method, Wittenkeller et al. demonstrated that the ion-pair of a cation complexed with valinomycin and a counter ion was distributed from the aqueous phase to the BLM [16,17]. In the previous report, we suggested that an ion-pair consisting of a cation and the counter ion were similarly distributed, and were continuously transferred across the BLM in the contrary direction [18].

As for the ion transport facilitated by the ion channel, this has been attributed to the transfer of the ion associated with the ion channel [1–5]. Gramicidin A (GA) is a linear polypeptide antibiotic and forms specific ion channels within the BLM phase for the transport of monovalent cations [8,19]. It has been reported that the influence of a counter anion on the transport of cations is negligible [8,9,20,21]. On the other hand, Watanabe et al. reported the anion and cation permeabilities of liposomes whose membrane phase contained GA could be changed by changing the anionic species [22]. Cohen also showed the influence of counter ions on the cation selectivity of liposomes with GA [23]. It is not clear, however, why the counter ion affects the ion permeability.

In the present work, the effect of the counter ion on facilitated transport of a potassium ion across the BLM containing GA was investigated, and the ion transport mechanism was elucidated by considering the hydrophobicity of both cations and counter ions and the formation constant of the GA-alkali metal ion complex.

2. Experimental

2.1. Chemicals

The lipids used to form the BLM were *L*-α-phosphatidylcholine, distearoyl (PC) (Wako Pure Chemical Industries, Ltd., Osaka; No. 161-12794), PC, and cholesterol (Ch) (Wako Pure Chemical Industries, Ltd., Osaka; No. 087-21). Gramicidin A was purchased from Sigma Chemical Co. (St. Louis, MO; No. 41K4005).

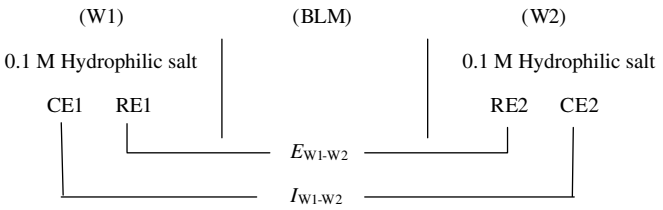
All other reagents were of reagent grade and used without further purification.

2.2. Preparation of BLMs

The electrochemical cell was essentially identical to that in our previous studies [13,14,18]. It contained two aqueous compartments that were filled with 15 ml of aqueous solution, respectively, and separated by a 0.2 mm thick tetrafluoroethylene resin sheet (a product of Du Pont-Mitsui Fluorochemical Co., Ltd, Shizuoka, Japan). The BLM used was obtained as a black lipid membrane by spreading the *n*-decane solution of lipids and GA over an aperture of 1 mm in diameter on a tetrafluoroethylene resin sheet. The formation of a BLM was confirmed by microscopic observation and capacitance measurement. The average value of the estimated bilayer area was evaluated as 0.00442 cm^2 (diameter: 0.075 ± 0.005 cm). The observed capacitance was 0.52 ± 0.05 μF cm^2, which was identical with the reported value (0.38–0.60 μF cm^2) [24]. Gramicidin A was dissolved in ethanol at a concentration of about 2×10^{-3} M, and this solution served as a stock solution. An adequate volume of the ethanol solution was added to a 1 ml flask. After the ethanol was evaporated, the BLM-forming solution was prepared by dissolving a mixture of GA, about 10 mg of PC and about 5 mg of Ch with *n*-decane in the flask. The amount of GA in the forming BLM was estimated from the molar ratio of BLM components in the forming solution.

2.3. Voltammetric measurement

The BLM system employed in the present study is indicated by Eq. (1). Here, M denotes mol dm^{-3}.



(1)

A hydrophilic salt such as KF, KCl, KBr, KI and $KClO_4$ was used as the supporting electrolyte in the aqueous phases.

The electrolytic cell used for the voltammetric measurement with a BLM system was essentially the same as that proposed by Tien [25]. It was placed in a Faraday cage during the measurement in order to decrease the background noise. The voltammogram for the ion transfer between W1 and W2 across the BLM was recorded by scanning the potential difference between W1 and W2, E_{W1-W2}, and by measuring the current between W1 and W2, I_{W1-W2}. Two silver–silver chloride electrodes, RE1 and RE2, and two platinum wire electrodes, CE1 and CE2, were used to apply E_{W1-W2} and to measure I_{W1-W2}, respectively.

All voltammograms were measured by scanning E_{W1-W2} at a rate of 0.01–0.20 V s^{-1} and at 25 ± 0.5 °C, unless otherwise described.

2.4. Apparatus

The potentiostat, the function generator and the X–Y recorder used for the voltammetric measurement were a Model HA-1010mM1A (Hokuto Denko Co., Tokyo), Model HB-105 (Hokuto Denko Co.) and Model F-5C (Riken Denshi Co., Tokyo), respectively.