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A dynamic method of measuring surface potential change due to binding of bitter substance at monolayer-coated liquid membrane surface

Kazuo Nomura *, Terumasa Yata

Chemistry Laboratory, Faculty of Science, Kyushu University Ropponmatsu, Ropponmatsu, Chuo-ku, Fukuoka 810-8560, Japan

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Abstract

A dynamic method of determining the membrane surface potential change due to a binding of a hydrophobic ion has been presented. The surface potential was determined from the time course of membrane potential under zero electric current during a transition between two steady states in a membrane filter impregnated with a phospholipid and 1-octanol. One of the alkaloids, quinine hydrochloride, was used as a hydrophobic electrolyte. Surface charge density and equilibrium constant for binding of quinine ions with ionizable groups of the phospholipids at the membrane surface were determined from the surface potential according to the Poisson–Boltzmann equation.

Keywords: Surface potential; Transient membrane potential; Monolayer; Liquid membrane; Quinine; Hydrophobicity

1. Introduction

Surface of the supported liquid membrane having phospholipids as ionic carrier species is covered with a charged monolayer of the lipid molecules. At the membrane surface, the electrostatic potential arises owing to the ionizable groups of the lipids in an analogous manner as for the surface of biological cell membrane [1], which plays an important role in the solute binding at the cell surface, interactions between cells, ion transport across the membrane and dynamic properties of cell in the field. At the interface between a solution and a charged membrane, charge separation is generated originating in the surface charges due to the ionized groups [2,3]. Potential distribution diffuses across the interface spreading toward the interior of the surface charge layer of the membrane [4,5] because the ionic species can diffuse across the surface layer. This surface phenomenon is common origin of the generation of the Donnan potential in a boundary region and the surface potential at the membrane/solution interface [6,7]. In the previous papers [8,9], the time course of the membrane potential in response to a bitter substance or a sour substance was resolved to obtain the Donnan potential difference and the intramembrane diffusion potential in the liquid membrane systems. In this study, the surface potential at the membrane surface has been determined from the rising transient curve from the initial steady potential to the peak potential. One of the bitter substances, quinine, has been used as a hydrophobic ion, which is monoprotonated (QH^+) in the aqueous electrolyte solution in the pH region studied. It has been suggested that especially in the case of the potential change due to binding of hydrophobic ions, this dynamic method is efficient to the measurement for the surface potential of charged membranes.

2. Experimental

Charged liquid membrane was prepared by soaking a membrane filter in 1-octanol (Sigma) containing water of 3.0% by weight and 5×10^{-4} mol dm^{-3} dihexadecyl hydrogen phosphate, DHP (Aldrich) under a reduced pressure. A polytetrafluoroethylene membrane filter with an average pore size of 1.0 μm and the thickness of 200 μm (Fluoropore, FP-100, Sumitomo Electric, Osaka) was used. The assembled measuring system is schematically illustrated in Fig. 1. Supported liquid membrane was horizontally mounted in the cell for the measurements. Initially, both external and internal solutions contained 1×10^{-1}

* Corresponding author. Tel.: +81-92-726-4744; fax: +81-92-726-4744.

E-mail address: k.nomrcc@mbox.nc.kyushu-u.ac.jp (K. Nomura).

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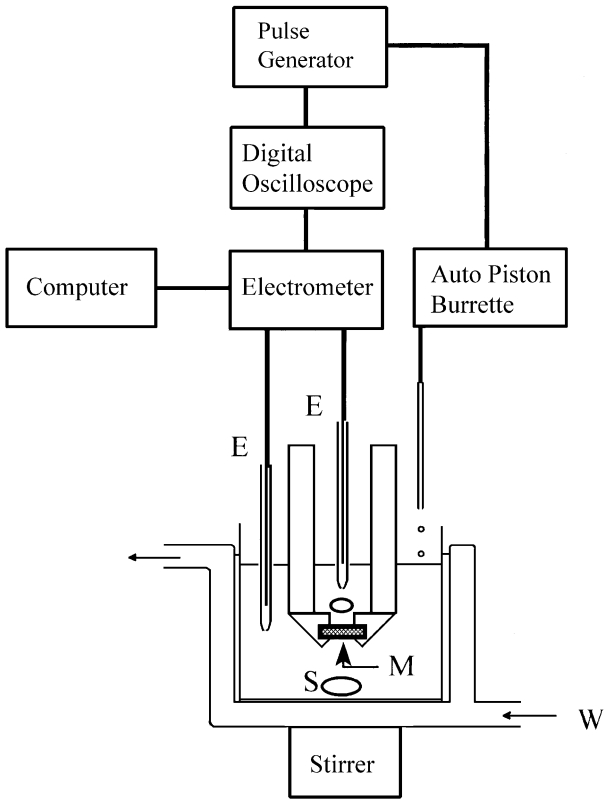

Fig. 1. Schematic diagram of assembled system for measurement of transient membrane potential in a model membrane system. M, supported liquid membrane; E, Ag–AgCl reference electrode; S, magnetic spin bar; W, thermostatted water.

mol dm^{-3} KCl. The area of the membrane surface adjacent to the electrolyte solution (diffusion area) was 0.20 cm^2. Immersing a pair of commercially available reference electrodes of Ag–AgCl type with a porous liquid-junction at the electrode tip (Model K-801, Radiometer, Copenhagen) in solutions, the membrane potential was measured by an electrometer (Model 614, Keithley, OH) and recorded on a digital oscilloscope (Model TDS430A, Tektronix, OR). A small amount of aqueous quinine hydrochloride (Fluka) was injected out of the nozzle of auto piston burette into the external solution while stirring by a magnetic stirrer at a speed of 675–700 rpm. Injection speed was controlled at 1 cm^3 s^{-1} so that the membrane potential started to change after the injection was completed. A trigger pulse of a level of voltage was produced from a pulse generator (Model SEN 3201, Nihon Kohden, Tokyo) to start to drive the auto piston burette. This pulse was also applied to the oscilloscope as a trigger source of a scan of time axis, linked with the injection of the solution. Reagents used in this study were the highest grade and were used without further purification. Water was purified by double distillation, of which once from an alkaline potassium permanganate solution. All the measurements were carried out at 25.0 °C.

3. Results and discussion

After the injection of quinine hydrochloride, the membrane potential rapidly increased to reach a peak that gradually relaxed to a level of the final steady potential (Fig. 2a). Based on the assignment that the earlier fast step and the subsequent slow step after the peak are respectively the generation processes of the potential at the interface (Donnan potential) and the intramembrane diffusion potential, the membrane potential has been divided into these constituents [8,9]. Moreover, the surface potential at the membrane surface, which is defined as the potential difference outside the membrane, can be separated from the Donnan potential, which includes the potential difference inside the membrane, because the fast process recorded on the oscilloscope was not monotonous as shown in Fig. 2b. Concentration dependence of the change in the surface potential, U_1, has been saturated at high concentration, where the binding of QH^+ ion is completed. This maximum change of U_1 can be regarded as the surface potential at the state of complete dissociation for ionizable groups at the membrane surface adjacent to the aqueous electrolyte solution ($\phi_{0,\infty}$), provided that the change in the contribution of dipoles at the surface [1,10] is negligibly small.

The potential difference across the interface between a liquid membrane that contains lipid molecules and an electrolyte solution is originated mainly from the electrostatic charges due to the monolayer adsorbed on the liquid membrane surface, although the ionizable groups are distributed throughout inside the membrane [2]. At the surface of the lipid monolayer spread at the air/water interface, similar generation of the surface potential due to the surface charges is observed. However, in the liquid membrane system, the potential distribution diffuses toward inside the membrane to generate the Donnan potential, which is widely observed for the charged membrane systems including the cell membranes because ions permeate across the membrane/solution interface. The surface charge density of the membrane, σ, was obtained from the surface potential, ϕ_0, according to the Poisson–Boltzmann equation [2,3]:

$$\sigma = \left(\frac{2\varepsilon_r\varepsilon_0\kappa kT}{e}\right) \sin h\left(\frac{e\phi_0}{2kT}\right) \quad (1)$$

$$\kappa = \sqrt{\frac{2e^2(n_E + n_{QH})}{\varepsilon_r\varepsilon_0 kT}} \quad (2)$$

where n_E, n_{QH} and ε_r denote the concentration of KCl in the external aqueous phase, the concentration of quinine hydrochloride in the external aqueous phase and relative permittivity, respectively; ε_0, k, T and e are the permittivity of vacuum, the Boltzmann constant, absolute temperature and elementary charge, respectively. The charge density

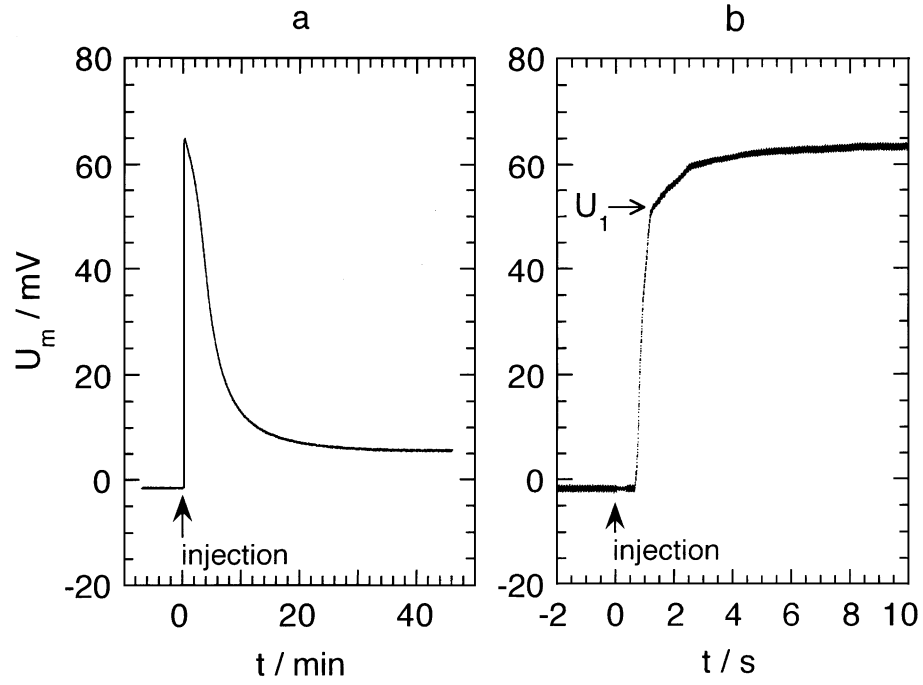

Fig. 2. (a) A typical time dependence of the membrane potential in a transition between two stationary states in response to the injection of quinine hydrochloride into the external solution. The concentration of potassium ions in the external solution was kept at 1×10^{-1} mol dm^{-3}. The concentration of quinine hydrochloride after the injection was 2×10^{-4} mol dm^{-3}. (b) Fast process in the time course of the membrane potential shown in (a).

varies depending on the degree of the dissociation of the ionizable groups at the surface. Accordingly, the dissociation constant for the reaction of quinine ion with ionizable groups of the lipid monolayer at the membrane surface, K_a, can be expressed in terms of the surface charge density as:

$$K_a = \frac{\frac{\sigma}{\sigma_\infty} n_{QH} \exp\left(-\frac{e\phi_0}{kT}\right)}{1 - \frac{\sigma}{\sigma_\infty}} \quad (3)$$

where σ_∞ denotes the surface charge density at complete dissociation, which has been obtained from $\phi_{0,\infty}$ to be -8.4×10^{-2} C m^{-2}. From this value, occupied area of ionizable group ($-PO_4^-$ group) per unit charge, $S(=-e/\sigma)$, at complete dissociation was calculated to be 1.9 nm^2. In comparison with the cross-section area of an oriented molecule for the monolayer of phospholipids at air/water interface (0.50 nm^2) [11] and for the bilayer of phosphatidyl choline (0.49 nm^2) [12], the monolayer at the liquid membrane surface is rather expanded because of the decrease in intermolecular cohesive forces between the lipid molecules as is to be expected from the values for lipid monolayers at oil/water interfaces [11]. In Fig. 3, σ and K_a obtained from the potential measurement in this work are plotted versus the concentration of quinine in the external solution.

Measuring the surface potential change by the dynamic method presented in this study is efficient especially for the

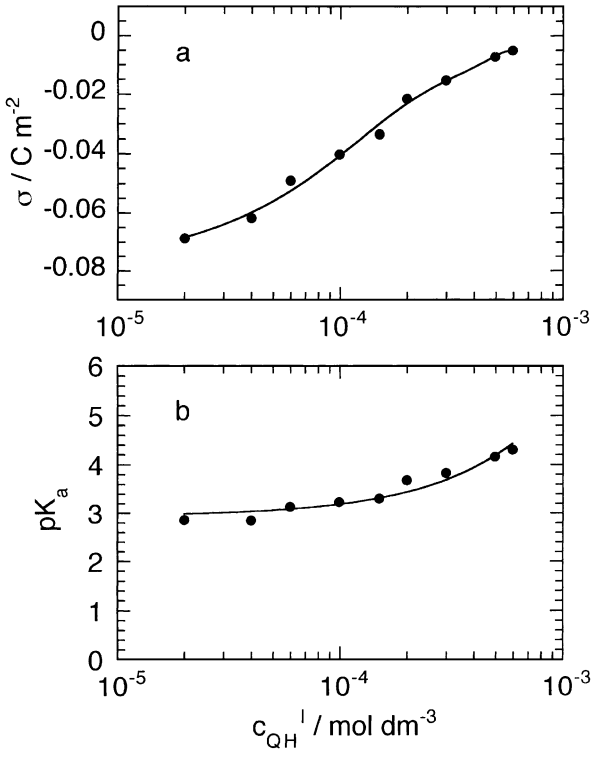

Fig. 3. (a) Surface charge density as a function of concentration of quinine hydrochloride in the external solution. (b) Surface pK_a for the dissociation reaction of ionizable group of DHP as a function of the concentration of quinine hydrochloride in the external solution.

hydrophobic electrolyte such as quinine because (1) the surface potential change is large even at low concentration of quinine hydrochloride (in the range of 10^{-3}–10^{-5} mol dm^{-3}) and (2) the potential jump due to the surface potential change is canceled out to a great extent by the change in the diffusion potential within the membrane in the slow step after the peak.

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