A NEW METHOD FOR MEASURING IONOPHORETIC ACTIVITY USING A GLASS-CELL APPARATUS EQUIPPED WITH ARTIFICIAL MEMBRANES

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A simple apparatus for measuring the ion-transport activity of a substance has been developed. The apparatus designated W-07 comprises three parts: two glass-cells of aqueous phase sandwiching one teflon cell of organic phase with two artificial membranes in between. Using the apparatus W-07, we have demonstrated the ion-transport activities (for Na^+, K^+, and Ca^{++} ions) of an oligopeptide-lactone, and resin-glycosides containing a macrocyclic lactone structure.

KEYWORDS ion-transport activity; liquid membrane apparatus; ionophore; crown ether; theonellapeptolide Id; merremoside a; merremoside h_1; oligopeptide-lactone ionophore; resin-glycoside lactone ionophore

Ionophores, which function in the ion-transport mechanism of biological membranes, have received much attention in recent years.[1] For this reason, development of a facile method for measuring ion-transport activity is important, especially for discovering new ionophores. The hitherto reported methods for measurement of ion-transport activity are classified into two categories: 1) methods using biological membranes[2] (e.g. mitochondria) and 2) methods using liquid membranes.[3,4] In 1980, Yamabe et al.[4a] successfully concentrated K^+ ions from Na^+-K^+ ion mixtures by the use of a membrane-separated organic phase containing dibenzo-18-crown-6 as an ion carrier. In our early surveys of naturally occurring new ionophores, we attempted to use Yamabe's apparatus[4a,5] but without satisfactory results. We have recently developed a new apparatus (designated W-07) for measuring ion-transport activity (Fig. 1). This paper reports three ionophoretic natural products of a new type found by using the apparatus W-07.[6]

Apparatus W-07 and Procedure for Ion-Transport Activity Test

The apparatus (Fig. 1) comprises two Pyrex glass-cells (350 ml of volume) side by side. One (left) contains ionic water (200 ml) of Na^+, K^+, or Ca^{++} ions (1 mol/l) and the other (right) contains deionized pure water (200 ml). In the center is a narrow teflon chamber for the organic phase (i.e. a liquid membrane of 4 mm thickness and 2.0 cm^3 volume). Two glass-cells sandwich the central teflon chamber with two sheets of artificial membrane (cellulose dialyzers) in between. A sample to be tested is dissolved in chloroform saturated with water (0.01-0.03 mol/l) and injected into the teflon chamber. The whole apparatus is kept at 25°C and the aqueous phases on both sides are gently stirred (200 times/min).

Every hour, the pure water phase is sampled (right) with a concomitant re-supply of deionized pure water of the same amount each time. The amount of ions transported from the ionic water phase (left) to the pure water phase (right) is analyzed quantitatively by atomic absorption spectrometry (Shimadzu AA-670 atomic absorption spectrophotometer). The molar ion concentration (Cn^*) in each sample taken from the pure water phase (right) is obtained by the following equation and the molar ion transport (m) of each cation per hour is calculated.

$$Cn^* = \frac{CnV + \sum_{k=1}^{n-1} (C_k - Co)\nu}{V}$$

where Cn = observed ion concentration (mol/l) in pure water phase (right) sampled at n time(s)

Co = initial ion concentration in pure water phase (mol/l)

V = initial amount of pure water (ml)

ν = the amount of sample (ml)

When the sample in the central organic pahse shows ion-transport activity, a metal cation in the left glass-cell moves to the right glass-cell, presumably by diffusion of the cation via complexation of the sample and the ion at the first cellulose dialyzer membrane between the ionic water (left) and the organic (center) phases and then decomplexation at the second cellulose dialyzer membrane between the organic (center) and the pure water (right) phases.

Materials

For positive standard samples, benzo-15-crown-5 (Aldrich), dibenzo-18-crown-6 (Aldrich), and Kryptofix 221D (Merck)[7] of reagent grade were used. Before use, cellulose dialyzer membranes of Visking 3787-F25 (0.09 mm thickness) were soaked for 12 h in deionized water which was prepared with a Milli-Q Labo apparatus (Millipore Co.). The ionic water of Na^+ or K^+ was prepared by dissolving NaCl or KCl in deionized pure water to give a final concentration of the salt at 1 mol/l, and subsequent saturation with chloroform. To prepare Ca^{++} ion water, $CaCO_3$ was dissolved in deionized water at 1 mol/l and neutralized with conc. aq. HCl, and the whole aqueous solution was saturated with chloroform.

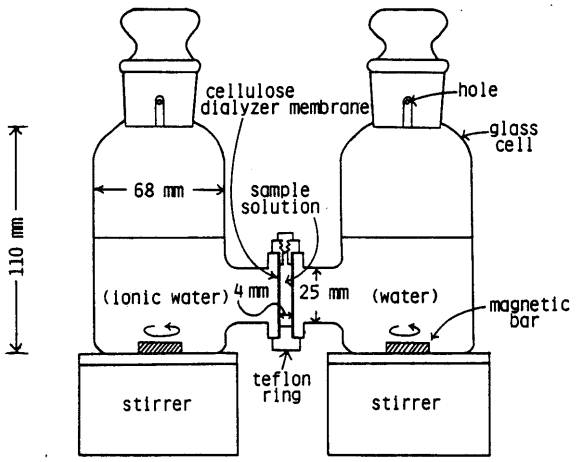

Fig. 1. Apparatus W-07 for Measurement of Ion-Transport Activity

Results and Discussion

The ion-transport activities of three synthetic ionophores (benzo-15-crown-5, dibebzo-18-crown-6, and Kryptofix 221D) at 0.03 mol/l (chloroform) for Na^+, K^+, and Ca^{++} ions were measured and linear correlations between the amount of ions transported (μmol) and time (h) were obtained as shown in Fig 2 (a,b,c). Benzo-15-crown-5, known as a Na^+-ionophore, transported Na^+ ions (m_{Na}= 9.91 x 10^{-8} mol/h) more efficiently than K^+ ions (m_K= 1.15 x 10^{-8} mol/h), but not Ca^{++} ions. Dibenzo-18-crown-6, a K^+-ionophore, transported both Na^+ and K^+ ions more efficiently than benzo-15-crown-5: m_{Na}= 2.26 x 10^{-7} mol/h and m_K= 7.33 x 10^{-7} mol/h, but not Ca^{++} ions. Kryptofix 221D, a cryptand compound, transported Na^+, K^+, and Ca^{++} ions highly efficiently: m_{Na}= 5.72 x 10^{-6} mol/h, m_K= 3.71 x 10^{-5} mol/h, and m_{Ca}= 4.08 x 10^{-6} mol/h.

The ion-transport activity of dibenzo-18-crown-6 was measured at different concentrations. It decreased m_K from 7.33 x 10^{-7} to 3.13 x 10^{-7} and 1.92 x 10^{-7} mol/h sequentially while changing the concentration of the ionophore from 0.03 to 0.03 x 0.5, then to 0.03 x 0.25 mol/l. In the absence of the ionophore, there was no ion-transport. HPLC analysis (UV detection for benzo-15-crown-5 and dibenzo-18-crown-6 and RI detection for Kryptofix 221D) also showed that the ionophore did not leak detectably out of the central organic phase through the cellulose dialyzer membranes into the aqueous phases.

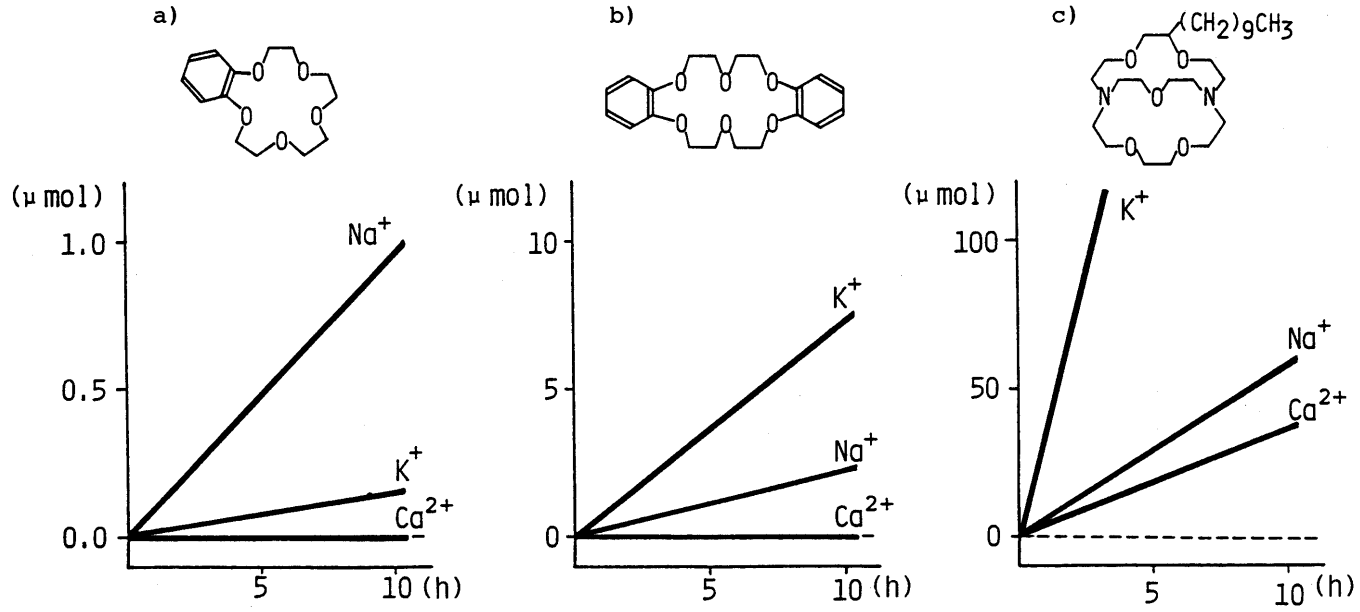

Fig. 2. Ion-Transport Activities of Synthetic Ionophores
a) Benzo-15-crown-5, b) Dibenzo-18-crown-6, and c) Kryptofix 221D (each 0.03 M in $CHCl_3$).

We have used the apparatus W-07 to test naturally occurring cyclic compounds isolated in our laboratory for their ionophoretic activities. We found that theonellapeptolide Id, an oligopeptide lactone isolated from the Okinawan marine sponge Theonella swinhoei,[8] and merremosides a and h_1, resin-glycosides having a macrocyclic lactone moiety isolated from the tuber of an Indonesian medicinal plant Merremia mammosa (Convolvulaceae),[6,9] showed ion-transport activities for Na^+, K^+, and Ca^{++} ions as shown in Fig. 3 (d,e,f). These ion-transport activities were completely lost when the macrocyclic lactone linkages in these compounds were cleaved by NaOMe-MeOH.

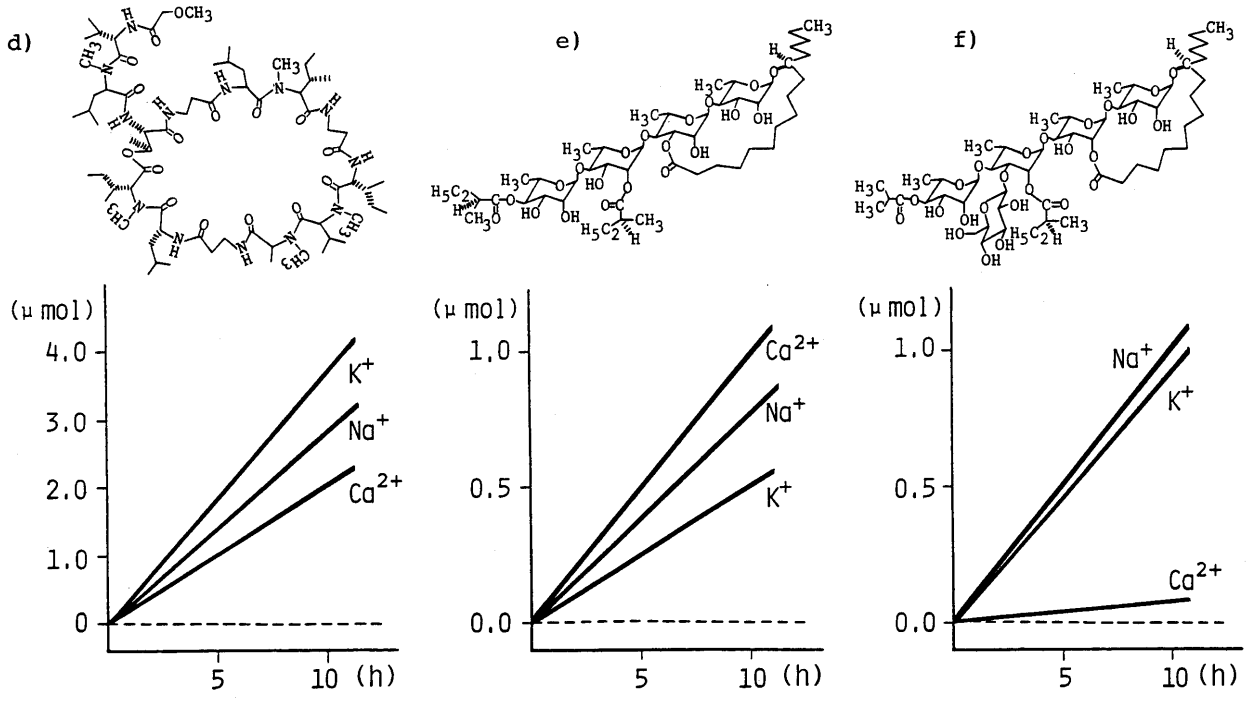

Fig. 3. Ion-Transport Activities of Naturally Occurring Macrocyclic Lactones
d) Theonellapeptolide Id (0.03 M in $CHCl_3$), e) Merremoside a (0.02 M in $CHCl_3$), f) Merremoside h_1 (0.02M in $CHCl_3$).

We are currently surveying either synthetic or naturally occurring ionophoretic substances using the W-07 apparatus.

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(Received February 27, 1989)