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Physico-Chemical Studies of Taste Reception

IV. Response of Individual Phospholipid Membrane to a Variety of Chemical Stimuli

Michihisa Miyake, Naoki Kamo, Kenzo Kurihara, and Yonosuke Kobatake

Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan

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Summary. Variations in the membrane potential across model membranes made of Millipore filter paper and various single phospholipids were measured in response to salt, acid and distilled water. The phospholipids used were phosphatidylcholine (PC), sphingomyelin (SM), phosphatidylethanolamine (PE), and phosphatidylserine (PS). Results were compared with those obtained with the model membrane made of the total lipids extracted from bovine tongue epithelium, which simulated well the receptor potential observed with intact taste organs. The membrane potential of PE- and PS-membranes increased monotonously with increase of the concentration of 1:1 type salts, while that of PC- and SM-membranes exhibited no appreciable change in 1:1 salt solutions. Application of $CaCl_2$ to the membranes brought about a variety of responses depending on the species of lipids used. PE- and PS-membranes showed a larger change in the membrane potential than PC- and SM-membranes when pH of the solution was varied. Fe^{3+} was strongly adsorbed on the surface of PC- and SM-membranes, while Fe^{3+} bound to PE- and PS-membranes was easily removed by an application of salt solution. A transient increase in the membrane potential was observed when distilled water was applied to the membrane adapted to an appropriate salt solution, which was similar to the water response observed in taste cells. PC- and SM-membranes responded to water when the membrane adapted to either NaCl or $CaCl_2$, but PS-membrane responded only when the membrane was adapted to a solution containing $CaCl_2$. PE-membrane did not respond to water in any cases examined. The membrane prepared with a mixture of two species of phospholipids responded neither to salt nor to water, while the membranes prepared with the total lipids or a mixture of three species of lipids in appropriate ratio responded to both. The water response of the total lipids membrane vanished in a high temperature medium, while the water response of PC-membrane retained in all temperature ranges examined, i.e. between 20° and 62 °C. The results obtained suggest that a mosaic structure, where each domain has different functions against various chemical stimuli, is formed on the surface of the model membrane made of the total lipids.

This series of papers is concerned with a model analysis for the physico-chemical mechanism of taste reception. In Part I [4], it was shown that

a model membrane composed of Millipore filter paper and the total lipids extracted from bovine tongue epithelium produced a change in membrane potential in response to salts, acids and distilled water, which was quite similar to the receptor potential observed with a living taste cell. Based on the experimental data obtained with the model membrane together with relevant data observed with intact taste organs, we proposed a theory of taste reception in Part II [5] stating that a change in the electric potential at the membrane-solution interface induced by taste stimuli is responsible for generation of the receptor potential.

The total lipids extracted from bovine tongue epithelium contain various kinds of phospholipids. Since individual phospholipids have different chemical and physical properties, it is not unreasonable to consider that each lipid responds to taste stimuli in a different manner. Hence, disclosure of the difference in the function of individual phospholipids against various stimuli is indispensable for detailed analysis of data obtained from the model membrane, and for a basic understanding of molecular interaction between taste stimuli and the receptor membrane. In the present paper, model membranes made of Millipore filter paper impregnated with single species of phospholipid and also with mixtures of lipids are prepared, and the responses to a variety of chemical stimuli are investigated. The phospholipids used are phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphigomyelin (SM), and phosphatidylserine (PS). These four lipids are the main components of the total lipids (T) from bovine tongue epithelium, i.e. weight fractions of PC, PE, SM, and PS in the total lipids are 36.1, 28.2, 18.0, and 8.8 percent, respectively [6].

Materials and Methods

PC and PE used in the present study were extracted from egg yolk and purified according to the method employed by Lea, Rhodes and Stell [7]; the extracts from fresh egg yolk with chloroform/methanol were passed through a column of cellulose, and PC and PE in the effluent were separated with a silicic column. PS was purified from cephalin extracted from beef brain by repetitive solvent partitioning with chloroform and ethanol. SM was purchased from K & K Laboratories, Inc. (Hollywood, Calif.) and used without further purification. The purity of these samples was established by thin-layer chromatography in two solvent systems, chloroform/methanol/water (65:25:4, by vol), and chloroform/methanol/acetic acid/water (5:15:4:2, by vol). The total lipids were extracted from bovine tongue epithelium as described in the previous paper [4].

The model membrane was prepared by the same procedure as that employed in our previous studies [4, 5]. SM was dissolved in chloroform/methanol (1:1, by vol) and other lipids in chloroform. A Millipore filter paper (type VSWP, pore size 25 nm) was soaked in PC or PE solution at room temperature and in PS or SM solution at 40 °C to prevent

precipitation of dissolved lipids. A model membrane made of a mixture of phospholipids was obtained by the same procedure by soaking the filter paper in a solution containing two or three lipids in an appropriate weight ratio. After about a 10-min soaking, the filter paper was air-dried. The amount of lipid adsorbed, which is designated by Q (mg/cm^2), was determined from the difference in weight of the filter paper before and after the adsorption of lipids. The Q values of the membranes used were between 10 and 20 mg/cm^2. The membrane thus prepared was immersed in 300 mM NaCl solution and conditioned overnight in a cold room before use. During the conditioning period, the hydrophilic groups of phospholipids in the membrane seem to face the aqueous bulk solution, and metal ions contaminating the lipids may be removed during the conditioning.

The membrane potential was measured by the same apparatus and procedures as described in Part I [4] of this series. The emf which arose between the stimulating solution and the reference solution (300 mM NaCl) separated by the membrane was measured with a high input-impedance electrometer through a pair of saturated KCl salt bridges and calomel electrodes. The variation of the composition and/or concentration of ions in the reference solution led to no appreciable difference in the conclusion described below.

The chemicals used in the present study were analytical grade without further purification. All measurements of the membrane potential were performed at 20 °C unless otherwise noted.

Results and Discussion

Response to Salt Stimuli

The steady potential of the model membrane made of PC, SM, PE or PS was measured when the membrane was subjected to various kinds of salt solutions. Fig. 1 shows the membrane potential observed with various membranes as a function of NaCl concentration. As seen in the Figure, the membrane potential obtained with PC- or SM-membrane did not change appreciably with increase of NaCl concentration, while a large potential change was observed with PE- or PS-membrane, which resembles the concentration dependence of the potential obtained with T-membrane (*see* solid line in Fig. 1). Application of KCl, LiCl, and NH_4Cl to various membranes brought about potential changes similar to those shown in Fig. 1.

As pointed out in the previous papers [4, 5], it is difficult to explain the above results obtained with the present model membranes under the assumption that the membrane potential stems from the permeation of ions across the membrane. The results can be interpreted in terms of the phase boundary potential at the interface between the membrane and the stimulating solution. The difference in the potential responses against salt stimuli between PC- or SM-membrane and PE- or PS-membrane is attributed to the difference in their surface charges exposed on

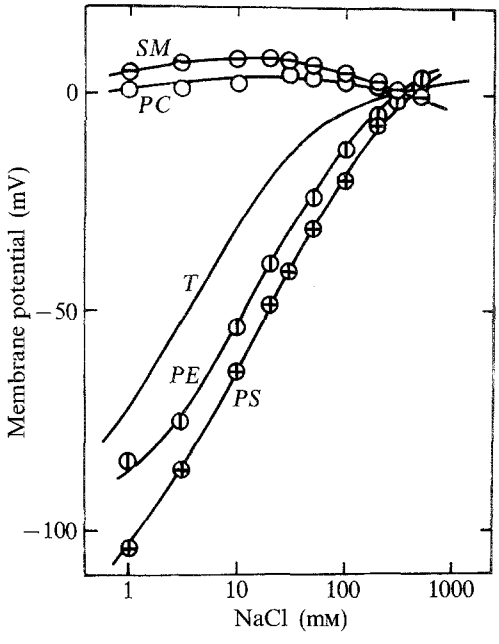

Fig. 1. Plots of membrane potential against NaCl concentration for a variety of single phospholipid membranes. ⊖ SM; ○ PC; ⊕ PE; ⊕ PS. The solid line represents the membrane potential of T-membrane

the membrane surface; PC- and SM-membranes carry almost zero net charge, while PE- and PS-membranes have negative surface charge. This is consistent with the results obtained with lipid monolayers or liposomes [2, 8, 11, 12].

Fig. 2 illustrates the dependence of the membrane potential on $CaCl_2$ concentration for various membranes. The curves for PC- and SM-membranes exhibit a plateau in a dilute solution lower than 10 mM and decrease monotonously with increase of $CaCl_2$ concentration. PS-membrane shows a monotonic increase in the membrane potential with increase of $CaCl_2$ concentration. The membrane potential of PE-membrane increases until $CaCl_2$ concentration becomes about 20–30 mM and then decreases with further increase of $CaCl_2$ concentration through a maximum. The curve obtained with T-membrane resembles that for PE-membrane as illustrated by a solid line in Fig. 2.

The decrease of the membrane potential with increase of $CaCl_2$ concentration as seen in PC- or SM-membrane is interpreted as follows. Ca^{2+} binds strongly to the negative groups of the membrane, and makes the sign of the surface charge of the membrane positive [5]. The phase

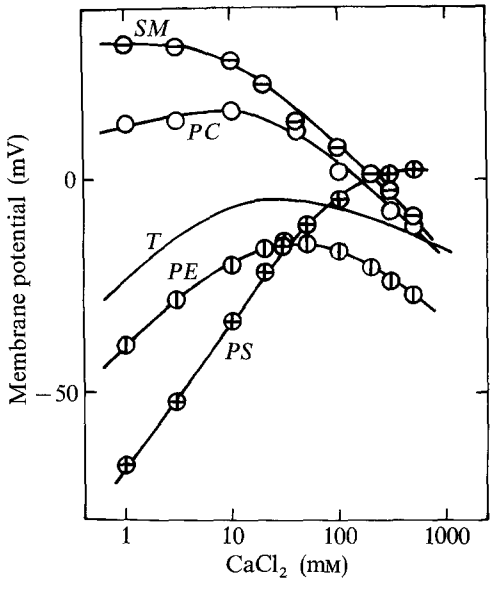

Fig. 2. Plots of membrane potential against $CaCl_2$ concentration for a variety of single phospholipid membranes. Notations are the same as in Fig. 1

boundary potential of a positively charged membrane decreases with increase of salt concentration, which contrasts to a negatively charged membrane like PS-membrane. In the case of PE- and T-membranes, the sign of surface charge changes from negative to positive at about 20 mM $CaCl_2$. This is consistent with the result obtained from the electrophoretic study of liposomes made of total lipids [5]. According to Papahadjopoulos [12], Ca^{2+} removes the proton from the primary amino group of the PS-molecule and the negatively charged nitrogen participates in the formation of a complex with Ca^{2+} by co-ordinating bond. This complex formation does not make the net charge at the membrane surface positive.

Response to Acid

Fig. 3 shows the dependence of the membrane potential for various phospholipid membranes on pH in the media which was changed by HCl. PS- and PE-membranes exhibit a large potential change with the variation of pH. It is noted that the curve in the Figure for PS-membrane increases with lowering of pH even below 0.5. PC- and SM-membranes exhibit much smaller potential changes compared with PS- and PE-mem-

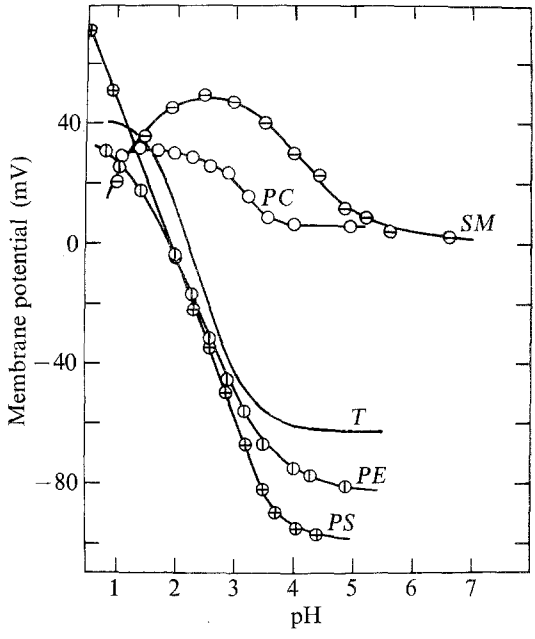

Fig. 3. Membrane potential-pH relation obtained by adjusting the pH of stimulating solution with HCl. Notations are the same as in Fig. 1

branes. The curve for PC-membrane reaches a plateau in a low pH region, while that for SM-membrane decreases with lowering of pH below 2.5. The pH dependence of the membrane potential obtained with T-membrane resembles that of PS- or PE-membrane.

Effect of $FeCl_3$

Tateda and Beidler [13] found that a treatment of the rat tongue with $FeCl_3$ or cocain made the steady potential of the taste cell positive, and subsequent application of NaCl solution produced negatively going potential deflection in the taste cell, which is the opposite direction to that observed with an untreated cell. As shown in the previous paper, the similar effect of $FeCl_3$ was observed with T-membrane [4]. In the present study, the model membranes made of various single phospholipids were treated with 60 mM $FeCl_3$ for 12 min. After the membranes were washed with water, NaCl solutions of various concentrations were applied successively. The results are shown in Fig. 4. PC- and SM-membranes exhibit a potential change similar to that of T-membrane, i.e. the potential

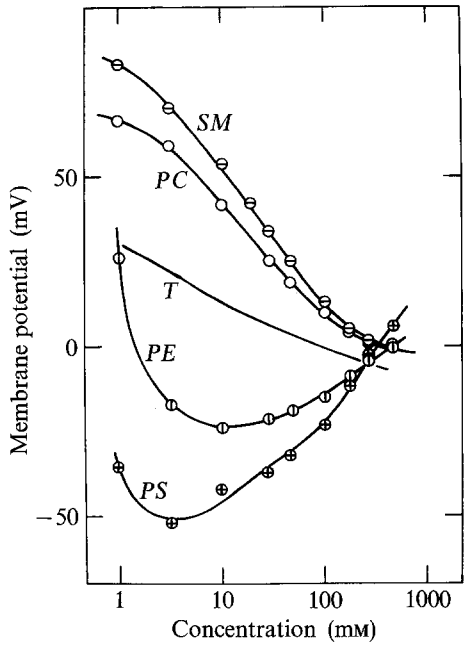

Fig. 4. Effect of $FeCl_3$ to various phospholipid membranes. After the membrane was treated with 60 mM $FeCl_3$ for 12 min and rinsed with water, NaCl solutions of various concentrations were applied to the membrane. The ordinate represents the membrane potential observed and the abscissa, the concentration of NaCl in logarithmic scale. Notations are the same as in Fig. 1

decreased monotonously with increase of NaCl concentration. The binding of Fe^{3+} to PC- and SM-membranes is so strong that Fe^{3+} bound on the membrane surface is not removed easily by washing with water or subsequent application of NaCl solution. Hence, application of NaCl solution to the Fe^{3+}-bound membranes, which are positively charged, brings about a decrease in the membrane potential with increase of NaCl concentration.

On the other hand, PS- and PE-membranes show a positive value of 60–90 mV under the presence of 60 mM $FeCl_3$ but the potential decreases sharply by washing the membrane with water. The membrane potential increases with increase of NaCl concentration through a minimum at about 10 mM NaCl. The above results indicate that Fe^{3+} is bound to PE- and PS-membranes with a rather weak interaction and can easily be removed from the membrane surface by washing and subsequent application of NaCl solution. Considering these results, it is concluded that the characteristic response of the $FeCl_3$-treated T-membrane is attributed to PC- and SM-components in the total lipids.

Response to Distilled Water

Application of distilled water to the membrane adapted to certain salt solutions induced the positive-going potential change, which corresponds to the water response observed with the gustatory organ [3]. In Part III of this series [9] dealing with the mechanism of the water response, we pointed out that the water response is attributable to the diffusion potential caused by movement of ions from the membrane surface to the bulk solution by application of water.

Fig. 5 represents the water response of the membrane made of various single phospholipids. The first column in the Figure illustrates the water response after adaptation of the membranes to Ringer's solution. As seen in the Figure, PC-, SM- and PS-membranes respond to distilled water, but PE-membrane does not. To examine the effect of the individual salt species in Ringer's solution on the generation of the water response, the response to water was observed when the membrane was adapted to 100 mM NaCl, 3.5 mM KCl and 2.5 mM $CaCl_2$, respectively. These salt concentrations are approximately equal to those of respective salts in Ringer's solution. When the membranes were adapted to 100 mM NaCl solution, PC- and SM-membranes responded to distilled water and PE-

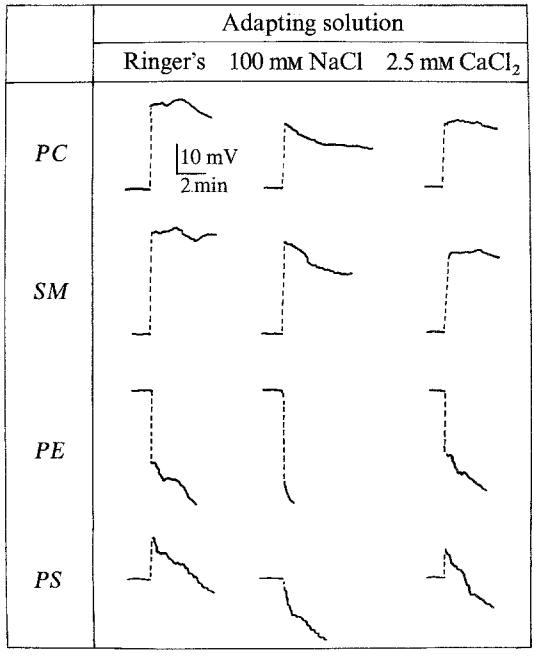

Fig. 5. The water response observed with various phospholipid membranes adapted to Ringer's solution, 100 mM NaCl and 2.5 mM $CaCl_2$

and PS-membranes did not. The water response was never observed for all membranes studied when the membranes were adapted to 3.5 mM KCl or to 100 mM KCl.

As shown in the previous paper [5], the surface charge density of the liposome made of the total lipids determined from the electrophoretic measurements did not increase with increase of concentration of 1:1 type salt. This implied that there is no specific binding of univalent cation to the membrane surface, and that the membrane potential is attributed to the long range interaction between the anion groups on the membrane surface and the ions in the bulk solution. It must be noted that the result described above does not always mean that the added 1:1 type salts are not adsorbed on or dissolved in the membrane surface, because the surface charge density is not varied by adsorption or dissolution of an equal amount of anion and cation of the salt in the membrane surface. Adsorbed salts on the membrane surface diffuse into the bulk solution when distilled water is applied, and hence a transient diffusion potential is observed as a cause of the water response [9]. The different behaviors in the water response between PE-(or PS-) membrane and PC-(or SM-) membrane observed after adaptation to NaCl solution can be attributed to the difference in the distribution of functional groups at the membrane surface. As mentioned above, the net surface charge of PE- or PS-membrane is negative. The adsorption of the added salts on the membrane surface is prevented by the electric repulsion between the anion of the salts and the negative charge in the membrane. On the other hand, the added salts are easily adsorbed on the surface of PC- or SM-membranes because there is no net charge on the surface of these membranes. Adaptation to KCl brought about no water responses for all membranes examined, since no potential is produced by the diffusion of KCl from the membrane surface into the bulk solution. When the membrane was adapted to 2.5 mM $CaCl_2$, PC-, SM-, and PS-membranes exhibited the water response, while PE-membrane did not. The different behaviors between PS- and PE-membranes in response to water (both PS- and PE-membranes have negative charge on the surface) may be attributed to the difference in affinities of Ca^{2+} against these two lipid molecules.

Effects of Mixing of Lipids

The membranes were prepared from the mixtures of phospholipids in a variety of mixing ratios, and the effect of mixing on the function

of the membrane to various stimuli was examined. As an example, Fig. 6 illustrates the effect of mixture of lipids on the relationship between the membrane potential and NaCl concentration. The membrane made of the mixture of three species of lipids (PC/PE/PS=6:4:3 in weight) responded well to NaCl as observed with the T-membrane. If one component is eliminated from the above lipid mixture, that is the membrane was prepared from the mixture of two lipid species, e.g. PC/PE=6:4, PC/PS=6:3, or PE/PS=4:3, the membrane produced only a small response to NaCl as shown in the Figure. It is important to note that PS/PE-membrane did not respond to NaCl, while the membrane made of PS or PE separately, responded well to NaCl (*cf.* Fig. 1). The above results implied that the lipid membrane made of two species of lipids manifested no or small net charge exposed on the membrane surface, and the negative charge on the membrane surface appeared when three components of lipids were mixed.

It is interesting to point out that the water response was not produced with a model membrane composed of two species of lipids as in the case of salt responses; the mixture of PC and PE with a ratio of 6:4

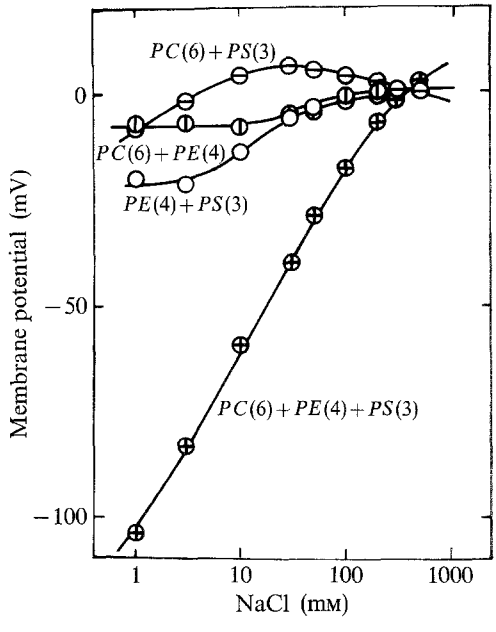

Fig. 6. Effect of mixing of phospholipids on the response to NaCl solution. Data marked by ⊕ represent the membrane potential observed with the membrane made of the lipid mixture, where PC, PE and PS are mixed in the ratio 6:4:3. Others are the membrane potential observed with the membrane made of two species of the lipids, where one species of phospholipids is eliminated from the above lipid mixture

or that of PE and PS with a ratio of 4:3 exhibited no water response even when the membrane was adapted to Ringer's solution. However, the model membrane composed of three lipids, i.e. PC/PE/PS, with a ratio of 6:4:3, showed an appreciable water response.

As shown above, the membrane made of three species of phospholipids or the total lipids responded both to salts and to water, while the membranes made of two species of lipids responded neither to salts nor to water. The detailed molecular picture underlying the above results is not clear to us at present, but one of the possible explanations to be considered is as follows: the lipids in the membranes made of three species of lipids or the total lipids are not in a homogeneously mixed state but each or similar species of lipids form clusters or domains in the membranes. It is noted that recent studies [1, 10, 14] suggest that the lipids in the artificial or biological membranes may not be in a homogeneously mixed state. The results described below support this view.

Effect of Temperature on Water Response

The water response of T-membrane adapted to Ringer's solution was examined when the membrane was subjected to the solutions at an elevated temperature. The results obtained are shown in Fig. 7. The membrane adapted to Ringer's solution of 20 °C was subjected to the solution of

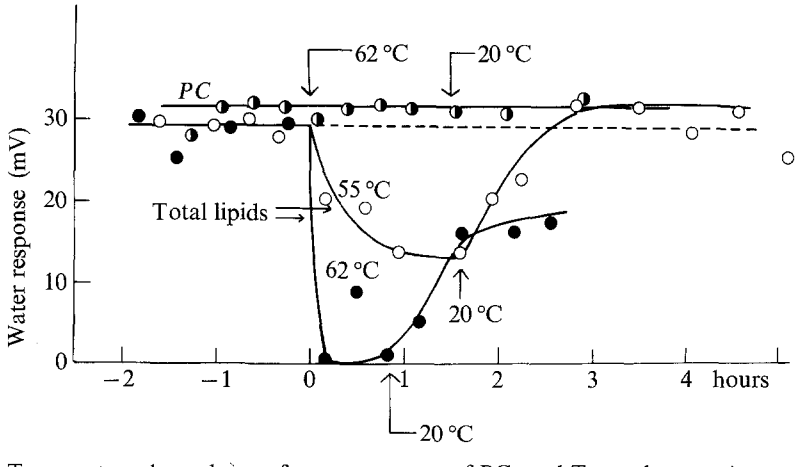

Fig. 7. Temperature dependence of water response of PC- and T-membranes. At zero time, the temperature of the system is suddenly increased from 20 °C to 55° or 62 °C as indicated. ◑ PC-membrane, 62 °C; ○ T-membrane, 55 °C; ● T-membrane, 62 °C. The arrows accompanied with 20 °C indicate the instance when the membrane was subjected to the solution of 20 °C. The membrane was allowed to stand at this temperature

55 °C at time zero in the Figure. As seen in the Figure, the magnitude of water response decreased immediately after application of the solution of high temperature. When the membrane was subjected to the solution of 62 °C, the function of the membrane to respond to distilled water disappeared completely. At the second arrow in the Figure, the membrane was subjected to the solution of 20 °C again and was allowed to stand at this temperature. The functions of the water response recovered gradually to the original level. The water response of PC-membrane was not affected at all by an elevation of temperature as shown in the Figure. These results can be explained as follows: during the conditioning process of the T-membrane in the salt solution, the clusters or domains, which are responsible for the generation of the water response, are formed on the surface of the membrane. With an elevation of temperature, the lipids in the membrane are mixed homogeneously and the domains disappear.

All the results obtained in the present study suggest that the model membrane made of the total lipids contains various domains having different functions. The domains may be composed of different lipid species and/or of single or similar species of lipids in the membrane. The protein molecules can also participate in the formation of domains in the actual biological membranes. Further study is necessary, however, to verify the existence of the mosaic structure in the lipid model membrane studied here and in the actual biological membranes.

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