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Change in electric characteristics of membranes in response to taste stimuli with increasing amount of lipids in membrane matrix of PVC and plasticizer

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Abstract

Changes in membrane electric potential in response to taste substances were studied for membranes containing differing amounts of negatively charged lipids in the membrane matrix of polyvinyl chloride and plasticizer. Responses to quinine showing bitterness decreased systematically with increasing the quantity of charged lipids contained in the membrane, whereas the response did not depend on differences in the hydrocarbon chains of lipids. The mechanism is discussed qualitatively in terms of hydrophobicity and hydrophilicity of the membranes.

Keywords: Lipid membrane; Polyvinyl chloride; Plasticizer; Taste substances; Electrochemical property; Chemical sensor

1. Introduction

In biological systems, taste substances are received at the biological membrane which covers taste cells of taste buds. As is well known, the biological membrane is composed of proteins and a lipid bilayer. Proteins, lipids or both are considered to act as receptor to chemical substances [1–3]. A recently-developed chemical sensor utilizes lipid membranes to transform taste information into electric potential changes [4–8], and is able to measure the taste quality and also the intensity as a whole.

The membranes of the taste sensor are prepared by mixing each lipid with polyvinyl chloride (PVC) and dioctylphenylphosphonate (DOPP) in tetrahydrofuran [4–8]. Lipids are incorporated into the membrane matrix of PVC and DOPP. The membrane composed of PVC and DOPP without lipids is known to show cationic selectivity due possibly to negatively charged impurities [9–12], and, therefore, it is important to understand the effects of lipids on the membrane characteristics from both the physicochemical and technological points of view. The results should also contribute to clarify the role of lipids in taste reception in biological systems.

Here we study the responses of the PVC/DOPP membrane containing lipids to taste substances NaCl and quinine by changing the kind and quantity of charged lipids. We show that the response to quinine decreases with increasing the amount of lipids in the membranes, and discuss the mechanism by focusing

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on hydrophobic and hydrophilic interactions between the membranes and taste substances. Quantitative explanation is made elsewhere [13].

2. Materials and methods

2.1. Materials

All the lipid analogues used were purchased from Tokyo Kasei. Eight kinds of lipids used so far in the taste sensor were as follows [4–8]: dioctylphosphate (DOP), trioctylmethylammonium chloride (TOMA), oleyl amine (OAm), decyl alcohol (DA), oleic acid (OA) and mixtures of DOP and TOMA in the molar ratio of 3:7, 5:5 and 9:1.

Other lipids were also used for comparison in this study: phosphoric acid diphenyl ester (PA6), phosphoric acid di(2-ethylhexyl) ester (PA8), phosphoric acid di-*n*-decyl ester (PA10), phosphoric acid oleyl ester (PA18), caproic acid (CA6), caprylic acid (CA8), capric acid (CA10), lauric acid (LA(12)), palmitic acid (PA(16)), stearic acid (C18-0), linoleic acid (C18-2). The numerical values following letters and hyphens indicate the number of carbons and the number of double bonds in the hydrophobic chain, respectively.

Taste substances, quinine hydrochloride and NaCl, were purchased from Wako Chemicals.

2.2. Lipid membranes

Lipid membranes were prepared as previously reported [4–8]. Each lipid analogue was mixed with 400 mg PVC (polymer) and 0.5 ml DOPP (plasticizer) in 10ml tetrahydrofuran (THF). The mixture was then dried on a glass plate, which was placed on a hot plate kept at 30°C. The lipid membranes thus prepared were a transparent colorless film about 200 μm thick. A PVC/DOPP membrane with no lipid was also prepared similarly. Membranes were preconditioned by immersing them in 1 mM KCl solution for one day or more before use.

2.3. Measurement system

The electric potential across the membrane was detected by a Ag/AgCl electrode in 100 mM KCl and a reference electrode (TOA, HS205C) in the taste solution. From analogy with the biological system, 100 mM KCl and taste solutions correspond to internal and external phases of the taste cell, respectively. The measured electric potential can be called the membrane potential. The same eight sets constructed one electrode made of acrylic board of a conventional taste sensor system [6–8]. The electric signal from each membrane was passed through a high-input impedance amplifier and an eight-channel scanner and was converted to a digital code by a digital voltmeter (ADVANTEST, R6551) and recorded in a computer (NEC, PC-9801). Experiments were carried out at room temperature (approximately 20°C).

3. Results

3.1. Response of lipid membranes to taste substances

Fig. 1 shows changes in membrane electric potentials in response to quinine hydrochloride and NaCl

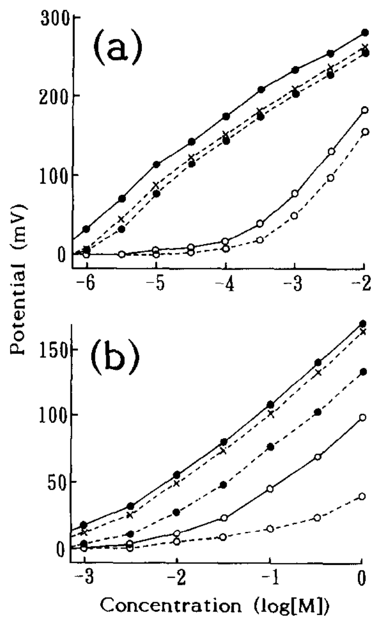

Fig. 1. Response of membranes to quinine hydrochloride (a) and NaCl (b): ○———○, DOP; ○---○, 9:1; ●---●, DA; ×---×, OA; ●———●, PVC/DOPP. Relative standard deviations with 5 measurements on each taste substance were 1–2%, similar to the cases reported previously [6,8]. The ordinate and abscissa are the change of electric potential response and the concentration of taste substances, respectively.

as a function of log concentration. Five kinds of membranes were tested, i.e. the PVC/DOPP membrane and the lipid membranes containing OA, DA, DOP or two lipid species of 9:1 ratio. The PVC/DOPP membrane responds to NaCl Fig. 1(b); it may originate from charged impurities, as suggested in Ref. [9–12].

The magnitude of the potential changes across the membrane was in the order PVC/DOPP ≈ OA ≈ DA > DOP > 9:1 for both quinine Fig. 1(a) and NaCl Fig. 1(b). The magnitude of the electric potential response for quinine is larger than that for NaCl for all the membranes. The difference in electric potential between the PVC/DOPP and the DOP membranes is larger for quinine than for NaCl. Thus, we studied the response to quinine.

3.2. *Characteristic change from lipid membrane to PVC / DOPP membrane*

Membranes were prepared by the addition of the same molar (200 μmol) phospholipids (PA6–PA18) or fatty acids (CA6 ~ C18-2) into THF containing PVC and DOPP, and responses to quinine were measured. Figs. 2 and 3 indicate that the magnitudes of the potential changes of the membranes containing phospholipids and fatty acids, respectively, for quinine are the same, if the membranes contain the same amount of lipids. The length of the hydrophobic chain has no effect on the response of the negatively charged membranes.

Next, the effect of the amount of negative-charge on quinine responses was investigated. For this purpose, PA18 and OA were selected as the representatives from phospholipids and fatty acids, respectively. The series of membranes PA18a–PA18h shown in Fig. 4 contain different amounts of lipids, but the kind of lipid is the same. The first membrane

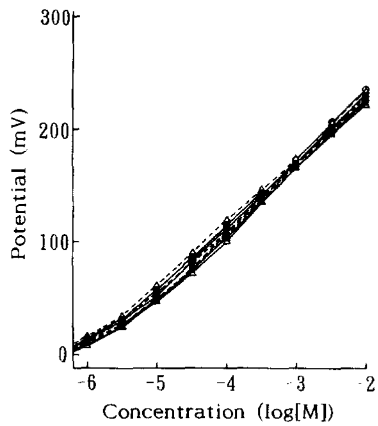

Fig. 3. Responses of membranes containing the same molar amount of fatty acids to quinine hydrochloride: ○——○, CA6; ○---○, CA8; ●——●, CA10; ●---●, LA(12); △——△, PA(16); △---△, C18-0; ▲——▲, OA; ▲---▲, C18-2.

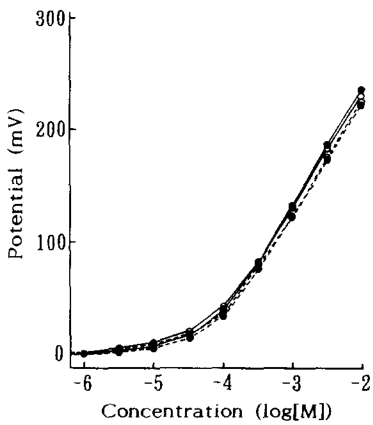

Fig. 2. Responses of membranes containing the same molar amount of phospholipids to quinine hydrochloride: ○——○, PA6; ○---○, PA8; ●——●, PA10; ●---●, PA18.

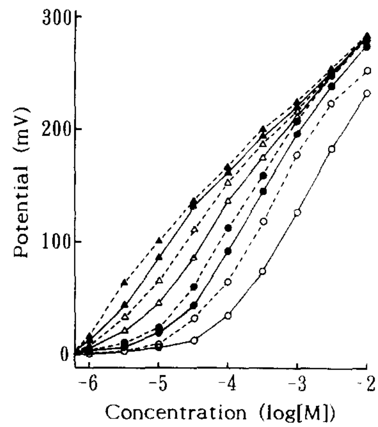

Fig. 4. Responses of membranes containing different molar amount of phospholipids to quinine hydrochloride. ○——○, PA18a; ○---○, PA18b; ●——●, PA18c; ●---●, PA18d; △——△, PA18e; △---△, PA18f; ▲——▲, PA18g; ▲---▲, PA18h.

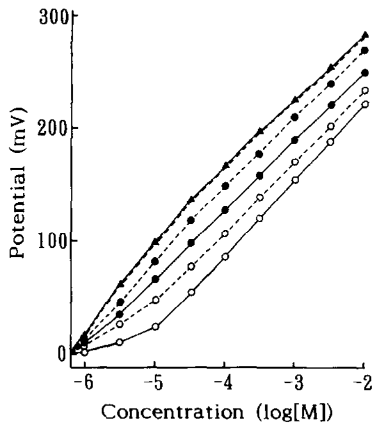

Fig. 5. Responses of membranes containing different molar amount of fatty acids to quinine hydrochloride. ○———○, OAa; ○- - -○, OAb; ●———●, OAc; ●- - -●, OAd; △———△, OAe; △- - -△, OAf; ▲———▲, OAg; ▲- - -▲, OAh. The curves for OAe–OAh, which contain the smaller amount of lipids, agree with each other.

in the series, P18a, was prepared by the addition of 200 μmol lipids. From PA18a through PA18h, the amount of lipid was decreased by one third. Hence, the amount of negative charge in the membranes decreased from PA18a to PA18h. The series of membranes OAa–OAh were also prepared in the same manner, except that the amount of fatty acid of OAa was 600 μmol.

Fig. 4 shows that the response to quinine hydrochloride increases with decreasing amount of phospholipids, and finally reaches the level of the PVC/DOPP membrane, while the form of response curves change depending on the amount of lipids. This result implies that the increase in negative electric charge of mixed lipids suppresses the response of membrane to quinine.

Fig. 5 shows the response of the OA series to quinine hydrochloride, which is saturated in the course of dilution of density of lipids. The OA membrane behaves like the PVC/DOPP membrane (Fig. 1), and hence the effect of dilution seems to be easily saturated.

4. Discussion

Lipid membranes are useful materials for transforming taste information to electric signals [14–16]. Based on these results, lipid membranes composed of PVC and DOPP (plasticizer) have been developed [3–8]. In the present paper, the basic characteristics of these membranes with different amounts of lipid molecules were studied. We found that the response to quinine increases with decreasing lipid amount irrespective of the length of hydrocarbon chains of lipid species used. This result is explained in terms of the change in hydrophobicity (and hydrophilicity) of the membrane. The PVC/DOPP membrane shows a Nernst-type response to quinine hydrochloride as in hydrophobic membranes for amphiphilic molecules [17]. If negatively charged lipids as phospholipids or fatty acids are mixed with the PVC/DOPP membrane matrix, the hydrophilicity may increase because of a hydrophilic charged group of lipid. Therefore, the relative magnitude of hydrophobicity may decrease and result in a decrease in adsorption of quinine, which is a hydrophobic molecule, to the membrane. It can be observed as a decrease in the membrane potential (Figs. 4 and 5).

The response to NaCl does not vary very much between these two kinds of membranes (PVC/DOPP and DOP). In this case, the degree of H^+ dissociation or the surface charge density is important for interacting with Na^+ ions. As shown in the accompanying paper [13], H^+ dissociation is rather low in the membrane containing anionic lipids because of its high packing density. Furthermore, the contribution of the diffusion potential within the membrane must be taken into account. Accordingly, the situation becomes difficult; quantitative discussion will be made elsewhere [13]. Nevertheless we may conclude that the PVC/DOPP membrane is a charged membrane with the strongly hydrophobic property and that the membranes containing anionic lipids in the PVC/DOPP matrix can be considered to have a moderately hydrophilic and hydrophobic property.

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