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A POSSIBILITY TO RECOGNIZE CHIRALITY BY AN EXCITABLE ARTIFICIAL LIQUID MEMBRANE

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Studies were made on oscillations across a liquid membrane consisting of an oil layer, nitrobenzene containing picric acid, between two aqueous layers: a solution of 1.5 M ethanol and 5 mM optically-active cationic detergent, the D- or L-form of *N*-α-methylbenzyl-*N*,*N*-dimethylmyristylammonium bromide, on the left and 0.1 M D- or L-form of various ligands, such as glucose, arabinose, alanine, glutamic acid, threonine, leucine, proline, or phenylalanine on the right. This system showed sustained rhythmic oscillations of electrical potential of 200–300 mV with intervals of the order of 1 min. The frequency of oscillations depended on the combination of chiralities of the detergent and ligand. This means that the two forms (D and L) of chiral ligands can be distinguished by differences in the electrical response of the liquid membrane.

1. Introduction

In living organisms, recognition of molecules, such as by taste, smell or immunity, is essential for the maintenance of life. There have been many studies on the recognition of molecules, but the molecular mechanisms involved are not well understood [1].

Dupeyrat and Nakache reported [2,3] quasi-periodic variations of a relaxation type in the interface tension and the electrical potential in an oil/water two-phase system. Stimulated by their pioneering study, we initiated studies on oscillatory phenomena between oil and aqueous solutions. Recently, we found [4,5] that rhythmic oscillations of electrical potential are generated in a liquid membrane consisting of water/oil/water phases and that the oscillatory responses of this liquid membrane to various chemical ligands added to one of the water phases resemble those of biological chemoreceptive membranes. Thus, we proposed that this system could be used as a model of taste reception.

As an extension of this work, in the present study we prepared a membrane containing a chiral surface-active reagent that had the ability to distinguish substances differing in chirality.

2. Materials and methods

2.1. Materials

The D- and L-forms of *N*-α-methylbenzyl-*N*,*N*-dimethylmyristylammonium bromide (D-I and L-I) were prepared by a similar procedure to that of Moss and Sunshine [6], as described below. D-Amino acids were obtained from the Peptide Institute, Osaka, Japan. Other reagents were commercial products of analytical grade. Before use, nitrobenzene was purified by distillation and picric acid was dried in vacuo.

D-*N*-α-Methylbenzyl-*N*,*N*-dimethylmyristylammonium bromide (D-I): A mixture of D-*N*,*N*-dimethyl-α-phenylethylamine (5.5 g), synthesized by the method of Clarke et al. [7], myristyl bromide (3.0 g) and ethanol (20 ml) was heated for 48 h at 80°C. Part of the ethanol in the reaction mixture was evaporated off. The resulting precipitate was recrystallized from ethanol/diethyl ether (1 : 2) as a white powder; m.p. 109–110°C; $[\alpha]_D^{25}$, +20.58; yield, 6.5 g (76%). Found: C, 67.55; H, 10.23; N, 3.01%. Calcd. for $C_{24}H_{44}NBr$: C, 67.66; H, 10.26; N, 3.02%.

L-*N*-α-Methylbenzyl-*N*,*N*-dimethylmyristylammonium bromide (L-I): This compound was synthesized by a similar method to that for D-I. White powder; m.p. 110–112°C; $[\alpha]_D^{25}$, −21.12; yield, 6.8 g (80%). Found: C, 67.54; H, 10.28; N, 3.03%.

2.2. Methods

Experiments were performed in the U-shaped glass tube (12 mm inner diameter) shown schematically in fig. 1. A solution (4 ml) of 1.5 mM picric acid in nitrobenzene was placed in the bottom of the U cell, and aqueous solutions (10 ml each) were introduced simultaneously into the arms of the U cell above the organic phase without stirring. Gentle stirring of the solutions was found to increase the frequency of oscillations. All measurements were carried out at 25°C. The voltage across the liquid membrane was measured with a Hitachi-Horiba F-7 pH/mV meter connected by two salt bridges to two Ag/AgCl electrodes batched in aqueous 3 M KCl. A positive electrical potential was defined as that observed when the potential of the right aqueous phase was higher than that of the left aqueous phase.

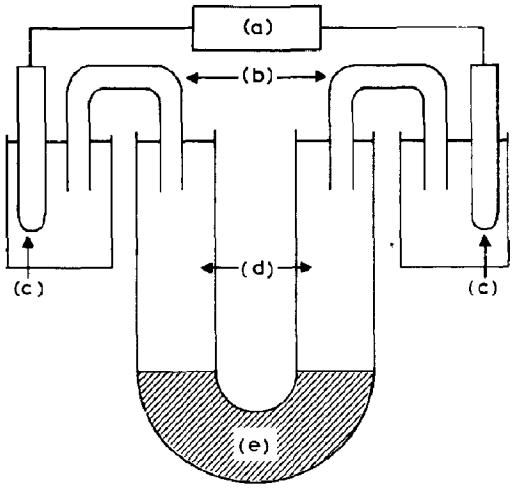

Fig. 1. Diagram of the experimental apparatus: (a) millivolt meter, (b) salt bridges, (c) Ag/AgCl electrodes, (d) aqueous layers, (e) organic layer.

All ^{1}H-NMR spectra were measured at 400 MHz with a Jeol GX-400 NMR spectrometer using the pulsed Fourier transform mode. Critical micelle concentrations (CMC) of cationic detergents were determined by measuring the specific conductance of their aqueous solutions in a glass cell with platinum-black electrodes (cell constant: 0.479 cm^{-1}), using a Kohlrausch bridge (Yokogawa Elect., type 2758) at 1 kHz.

3. Results and discussion

3.1. Frequencies of oscillations induced by chiral chemicals

Fig. 2 shows the oscillations of voltage across a liquid membrane consisting of an oil layer, nitrobenzene containing 1.5 mM picric acid, between two aqueous phases: a solution of 5 mM optically active detergent (D-I or L-I) plus 1.5 M ethanol on the left, and a solution of 0.1 M D- or L-glucose on the right. Fig. 3 shows the oscillations with 0.1 M D- and L-alanine in the right arm of the cell. The results of three independent experiments for combinations of D-alanine (c-1,2,3) and L-alanine (d-1,2,3) in the right arm and L-I in the left are presented to show the reproducibility. The oscillations started several minutes after the organic phase came into contact with the two aqueous solutions and continued at almost constant amplitude for 10 min to 2 h. It is noteworthy that the frequencies of oscillations differed depending on the chiralities of both the detergent (D-I or L-I) and the ligand, whereas the amplitudes of the oscillations were scarcely affected by the chiralities of the two. No oscillations occurred in the

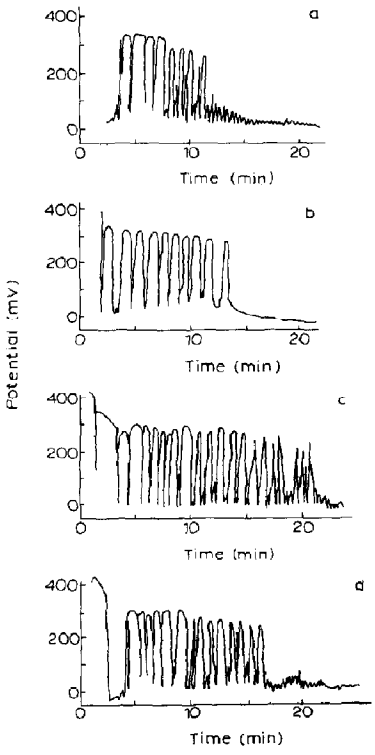

Fig. 2. Oscillations of electric potential across the organic phase. Right aqueous phase: D-glucose (a, c); L-glucose (b, d). Left aqueous phase: D-I (a, b); L-I (c, d). In the record of the electrical potential, an upward change denotes an increase in relative positive charge in the right aqueous phase.

absence of either picric acid or the cationic detergent. The presence of the detergent apparently affected the migration of picric acid. The left aqueous phase remained colourless throughout the measurements, but the right aqueous phase gradually became yellow, suggesting migration of picric acid into the right aqueous phase.

On inspection of figs. 2 and 3, one can observe that the frequency does not remain constant and changes with time. At present, it is quite difficult to determine the conditions for inducing oscillations with constant frequency. This is because the concentrations of picric acid and the detergent in the organic and aqueous phases, as well as at the interface, change gradually with time. Thus, we would like to discuss the mean values of the frequencies.

Table 1

Frequencies (min^{-1}) of oscillations of electrical potential

Average frequencies and standard deviations in at least three runs for oscillations with an amplitude of over 200 mV are given.

Substrate	Cationic detergent	
	D-I	L-I
D-Glucose	1.62 ± 0.53	1.70 ± 0.25
L-Glucose	1.02 ± 0.08	1.25 ± 0.31
D-Arabinose	0.78 ± 0.04	1.70 ± 0.06
L-Arabinose	1.63 ± 0.12	1.24 ± 0.11
D-Alanine	0.71 ± 0.17	1.77 ± 0.17
L-Alanine	1.92 ± 0.16	0.35 ± 0.03
D-Glutamic acid	0.78 ± 0.12	0.94 ± 0.13
L-Glutamic acid	1.44 ± 0.26	0.67 ± 0.20
D-Threonine	1.23 ± 0.30	0.73 ± 0.19
L-Threonine	0.60 ± 0.13	0.99 ± 0.19
D-Leucine	0.94 ± 0.23	1.18 ± 0.30
L-Leucine	0.83 ± 0.12	1.06 ± 0.18
D-Proline	1.21 ± 0.45	0.76 ± 0.19
L-Proline	0.39 ± 0.11	0.71 ± 0.22
D-Phenylalanine	1.01 ± 0.16	0.99 ± 0.35
L-Phenylalanine	0.95 ± 0.33	0.70 ± 0.18

Table 1 summarizes the mean values and standard deviations of the frequencies of oscillations observed with various sugars and amino acids, and optically active detergent, D-I or L-I. The results are means for oscillations with an amplitude of more than 200 mV in three experimental runs. The procedure used for calculations of the mean values of the frequency was as follows: All the spikes with amplitudes of over 200 mV were taken into account. The periods between these spikes were measured from traces in three experimental runs, and the mean value and standard deviation were calculated from the reciprocal values of the periods. At the liquid membrane containing the chiral detergent, the chiralities of substances could be distinguished by their effects on the frequency of oscillations. It is also noteworthy that diastereomeric pairs, such as L-I and L-alanine, and L-I and D-alanine, exhibited different oscillatory frequencies, suggesting that the pattern of oscillations reflects differences in interaction between the detergent and chiral molecules.

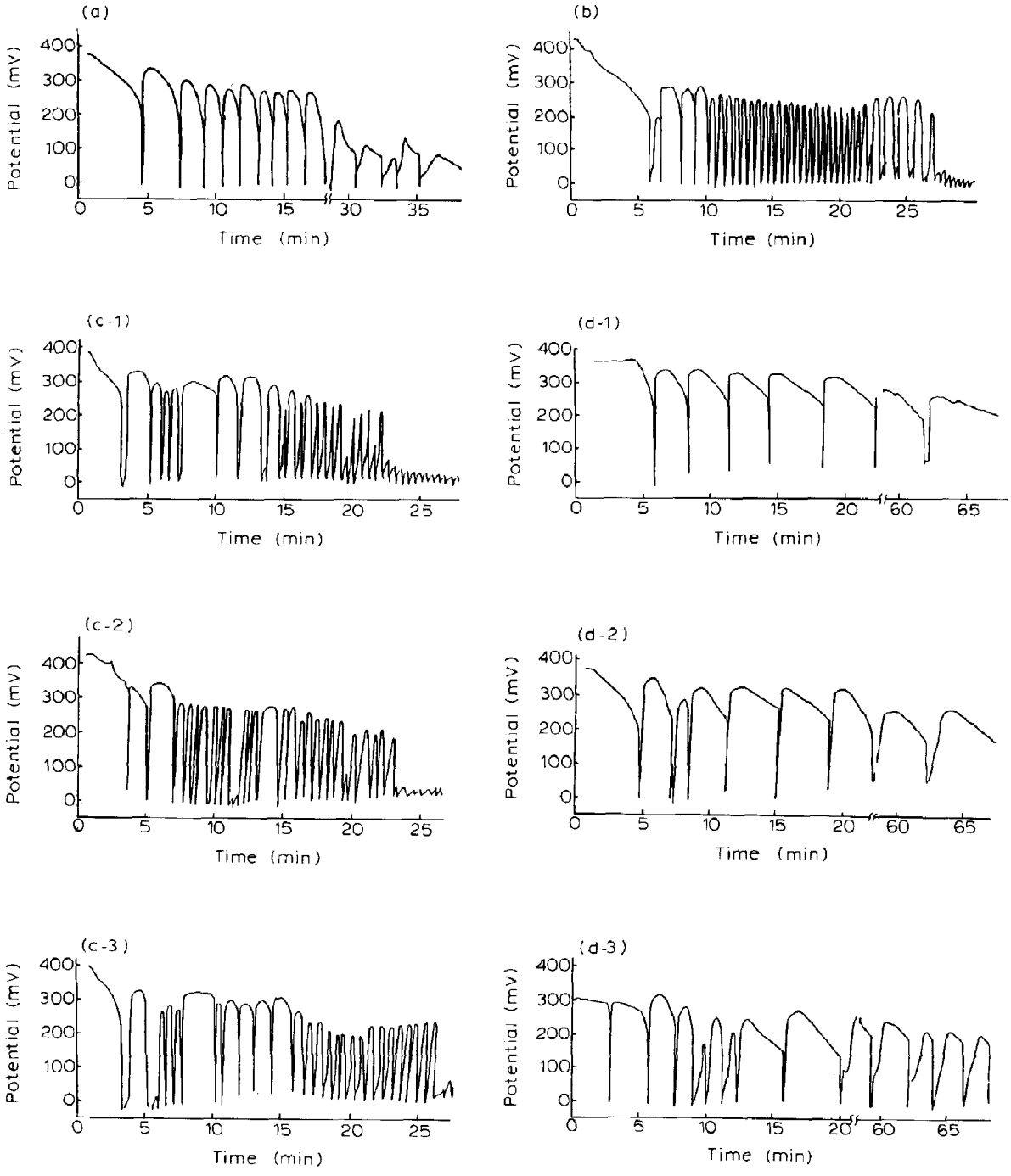

Fig. 3. Oscillations of electric potential. Right aqueous phase: D-alanine (a and c-1,2,3); L-alanine (b and d-1,2,3). Left aqueous phase: D-I (a and b); L-I (c-1,2,3 and d-1,2,3).

3.2. Dependence of the frequency of oscillations on the concentration of chiral ligands

The frequency of electrical oscillations depends on the concentration of the ligand added to the right aqueous phase. As an example, fig. 4 shows the dependence of the frequency of oscillations on the concentration of alanine when 5 mM L-I plus ethanol was used as the left aqueous phase. In the absence of alanine, the frequency was 1.08 min^{-1} (log $f = 0.03$). On addition of D-alanine, the frequency increased progressively. On the other hand, on addition of L-alanine, the frequency first decreased and then increased at concentrations of L-alanine above 0.1 M. Similar concentration-dependent changes in frequency were observed with other chiral ligands in place of alanine.

3.3. Interaction of the detergent with chiral ligands

Next, the specific conductance of the aqueous solution of the cationic detergent was measured. In the absence of a chiral ligand (sugar or amino acid), the CMC values for both L-I and D-I were 1.05 mM, as determined from the point of inflection in the graph of the specific conductance vs. the concentration of detergent. Fig. 5 shows the specific conductances of aqueous solutions of the detergent (L-I) in the presence of 0.1 M L- and D-alanine, respectively. The inflections in the presence of L- and D-alanine are 0.87 and 1.02 mM, respectively. This result clearly indicates that L- and D-alanine have different effects on the aggregation of the cationic detergent L-I.

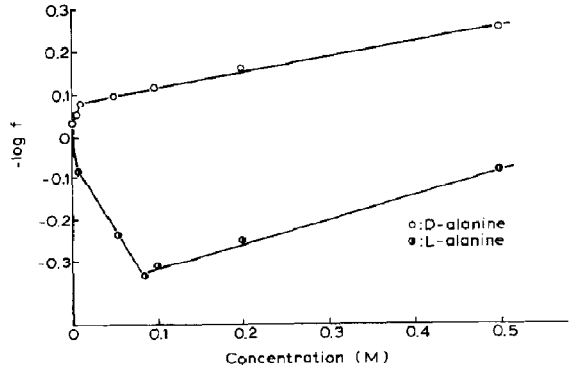

Fig. 4. Dependence of frequency on the concentrations of D- and L-alanine. Experimental conditions were as for figs. 2 and 3.

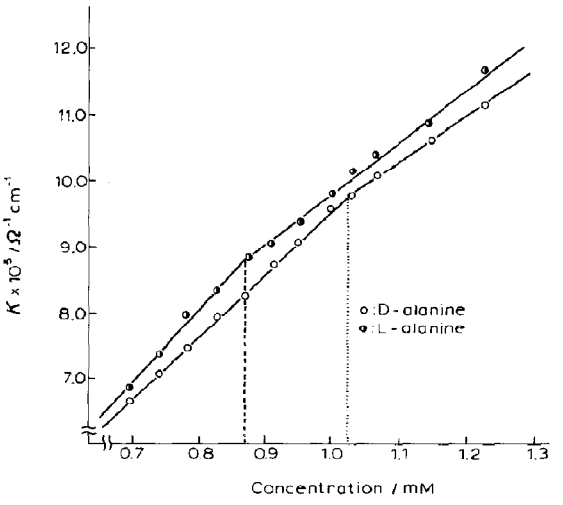

Fig. 5. Concentration dependence of the specific conduction of the aqueous solution of L-I in the presence of 0.1 M L- or D-alanine.

Fig. 6 shows the 400 MHz ^{1}H-NMR spectrum of the cationic detergent L-I in d_5-nitrobenzene. Note that the chemical shifts of the two *N*-methyl groups are different (e and e'). Similarly, the two hydrogen atoms on the C_1-carbon of the myristyl chain show a split signal (d and d'). This suggests that the intramolecular rotation around the N–C bond between the nitrogen atom and the 1-phenylethyl moiety is restricted. The chirality would determine the extent of H-bonding and hydrophobic interactions between the picrate ion and the chiral ligand in the zone around the quaternary nitrogen atom. The rigidity around the nitrogen may be favorable for specific interaction with chiral chemicals or for recognition of chiral chemical ligands.

3.4. Mechanism of oscillations

The tastes of the D- and L-forms of chiral substances are reported to be different [8]. This difference has been attributed to differences in the specific interactions of the chiral molecules with

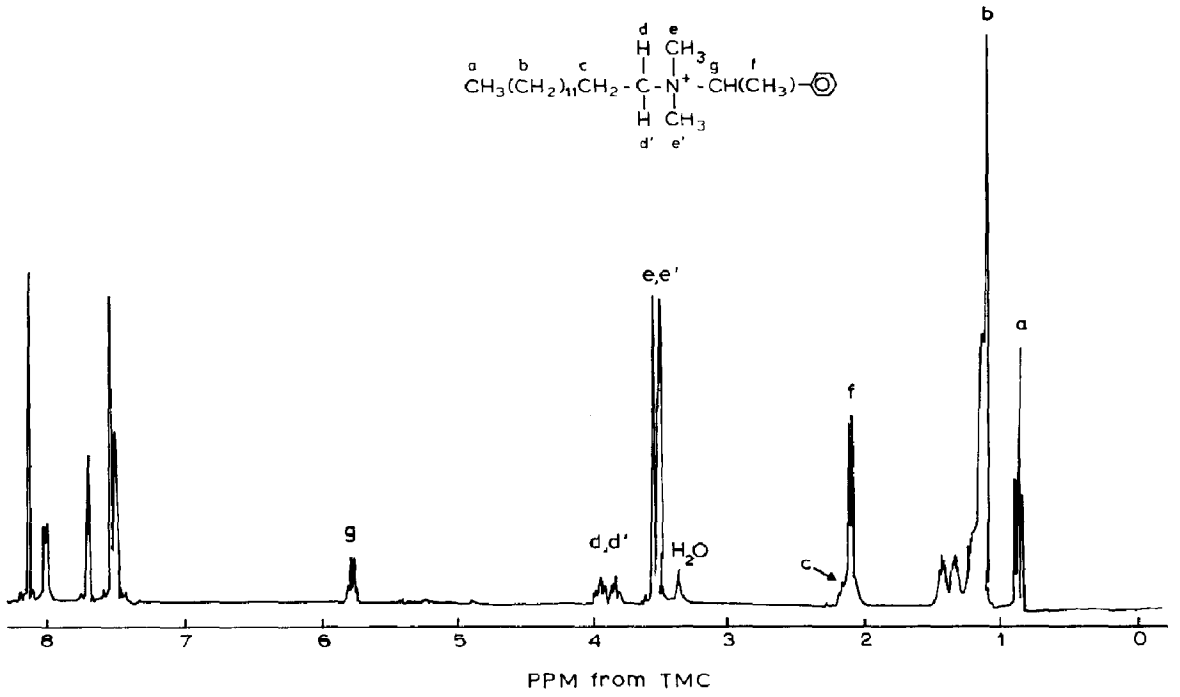

Fig. 6. 400 MHz ^{1}H-NMR spectrum of a d_5-nitrobenzene solution of L-1.

receptor proteins. In the present work, we found that differences in chirality could be recognized with a simple liquid membrane in the absence of any receptor protein. We also found that the oscillations in the electrical potential could be generated directly and that they differed according to chiral ligands. These experimental findings are interesting in relation to the sense of taste in animals.

Recently, we reported [5] oscillatory phenomena across a liquid membrane consisting of an oil layer, nitrobenzene containing picric acid, between two aqueous layers: a solution of 5 mM CTAB (hexadecyltrimethylammonium bromide) plus alcohol at various concentrations on the left and a solution of 0.1 M sucrose on the right. We found that this system exhibited rhythmic oscillations of electrical potential of 200–400 mV at intervals of the order of 1 min. We also found that the frequency of oscillations increased with increase in the concentration of alcohol, and that the threshold concentrations of alcohols needed to induce oscillations decreased with increase in their hydrophobicity. We suggested that the response of the liquid membrane to alcohol resembled that of taste. As an extension of this work, in the present study we constructed a liquid membrane with the ability to distinguish differences in chirality.

Here the mechanism of the oscillation should be discussed briefly. The oscillations observed in this work may be explained in a similar way to those in our previous study [5]. Namely, as a first step, D-I or L-I cations, which are mainly present as micelles in the aqueous phase, move toward the interface and become situated at the interface. Simultaneously, molecules of picric acid move toward the interface and dissolve in the aqueous phase. Thus, the concentrations of the cationic detergent (D-I or L-I) and picric anion at the interface increase gradually, and the surfactant monomers of the detergent tend to form a monolayer structure at the interface. When the concentration of the cationic detergent at the interface reaches a critical value, these surfactant

monomers are abruptly transferred to the organic phase by formation of inverted micelles, and this transfer is associated with an abrupt decrease of the membrane potential. Then, when the concentration of the cationic detergent at the interface decreases to a lower critical value, accumulation of the detergent at the interface begins again and the cycle is repeated. Changes in state thus occur repeatedly.

Chiral detergents may also be expected to migrate into the organic phase, flow toward the the right interface and cross the interface into the right aqueous phase. This process may occur in the induction period. However, this process does not seem to affect the observed change of electrical potential directly. If the detergents form a monolayer at the right interface, this should induce a 'negative' potential due to the electric double layer at the right surface [9]. However, no region of negative potential was observed throughout the measurements.

Ligands with different chiralities probably have different effects on the structure of the monolayer of the chiral detergent at the interface depending on the combination of their chirality and that of the detergent. These chiralities may also affect the rates of migration of the aggregates of the detergent from the aqueous phase to the interface and from the interface to the organic phase. These effects due to differences in interactions of chiral substances may result in differences in frequency of oscillations.

NMR spectroscopy was used to obtain further information on the mechanism of oscillations. Fig. 7 shows the 400 MHz ^{1}H-NMR spectra of the nitrobenzene solution sampled from the organic layer (a) before contact with the aqueous solutions and (b) after 15 oscillations (~ 50 min after contact of the solutions). The samples were collected through a pipette inserted to near the center of the organic phase. Other experimental conditions were as for fig. 3 (d). Dioxane (2.1 mM) was added to the sample tube as a standard of chemical shift and signal intensity. The spectra show that water, the cationic detergent and ethanol, initially present in the left aqueous phase, move toward the organic phase after construction of the aqueous-organic-aqueous system. This result supports the above mechanism including inverted-micelle formation in the organic phase.

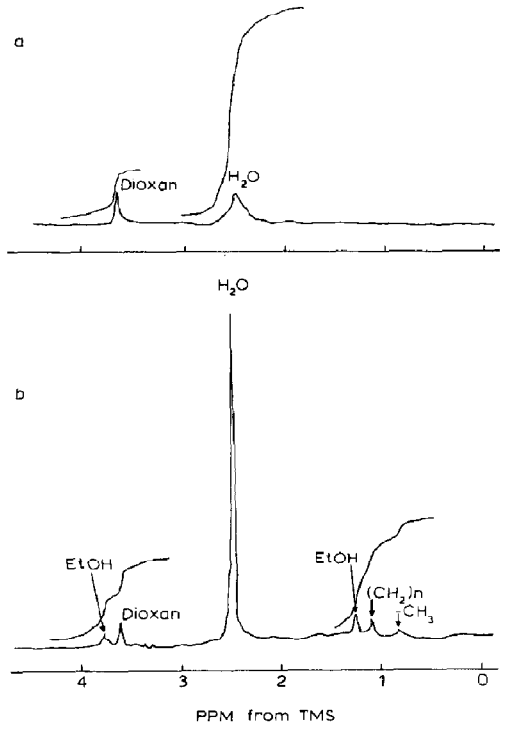

Fig. 7. 400 MHz ^{1}H-NMR spectra of a nitrobenzene solution of L-I. Experimental conditions were as for fig. 3d. (a) Before construction of the liquid membrane. (b) After 15 oscillations (~ 50 min after construction of the membrane).

The concentrations of water, L-I, and ethanol were 66.4, 0.4 and 3.6 mM, respectively, in fig. 7b, whereas the concentration of water was 23.3 mM and those of L-I and ethanol were zero in fig. 7a. These concentrations were calculated from the relative integrated intensity of each NMR signal. Because of the low resolution due to the inhomogeneity of the sample and to the high decoupling power, it is possible that the methyl resonance of alanine overlaps that of ethanol. The increases in the concentrations of water, L-I, and ethanol were thus 43.1, 24 and 3.6 mM, respectively. As the volume of the organic phase was 4 ml and the number of oscillations 15, the transfers of water, L-I, and ethanol were calculated to be 11.5, 0.11 and 0.96 μmol, respectively, per oscillation. This

result indicates that about 100 molecules of water and nine molecules of ethanol were transferred to the organic phase with transfer of one detergent molecule. However, it is probably more realistic to consider that water and ethanol are also transferred across the interface by simple diffusion.

Let us now estimate the amount of detergent in a monolayer covering the interface between the aqueous and organic phases. The area of the oil/water interface is 1.13 cm^2 in the U-shaped cell. If the area occupied by each surfactant molecule is tentatively assumed to be 100 $Å^2$, the amount of the surfactant forming a monolayer is calculated to be 0.19 nmol. On the other hand, 0.11 μmol of detergent was moved toward the organic phase in one oscillation, as described above. This suggests that only a small portion of the detergent molecules transferred across the interface contribute to the observed oscillation of the electrical potential. In other words, continuous movement of the detergent molecules from the aqueous to the organic phase may proceed simultaneously with the periodic movement accompanied by formation and destruction of the monolayer. The change of the frequencies with time, as is shown in figs. 2 and 3, is also attributable to the change of the concentrations of these chemical species caused by their continuous movements through the interface. It is interesting to note that chaotic changes in the frequency were observed sometimes (for example, see fig. 3, c-1,2,3). This may be also related to the change of the concentrations with time.

Quite recently we obtained an elaborate model for simulating the oscillations by taking account of the conditions inducing 'hard-mode instabilities' in a set of nonlinear differential equations [10]. We are now trying to analyze the chaotic change by the application of these nonlinear equations.

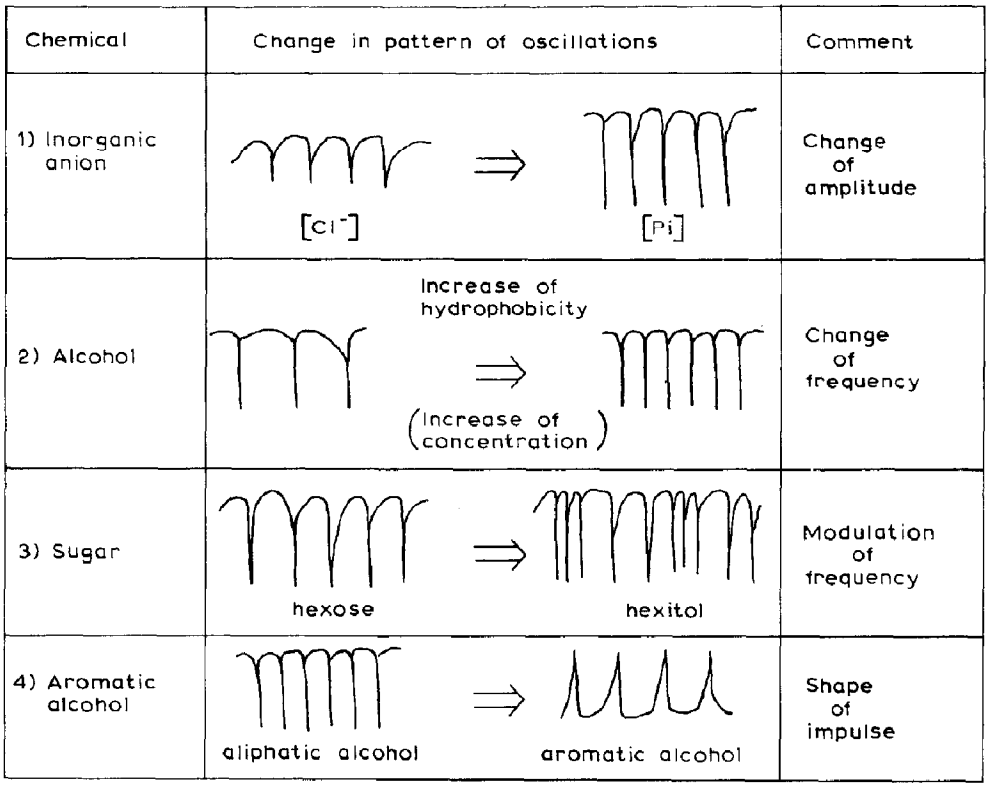

Fig. 8. Schematic representation of specific responses of the excitable liquid membrane.

3.5. Chemoreception by a liquid membrane

We have reported [4] that the amplitude of oscillations in a liquid membrane increases on addition of inorganic phosphate to the aqueous phase. More recently, we found [5] that the frequency of oscillations increases with increase in the concentration of alcohol added to the aqueous phase. We also found that the frequency increases with increase in the hydrophobicity of the alcohol, and that the pattern of oscillations in the presence of an aromatic alcohol is quite different from that in the presence of an aliphatic alcohol. Furthermore, quite recently we found that the frequency of the oscillation is modulated by the addition of ribose, arabinose or xylose, but not affected appreciably by addition of glucose, sorbitol or mannitol. The changes in patterns of oscillation of the liquid membrane in response to various chemical substances [4,5] are shown schematically in fig. 8. These results suggest that by studies on excitable phenomena in liquid membranes it may be possible to develop a new type of chemical sensor capable of distinguishing various chemical substances on the bases of information on the amplitude, frequency and shape of impulses.

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