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SELF-SUSTAINED OSCILLATIONS OF ELECTRIC POTENTIAL IN A MODEL MEMBRANE

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A model membrane constructed from a Millipore filter, whose pores are filled with dioleyl phosphate molecules, exhibits a self-oscillation of the electric potential with a period of about a few seconds in the presence of a salt-concentration difference, pressure difference and/or electric current across the filter. In this paper, the effects of chemicals such as KCl, $CaCl_2$, pH and sucrose on the self-oscillation are investigated experimentally. These chemical substances are shown to alter the characteristic properties as the frequency of oscillation. Theoretical consideration of electrochemical interaction between these substances and DOPH molecules gives a fairly good explanation of the observed results.

1. Introduction

A dioleyl phosphate (DOPH)-Millipore membrane is a model membrane composed of a Millipore filter whose pores are choked up with a lipid analogue, DOPH. This model membrane shows a self-sustained oscillation of membrane potential under an electric current I_a and pressure difference ΔP_a in the presence of a salt-concentration difference, Δn_a. Previous papers have revealed that the self-sustained oscillation appears as a result of the repeat of an accumulation and release of salt in a pore where the repetition process is coupled strongly with the phase transition of DOPH molecules [1,2].

Oscillatory behavior in another type of artificial membrane was found by Teorell [3]. Those kinds of oscillations are analogous to the nerve excitation in real systems. Chemical reception in biological systems is made by translating the d.c. change in membrane potential to the periodic response of excitation [4]. Therefore, the study of self-oscillation in model membrane systems has a biological significance.

In the present paper, we pursue detailed properties of self-oscillations by studying the effects of various chemicals such as KCl, $CaCl_2$, pH and sucrose on the frequency of oscillation. From the viewpoint of taste sensation, salt, Ca^{2+}, H^+ and sucrose correspond to salt, bitter, sour and sweet, respectively. Consideration of electrochemical interaction between these chemicals and DOPH molecules gives a fairly good explanation of the obtained experimental results. Section 2 is concerned with the experimental procedure and results, and the theoretical description based on the previously presented theory [1,2] is made in section 3. Discussion of the possibility of self-sustained oscillations in model membranes using other kinds of lipids than DOPH is made in section 4.

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2. Experimental

2.1. Materials and methods

DOPH-Millipore membranes used in the present experiments were prepared by the same methods as reported previously [1,2,5]. Millipore filters of cellulose ester with a normal pore size of 8 μm were immersed for a few minutes in a solution of DOPH in benzene, and then dried in air. The quantity of DOPH adsorbed within the filter paper was adjusted to about 3 mg/cm^2 by controlling the concentration of the DOPH-benzene solution. The DOPH-Millipore membranes were conditioned in 100 mM KCl aqueous solution over 12 h, and later immersed in 5 mM KCl solution for a few hours. Each membrane was placed between two cells with two pairs of Ag-AgCl electrodes, one of which was used for the measurement of membrane potential, and the other for the constant current supply. The cells were also manufactured so that a pressure difference could be imposed on the membrane.

One cell is occupied by the 5 mM KCl solution and the other by the 100 mM solution for investigating the influence of chemicals such as $CaCl_2$, pH and sucrose on the oscillation in DOPH-Millipore membranes. Chemicals were added to the 5 mM KCl solution, which was stirred throughout the experiment. The temperature of the solution was kept at 25 ± 0.5°C. KCl, $CaCl_2$, HCl and sucrose were purchased from Nakari Chemicals, Ltd. In the pH experiment, dilute HCl was added stepwise and the concentration of H^+ was measured with a pH electrode (Iwaki Glass, model IW202). The effect of KCl in the cell with the lower concentration was also studied by increasing it. The measurements were made for at least three samples, and similar properties were obtained for the effect of one chemical species on the self-oscillation. The pH in the aqueous solution was approx. 5.8 unless states otherwise. Chemicals are added in the 5 mM solution because the self-oscillation occurs within a pore near the side of the 5 mM solution, as mentioned later.

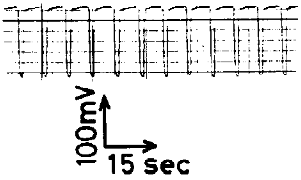

Fig. 1. An example of short-period self-sustained oscillations.

2.2. Results

Fig. 1 shows an example of self-oscillation with a period of about 7 s, when a pressure difference of 35 cmH_2O and an electric current of 0.3 μA are imposed on the membrane from the 100 mM to 5 mM side. Under constant conditions, these oscillations continued stably for a few hours. The frequency of oscillation in one membrane, however, usually differs from that in the other even if the quantities of adsorbed DOPH are equal to each other. This may reflect a semi-macroscopic local property of self-oscillation rather than the average characteristics of a membrane, as pointed out previously [1]: DOPH molecules in only one pore repeat phase transitions and are responsible for the self-oscillation of the membrane potential. The assembled form of DOPH molecules in each pore cannot be regarded as uniform in each membrane, as it leads to different frequencies of oscillation.

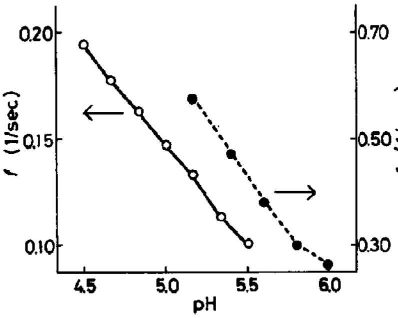

Fig. 2. Effect of pH on self-oscillation. Experimental data (O——O) for $\Delta P_a = 22$ cmH_2O and $I_a = 0.28$ μA are shown, and data (●------●) for $\Delta P_a = 39.9$ cmH_2O and $I_a = 0.66$ μA are from ref. 8.

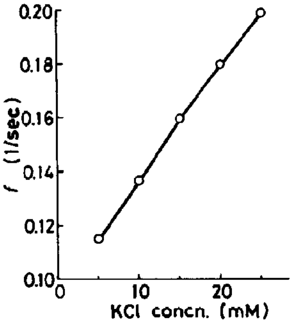

Fig. 3. Effect of KCl on self-oscillation. Conditions: $\Delta P_a = 30$ cmH_2O and $I_a = 0.25$ μA.

The oscillation is attributable to the phase transition of DOPH molecules between hydrophobic oil droplets and hydrophilic multilayers [1,2,6,7]. The change in hydrophobicity or hydrophilicity surrounding DOPH molecules may affect the oscillation. Fig. 2 shows the frequency f as a function of the pH of 5 mM KCl solution, when the pH is changed from 6 to 4.5 under $\Delta P_a = 22$ cmH_2O and $I_a = 0.28$ μA. The increase in H^+ enhanced the frequency; this result supports the tendency of previous observations [8] shown by black circles for the membrane with the quantity of adsorbed DOPH being 2.8 mg/cm^2 for $\Delta P_a =$ 39.9 cmH_2O and $I_a = 0.66$ μA. Excess H^+ below pH 4.5 stopped the oscillation. While the oscillation in fig. 2 ceased at pH 4.5, the change in external forces such as pressure and/or electric current can cause a renewed oscillation.

As shown in fig. 3, the increase in KCl also accelerated the oscillation. Further addition of salt stopped the oscillation. This result suggests a participating role of the phase transition of DOPH molecules, because the critical concentration of KCl for the occurrence of the phase transition is 30 mM [5–7].

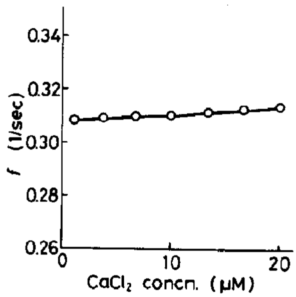

Fig. 4. Effect of $CaCl_2$ on self-oscillation. $\Delta P_a = 30$ cmH_2O, $I_a = 0.1$ μA.

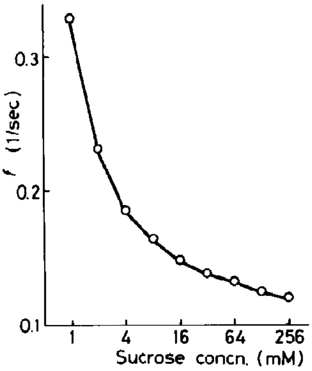

Fig. 5. Effect of sucrose on self-oscillation for $\Delta P_a = 40$ cmH_2O and $I_a = 0.18$ μA.

$CaCl_2$ exerted a pronounced effect as shown in fig. 4. It stopped the oscillation at very low concentration (20 μM) at which only a slight increase in frequency was observed. This may be brought about by the strong adsorption or bridging ability of Ca^{2+} to lipids such as DOPH [9,10] causing the formation of spherical inverted micelles rather than a multilayer. Thus, the phase transition between oil droplets and multilayers can hardly occur.

The effect of sucrose distinctly differed from those of the above-mentioned chemicals. It suppressed the oscillation resulting in a decrease n frequency (see fig. 5). However, the self-oscillation continued stably while the concentration of sucrose was changed over 2 orders of magnitude. Even at 256 mM, the oscillation did not stop. The carbohydrate ring of sucrose apparently slows the repeat of the phase transition of DOPH.

3. Theory

3.1. Theoretical model

In this section, we mention a previously presented model [1,2], and improve it slightly so as to describe the electrochemical effect of H^+, KCl and $CaCl_2$ on self-sustained oscillations.

3.1.1. Phase transition of DOPH molecules

Fig. 6 illustrates possible conformations of DOPH molecules and salt concentration within a pore for the salt-concentration difference Δn_a ($= n_h - n_l$), pressure difference ΔP_a and electric current I_a. The salt concentration inside a pore increases gradually from the side of the left cell with a lower salt concentration, n_l, to that of the right cell with a higher concentration, n_h. A phase-transition region is defined as that in which the stationary concentration is lower than the critical concentration n_t ($= 30$ mM), above which the major fraction of DOPH is transformed into multilayers. The length of this region l is given by [1]

$$l = L + \frac{\tilde{D}}{U_m} \ln\left[\left(1 - \frac{\Delta n_t}{\Delta n_a}\right) \exp\left(-\frac{U_m L}{\tilde{D}}\right) + \frac{\Delta n_t}{\Delta n_a}\right], \tag{1}$$

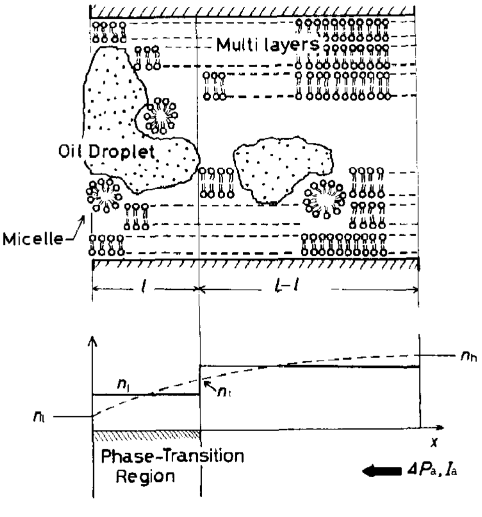

Fig. 6. Theoretical model. The dashed line in the lower figure is the average KCl concentration calculated from the stationary equations for flux [1]. The phase-transition region is defined by the region where the average concentration is below n_t ($= 30$ mM). The region near the right cell with high salt concentration ($= 100$ mM) contains a higher concentration than n_t on average, and hence is mostly composed of the multilayer phase. This region does not play a significant role in the self-oscillation. The salt concentration in each region is approximated as a uniform distribution shown by the solid line.

where L is the thickness of the filter paper, $\tilde{D}$ the effective diffusion constant of salt, and Δn_t is defined by

$$\Delta n_t = n_t - n_l. \tag{2}$$

The effective diffusion constant $\tilde{D}$ arises from the combination of three equations for mass flow, electric current and salt flow [1]. It concerns the average behavior of salt flow within one pore. The value can be estimated as about 10^{-6} cm^2/s; this is a little smaller than that in aqueous solution of 10^{-5} cm^2/s. The mass flow U_m is expressed by ΔP_a and the electric voltage difference V with the relevant phenomenological positive coefficients L_p and L_v, respectively:

$$U_m = -L_p \Delta P_a - L_v V. \tag{3}$$

Eqs. 1–3 show that the length l decreases for increasing ΔP_a or V.

DOPH molecules make an assembly form of hydrophobic oil droplets at lower salt concentrations but hydrophilic micelles or multilayers at higher salt concentrations [5–7]. Oil droplets can be considered as large aggregates composed of many loosely packed lipids in random phase. Spherical micelles are, on the other hand, formed from about 100 lipids, and multilayers have planar lamellar structures with tight packing, while all polar head groups are in contact with the aqueous phase.

As shown in fig. 6, the major fraction of DOPH in the phase-transition region is oil droplets because of the lower salt concentration. The repeat of the phase transition among three kinds of conformations occurs in this region, where salt accumulation and release occur [1]. The region of higher salt concentration does not contribute to the oscillation due to the impossibility of transformation from multilayers or micelles to oil droplets. Even if the majority of DOPH molecules remain in the oil-droplet phase for several hours after the membrane has been placed between two cells, they cannot repeat phase transitions among three phases; they tend to change slowly to multilayers in the presence of high salt concentration.

Let us then give kinetic equations for the phase transition of DOPH molecules in the phase-transition region (see fig. 7). We define η, η_m and η_s as

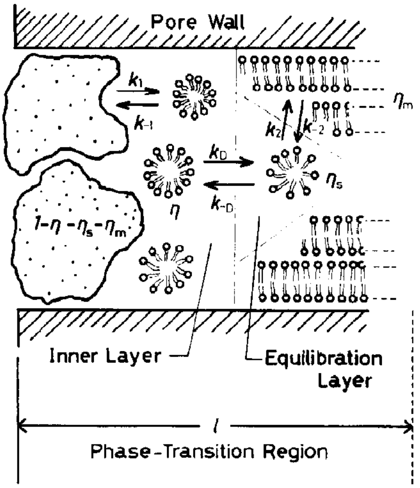

Fig. 7. Illustration of the phase-transition region. This region touches the left cell with low salt concentration (= 5 mM), and is mainly composed of the oil-droplet phase. Salt flow causes self-oscillation of the phase transition of DOPH molecules in this region.

the fractions of DOPH molecules in micelles in the inner bulk layer far from the pore wall, multilayers near and parallel to the wall and micelles in the equilibration layer in contact with each phase, respectively:

$$\eta = [\text{micelles in inner layer}]/\hat{N},$$
$$\eta_m = [\text{multilayers}]/\hat{N},$$
$$\eta_s = [\text{micelles in equilibration layer}]/\hat{N}, \qquad (4)$$

where [M] denotes the number of DOPH molecules with the structure specified by M, and $\hat{N}$ is the total number of DOPH in the phase-transition region.

Kinetic equations for DOPH may be given by [1,2,5]

$$\frac{d\eta}{dt} = k_1(1-\eta-\eta_s-\eta_m)n_i - k_{-1}\eta + k_{-D}\eta_s - k_D\eta,$$
$$\frac{d\eta_s}{dt} = -k_{-D}\eta_s + k_D\eta + k_{-2}\eta_m - k_2\eta_s,$$
$$\frac{d\eta_m}{dt} = -k_{-2}\eta_m + k_2\eta_s, \qquad (5)$$

where the coefficients, k_1, k_{-1}, k_2, k_{-2}, k_D and k_{-D} designate the relevant rate constants. These depend on the length of the phase-transition region l, and their explicit forms are given in ref. 1. The fraction of DOPH in oil droplets corresponds to $(1-\eta-\eta_s-\eta_m)$. The salt concentration n_i refers to that in the phase-transition region.

An expression for the rate constant k_{-2}, which concerns the multilayer stability, can be obtained by taking account of the electrochemical free energy of multilayers in the presence of cations such as K^+, Ca^{2+} and H^+ and anions like Cl^-. Since DOPH is negatively charged under normal conditions, the electrochemical interaction with cations proves to be significant. The effects of KCl, $CaCl_2$ and pH on self-oscillations can be considered as being caused by cations such as K^+, Ca^{2+} and H^+, respectively.

When Ca^{2+} is not contained in the aqueous solution, the electrochemical energy per DOPH molecule is given by [5,9,11]

$$F_{el}^{H} = k_B T \int_0^{\alpha} \ln\left(\frac{[H^+]}{K_d}\frac{\alpha'}{1-\alpha'}\right) d\alpha' + A\int_0^{\sigma} \psi \, d\sigma', \qquad (6a)$$

where the surface charge density σ is given by

$$\sigma = -(e/A)\alpha \qquad (7)$$

with α designating the degree of dissociation of H^+ given by

$$\alpha = -2(A/q)\sqrt{n_i}\,\sinh(\phi/2); \qquad \phi \equiv e\psi/k_B T. \qquad (8a)$$

In eqs. 6–8, $[H^+]$ is the proton concentration in the left cell, K_d the dissociation constant of H^+, A the molecular surface area of DOPH, ψ the surface electric potential, e the positive elementary charge, k_B the Boltzmann constant, T the absolute temperature and q is defined by

$$q = \sqrt{2\pi e^2/\varepsilon_W k_B T}, \qquad (9)$$

where ε_W is the dielectric permeability of water. A high potential approximation of $e^{-\phi} \gg 1$ at $\alpha \simeq 1$ and $n_i \geq 1$ mM gives an approximate expression for the electrochemical energy as

$$F_{el}^{H} = k_B T\left\{\ln([H^+]/K_d n_i) - \ln(A^2/q^2) - 2 + 4A\sqrt{n_i}/q\right\}. \qquad (10)$$

Since the rate constant k_{-2} is proportional to $\exp F_{\mathrm{el}}$ and the last term is much smaller than unity, $k_{-2\mathrm{H}}$ ($\equiv k_{-2}$) is given by

$$k_{-2\mathrm{H}} = \tilde{k}_{-2}[\mathrm{H}^+]/K_{\mathrm{d}} n_{\mathrm{i}} = k^0_{-2\mathrm{H}}[\mathrm{H}^+]/n_{\mathrm{i}}, \qquad (11\mathrm{a})$$

with $\tilde{k}_{-2}$ and $k^0_{-2\mathrm{H}}$ the numerical constants. This expression is improved by taking account of the effect of H^+ in that used previously [1].

When Ca^{2+} exceeding about 10 μM is contained in the cell, on the other hand, the effect of 1 μM H^+ (pH 6) can be disregarded. Hence the electrochemical energy is given by [9,10]

$$F_{\mathrm{el}}^{\mathrm{Ca}} = k_{\mathrm{B}}T\int_0^{\alpha} \ln\left(K_2 n_2 \frac{\alpha'}{1-\alpha'}\right) \mathrm{d}\alpha' + A\int_0^{\sigma} \psi \,\mathrm{d}\sigma', \qquad (6\mathrm{b})$$

where K_2 is the adsorption constant of Ca^{2+} and n_2 the Ca^{2+} concentration in the cell. The relation between α and ψ is expressed by [9–11]

$$\alpha = -2(A/q)\sinh(\phi/2)\left[n_{\mathrm{i}} + n_2(e^{-\phi}+2)\right]^{1/2}. \qquad (8\mathrm{b})$$

Through a procedure similar to the above, we can obtain $k_{-2\mathrm{Ca}}$ as a function of n_{i} and n_2:

$$k_{-2\mathrm{Ca}} = \tilde{k}_{-2}\sqrt{K_2 n_2}/n_{\mathrm{i}} = k^0_{-2\mathrm{Ca}}\sqrt{n_2}/n_{\mathrm{i}}, \qquad (11\mathrm{b})$$

with $k^0_{-2\mathrm{Ca}}$ the numerical constant.

The rate constants $k_{-2\mathrm{H}}$ and $k_{-2\mathrm{Ca}}$ should be used for the respective absence and presence of Ca^{2+} in the KCl aqueous solution within the cell.

We now simplify eqs. 5 for the convenience of numerical calculation. Since the values of k_2 and k_{-2} are much larger than those of other rate constants, we can adopt a quasi-stationary approximation or an adiabatic elimination. Thus eqs. 5 lead to [1,2]

$$\frac{\mathrm{d}\zeta}{\mathrm{d}t} = k_1(1-\zeta-\eta_{\mathrm{m}})n_{\mathrm{i}} - k_{-1}[\zeta - (k_{-2}/k_2)\eta_{\mathrm{m}}],$$
$$\frac{\mathrm{d}\eta_{\mathrm{m}}}{\mathrm{d}t} = k_{\mathrm{D}}[(k_2/k_{-2})\zeta - (1+v)\eta_{\mathrm{m}}], \qquad (12)$$

where ζ is the total fraction of spherical micelles defined by

$$\zeta = \eta_{\mathrm{s}} + \eta. \qquad (13)$$

The volume ratio v of the equilibration layer to the inner one appears in eq. 12 through the relation $vk_{\mathrm{D}} = k_{-\mathrm{D}}$.

3.1.2. Salt concentration n_{i}

If a salt-concentration difference Δn_{a} is imposed across a Millipore membrane, the KCl concentration n_{i} becomes dependent on the conformational states of DOPH [1,2]. When the greater part of the DOPH molecules form multilayers, n_{i} may be reduced to nearly n_{l}. This phase may be packed compactly to result in empty space in the pore, and hence the phase-transition region adjoins the left cell with n_{l} in fig. 6. In fact, the DOPH-Millipore membrane at higher salt concentrations shows lower electric resistance [5–7]. If micelle and oil-droplet phases develop as a consequence of depletion of the multilayer phase, n_{i} may increase almost to n_{h}; this is because the phase-transition region and the left cell break off by the interruption through hydrophobic oil droplets, so that salt is accumulated in the hydrophilic micelle phase by penetration from the side of the right cell with n_{h}.

The equation for n_{i} can therefore be written as [1]

$$\frac{\mathrm{d}n_{\mathrm{i}}}{\mathrm{d}t} = \hat{D}[\hat{n} - n_{\mathrm{i}}], \qquad (14)$$

where $\hat{D}$ is the diffusion constant and $\hat{n}$ is defined by

$$\hat{n} = X_{\mathrm{N}}[X_{\mathrm{A}} + (1-X_{\mathrm{A}})\hat{f}_1(\zeta)]$$
$$\times[1-(1-X_{\mathrm{B}})\hat{f}_2(\eta_{\mathrm{m}})], \qquad (15)$$

with X_{N}, X_{A} and X_{B} denoting parameters dependent on n_{l} and n_{h}. We have omitted the term expressing salt accumulation by the electric current, since it has only negligible values in the relevant self-oscillation [1]. The diffusion constant $\hat{D}$ refers only to the phase-transition region in one pore, different from $\tilde{D}$ concerning the average flow through the pore. Therefore, $\hat{D}$ depends strongly on the conformational states of DOPH molecules in this region.

The functions $\hat{f}_1$ and $\hat{f}_2$ are given by

$$\hat{f}_1(\zeta) = (\zeta/\zeta_{\mathrm{c}})^{\gamma}/[1+(\zeta/\zeta_{\mathrm{c}})^{\gamma}],$$
$$\hat{f}_2(\eta_{\mathrm{m}}) = (\eta_{\mathrm{m}}/\eta_{\mathrm{mc}})^{\gamma'}/[1+(\eta_{\mathrm{m}}/\eta_{\mathrm{mc}})^{\gamma'}], \qquad (16)$$

where γ, γ', ζ_c and η_{mc} are numerical constants. The functions f_1 and f_2 are constructed so as to express the property that the multilayer phase releases salt while the spherical-micelle phase accumulates salt. The numerical constants γ and γ' express the nonlinearity of salt accumulation and release. The phase transition occurs cooperatively coupled with the electrochemical interaction between salt and DOPH molecules. Therefore, we can expect a drastic change in salt concentration within the pore. The selection of values of γ and γ' can affect the details of the oscillatory wave form significantly. We chose $\gamma = 3.5$ and $\gamma' = 3.0$ so that they might not result in an unreasonably strong cooperativity.

For describing the effect of lower salt concentration n_l on self-oscillations in fig. 3, we must express X_N, X_A and X_B in terms of n_l. A maximum value of $\hat{n}$ is X_N and a minimum is $X_N X_A X_B$. We can, therefore, roughly estimate X_N and $X_N X_A X_B$ as n_h and n_l, respectively. It may be adequate to assume the following relation:

$$X_A = X_A^0\sqrt{n_l}, \quad X_B = X_B^0\sqrt{n_l}, \tag{17}$$

where X_A^0 and X_B^0 are the numerical positive quantities.

3.1.3. Self-sustained oscillations

Since eq. 1 for I contains the electric voltage difference V, a strict consideration of self-oscillation in DOPH-Millipore membranes requires the electric circuit for connecting the internal variables ζ, η_m and n_i with the electric characteristics as V. Consideration of the electric circuit, however, is not essential in understanding the mechanism of self-oscillation, when the discussion is limited to short-period oscillations under Δn_a, ΔP_a and I_a; hence we shall discuss the electric circuit no longer, which is detailed elsewhere [1,12]. It becomes important in discussing a relationship between long-period oscillations appearing under only Δn_a and short-period oscillations under Δn_a, ΔP_a and I_a.

Eqs. 12 and 14 are relationships describing the phase transition of DOPH and salt concentration under Δn_a, ΔP_a and I_a. A numerical example of self-sustained oscillations is shown in fig. 8. It can be seen that η_m and n_i change periodically so as to produce the oscillation of the membrane potential V, the wave form of which resembles quite well the observed data in fig. 1. The amplitude also agrees with experimental data, usually amounting to one to several hundred millivolts.

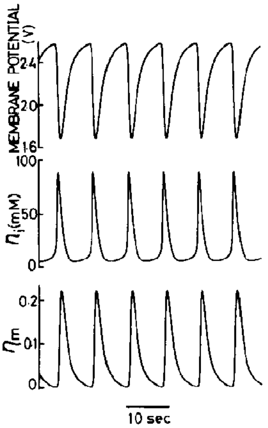

Fig. 8. A numerical example of self-oscillation of membrane potential for $\Delta n_a = 95$ mM, $\Delta P_a = 20$ cmH$_2$O and $I_a = 0.3$ μA.

Fig. 9 gives a schematic illustration of the conformational states of DOPH coupled with salt release and accumulation.

3.2. Results

A numerical result concerning the pH effect is shown in fig. 10. The values of the numerical

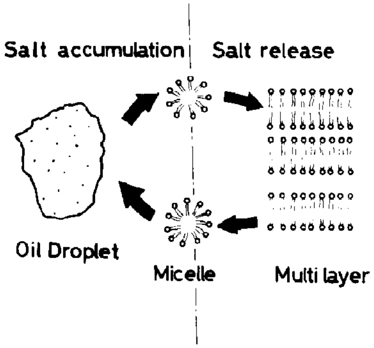

Fig. 9. Schematic illustration of self-oscillation. Repeat of phase transitions among oil droplets, micelles and multilayers is brought about by intimate coupling to salt accumulation and release.

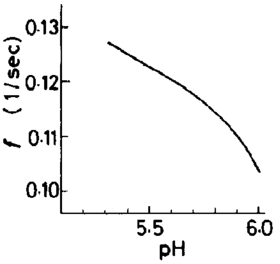

Fig. 10. Theoretical result of pH effect. Conditions as in fig. 8. The self-oscillation stopped at pH 5.325.

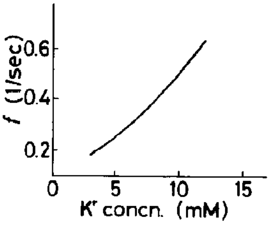

Fig. 11. Theoretical result of KCl effect. The oscillation stopped at 13 mM KCl for $\Delta P_a = 25$ cmH$_2$O and $I_a = 0.6$ μA.

parameters adopted here are mostly due to the previous results [1] and are summarized in table 1. The tendency of the frequency f to decrease for decreasing pH agrees with the observed data in fig. 2. The observed property that too low pH values stop the oscillation is also described. The enhancement of frequency can be understood as being due to the increase in the rate constant k_{-2H} given by eq. 11a: It raises the rate of the phase transition to result in an increase in the repeating rate of phase transition among multilayers, micelles and oil droplets. The too large rate constant k_{-2H}, however, may deviate from the condition adequate for the self-oscillation, since the delicate balance of these kinetic rates related to the phase transition and salt flow is essential for the occurrence of oscillation.

Fig. 11 shows the numerical result of the theoretical model concerned with KCl. The experimental tendency may be explained well by the present theory: The increase in frequency is accompanied by a decrease in the length of the phase-transition region l, as expressed by eq. 1, for decreasing Δn_t and Δn_a. This reduces the number of DOPH molecules participating in the self-oscillation. Rate constants such as k_1 and k_{-1} are decreasing func-

Table 1

Values of numerical parameters adopted in the theory

Symbols as in ref. 1, from which most of the parameter values were chosen. Parameters D_0^* and $\hat{D}_0^*$ are coefficients independent of both the length of the phase-transition region and the conformational states of DOPH [1]: $k_D = D_0^* \exp[-d_1/(1+d_2\eta_m^2)]/(l/L)$, $\hat{D} = \hat{D}_0^* \exp\{-\hat{d}_1/[1+\hat{d}_2(\zeta+\eta_m)^2]\}/(l/L)^2$. The diffusion constant of salt in the phase-transition region amounts to approx. 10^{-8} cm^2/s for the value shown here, which is acceptable if we note the order of 10^{-5} cm^2/s for KCl in the aqueous solution. The value of k_D for expressing diffusion of DOPH is chosen by considering the results obtained on the kinetics of the phase transition [5] with the large adsorbed quantity of DOPH as 3 mg/cm^2 in the experiment for self-oscillation. Kinetic parameters k_1^* are defined by $k_1 = k_1^*/(l/L)$, etc. [1]. The values are based on previous experiments on kinetics [5], showing a slow phase transition amounting to 1 h or more.

Kinetics of DOPH molecules		Kinetics of salt	
D_0^*	10^{-4} (s^{-1})	$\hat{D}_0^*$	2.1×10^{-4} (s^{-1})
d_1	4	$\hat{d}_1$	4
d_2	50	$\hat{d}_2$	50
k_1^*	2.63×10^{-3} (s^{-1})	$L_p/\tilde{D}$	100 (cmH$_2$O^{-1} cm^{-1})
k_{-1}^*	0.526 (s^{-1})	$L_v/\tilde{D}$	1000 (V^{-1} cm^{-1})
k_2/k_{-2H}^0	4.2×10^{-5} (M^{-1})	X_N	324 (mM)
k_2/k_{-2Ca}^0	3.97×10^{-2} (M$^{-1/2}$)	X_A^0	1.79×10^{-2} (mM$^{-1/2}$)
v	100	X_B^0	8.94×10^{-2} (mM$^{-1/2}$)
		γ	3.5
		γ'	3.0
		ζ_c, η_{mc}	0.105

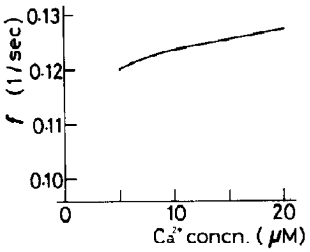

Fig. 12. Theoretical result of $CaCl_2$ effect. Same conditions as in fig. 8, and addition of 20 μM $CaCl_2$ stopped the self-oscillation.

tions of l. Addition of KCl to reduce l, therefore, results in enhancement of the frequency of self-oscillation due to the facilitated phase transition of DOPH.

The effect of $CaCl_2$ is shown in fig. 12, which explains the experimental observation in fig. 4. While K^+ stops the self-oscillation near 30 mM, Ca^{2+} is effective only by 20 μM. This quantitative difference may originate in the strong ability of Ca^{2+} to bind or bridge DOPH molecules, as in other lipid molecules [9]. In fact, addition of Ca^{2+} drastically increases the electric resistance of DOPH-Millipore membranes [13]. The bridging ability of Ca^{2+} is incorporated into the rate constant k_{-2Ca} in eq. 11b for the phase transition.

While the experimental tendencies concerned with pH, KCl and $CaCl_2$ can be explained directly by the theoretical model, description of the effect of sucrose on the self-oscillation may be beyond the present theory. Therefore, we can only suggest a probable mechanism: Sucrose may be considered to interact with the hydrocarbon chains of DOPH molecules. The phase transition of DOPH can be broadened thus becoming obscure because of the weakened cooperativity. Furthermore, the presence of sucrose in the aqueous solution may prevent the smooth diffusion of K^+ and Cl^-. Consequently, the values of the rate constants included in the present theory are expected to decrease on average. We can therefore anticipate that the self-sustained oscillation is retarded on addition of sucrose.

4. Discussion

In the present paper, the effects of chemicals such as KCl, $CaCl_2$, pH and sucrose on the self-oscillation were studied. Theoretical consideration of electrochemical interaction between these and DOPH molecules was shown to give a fairly good explanation of the experiments. Self-sustained oscillations are typical nonequilibrium, nonlinear phenomena: The phase transition of DOPH molecules is intimately coupled with salt flow. A repeat of the phase transition is induced by accumulation and release of salt within the pore. In relation to this fact, the effective charge density [14] within the pore is expected to oscillate synchronously. This point will be discussed in a separate paper [12].

As for the effects of H^+ and Ca^{2+}, we must give an explanation for the parameter values of k^0_{-2H} and k^0_{-2Ca} in eqs. 10. The value of k^0_{-2Ca} is chosen so that k_{-2} may nearly equal that adopted previously [1] when the pH is 6. The ratio k^0_{-2H}/k^0_{-2Ca} is calculated as $1/K_d\sqrt{K_2}$ from eqs. 11; if we adopt 10^{-2} and 10^{-2}–1 as K_d and K_2 [9,15,16], respectively, we obtain the value 10^2–10^3. The parameter k^0_{-2Ca} in fig. 12 is chosen within this range.

The self-sustained oscillations studied here are caused by the phase transition of the lipid analogue, DOPH in connection with salt accumulation and release within the filter pore. As is well known, a phase transition showing macroscopic conformational changes can be induced electrically by ions in other lipids [17,18]. Self-sustained oscillations of membrane potential are therefore expected to occur in systems containing these kinds of lipids. In fact, a recent experiment [19] demonstrates the self-oscillation of the electric potential in a porous membrane doped with glycerol α-monooleate under an Na^+/K^+ concentration gradient. A self-oscillation caused by a phase transition strongly coupled with salt flow can also be observed in biological membranes reconstituted on a *Nitella* protoplasmic droplet [20,21]. We can therefore infer that self-oscillations occur in a wider range of simple model systems or biological systems [22].

The chemicals studied here such as KCl, $CaCl_2$,

H^+ and sucrose may correspond to salt, bitter, sour and sweet, respectively, from the viewpoint of taste sensation. The DOPH-Millipore membrane transforms the change in each concentration to that in the frequency of self-oscillation of the membrane potential. This model membrane can be considered as a prototype of a taste sensor. While the actual use as a sensor requires further improvement of membrane systems, a basic property may rely on the phase transition with nonlinear coupling to salt flow.

References

1 K. Toko, M. Tsukiji, S. Ezaki and K. Yamafuji, Biophys. Chem. 20 (1984) 39.
2 K. Toko, K. Ryu, S. Ezaki and K. Yamafuji, J. Phys. Soc. Jap. 51 (1982) 3398.
3 T. Teorell, J. Gen. Physiol. 42 (1959) 831.
4 K. Kurihara, N. Kamo and Y. Kobatake, Adv. Biophys. 10 (1978) 27.
5 K. Toko, J. Nitta and K. Yamafuji, J. Phys. Soc. Jap. 50 (1981) 1343.
6 N. Kamo, T. Yoshioka, M. Yoshida and T. Sugita, J. Membrane Biol. 12 (1973) 193.
7 Y. Kobatake, Adv. Chem. Phys. 29 (1975) 319.
8 K. Urahama and K. Yamafuji, Trans. IECE Jap. 65 (1982) 185.
9 K. Toko and K. Yamafuji, Biophys. Chem. 14 (1981) 11.
10 K. Toko and K. Yamafuji, J. Theor. Biol. 99 (1982) 461.
11 H. Träuble, M. Teubner, P. Woolley and H. Eibl, Biophys. Chem. 4 (1976) 319.
12 K. Yamafuji and K. Toko, Mem. Fac. Eng. Kyushu Univ. 45 (1985) 179.
13 J. Arisawa and T. Furukawa, Trans. IECE Jap. 62 (1979) 645.
14 Y. Kobatake and N. Kamo, Prog. Polym. Sci. Jap. 5 (1973) 258.
15 K. Toko and K. Yamafuji, Chem. Phys. Lipids 26 (1980) 79.
16 F. Lakhdar-Ghazal, J.L. Tichadou and J.F. Tocanne, Eur. J. Biochem. 134 (1983) 531.
17 V. Luzzati, Nature 218 (1968) 1031.
18 C. Tanford, The hydrophobic effect – Formation of micelles and biological membranes (Wiley, New York, 1973).
19 K. Yoshikawa, K. Sakabe, Y. Matsubara and T. Ohta, Biophys. Chem. 20 (1984) 107.
20 K. Toko, M. Nosaka, M. Tsukiji and K. Yamafuji, Biophys. Chem. 21 (1985) 295.
21 Y. Kobatake, I. Inoue and T. Ueda, Adv. Biophys. 7 (1975) 43.
22 A.M. Monnier, J. Membrane Sci. 2 (1977) 67.