Biophysical Chemistry 29 (1988) 293–299
Elsevier

BPC 01239

Electrical oscillation and fluctuation in phospholipid membranes

Phospholipids can form a channel without protein

K. Yoshikawa [a], T. Fujimoto [b], T. Shimooka [b], H. Terada [b], N. Kumazawa [c] and T. Ishii [d]

[a] *College of General Education, University of Tokushima, Minami-Josanjima, Tokushima 770,*
[b] *Faculty of Pharmaceutical Sciences, University of Tokushima, Shomachi-1, Tokushima 770,*
[c] *Wakayama Medical College, Wakayama 640*
and [d] *School of Dental Medicine, Tsurumi University, Tsurumi, Yokohama 230, Japan*

Received 20 March 1987
Revised manuscript received 5 October 1987
Accepted 19 November 1987

Excitation; Spontaneous oscillation; Langmuir-Blodgett film

Fluctuations and/or step-wise changes in membrane potential and electrical current were observed in bilayer membranes of dioleoylphosphatidylcholine (DOPC) in the absence of any channel protein. The DOPC membranes consisted of three types: black lipid membranes, pipette-clamp membranes and lipid membranes transferred to porous filter paper by conventional Langmuir-Blodgett techniques. This finding is significant since phospholipids are the main constituents of biomembranes. Lipid molecules with a *cis* double bond in their carbon skeleton are suggested to be important in the gating or excitation of biomembranes.

Introduction

Excitability is one of the most important properties of cell membranes. The excitatory phenomenon has been attributed to the gating of 'channel' proteins present in the lipid bilayer membrane. Ion channels are widely believed to be transmembrane proteins that can undergo conformational changes, resulting in the opening and closing of an aqueous pore. There have been many reports of studies on the mechanism of the gating process in channel proteins by reconstitution of channel proteins into bilayers [1–4]. In the last 20 years black lipid membranes (BLM) have been used most frequently as models of biomembranes [5–7], but recently, studies using the gigaohm-seal 'pipette-clamp' or 'patch-clamp' method have increased in number [8–11]. Implicit in most of these studies have been the assumptions that the lipid bilayer serves as an essentially inert matrix of high resistance and that the channels observed occur within the pore-forming protein that is present in the bilayer. However, there is also recent evidence suggestive of the direct involvement of lipid molecules in membrane permeability [12,13]; namely, ion channels or current fluctuations were observed at the phase transition temperatures, in bilayer membranes composed entirely of synthetic saturated phospholipid without a channel protein [14–16].

The present article reports fluctuations and/or pulsing of the membrane potential and electrical current in bilayer membranes composed entirely of dioleoylphosphatidylcholine. The thin films of

Correspondence address: K. Yoshikawa, College of General Education, University of Tokushima, Minami-Josanjima, Tokushima 770, Japan.

phospholipid were of three different types; i.e., black lipid membranes (BLM), pipette-clamp membranes and lipid membranes supported on porous filter paper, obtained by conventional Langmuir-Blodgett techniques (LB films) [17–20].

2. Materials and methods

2.1. Materials

Synthetic L-α-dioleoylphosphatidylcholine (DOPC) was obtained from Avanti Polar Lipids. Essentially the same results as regards electrical oscillations have been observed for DOPC from Nihonseika (Tokyo). All other reagents were of analytical grade. *n*-Decane was distilled and passed through a column of activated alumina before use. Fresh twice-distilled water was used.

2.2. Formation of a BLM

A solution of 10 mg DOPC/5 mg cholesterol/1 ml *n*-decane was used to form a planar bilayer membrane (BLM) across a hole of about 1 mm diameter in the wall of a Teflon cell. Cholesterol and *n*-decane were used in order to form a mechanically stable membrane [7]. The aqueous medium was 0.5 M KCl solution without buffer ions. The electrical conductivity of the BLM was measured at 20 °C, as described previously [21–23]. The actual area of the black film was measured in each experiment with a microscope equipped with a TV camera.

2.3. Formation of a pipette-clamp membrane

Experiments with bilayer membranes of DOPC on pipettes were carried out by using the method of Coronado and Latorre [10,11]. The pipettes were made by the two-pull method without heat polishing from glass capillaries (2 mm diameter, Narishige, Tokyo). The tip diameters were 2–3 μm, the electrical resistance of the pipettes being 5–8 MΩ. A pipette and petri dish (50 mm diameter) were filled with aqueous 0.5 M KCl. First, the tip of the pipette was immersed in the solution in the dish. Then, a DOPC monolayer was spread within a ring of silk fiber floating on the air/water interface by the introduction of approx. 5 μl of a chloroform solution of DOPC (0.2 mg/ml) onto the surface of the solution. The monolayer surrounded by the silk fiber was maintained at a constant surface pressure of 0.03 N/m by application of oleic acid as a piston oil [17,18] to the surface outside the silk fiber. A bilayer was formed by raising and lowering the pipette. Electrical measurements were carried out in the same petri dish. A potential gradient of 0–100 mV was applied across the membrane and the resulting current was monitored at 20 °C with a digital picoammeter (model AM271-A, TOA Electric, Tokyo) connected with a recorder. The electrical resistance of the bilayer was 2–6 GΩ.

2.4. Formation of a membrane by the LB method

A monomolecular layer of DOPC was deposited on a porous membrane, triacetylcellulose filter paper (FM-22) of 0.22 μm nominal pore size and 135 μm thickness (Fuji Photo Film, Tokyo), by the Langmuir-Blodgett technique [17,18], as described previously [24,25]. A stable membrane with a pore size of more than 0.5 μm could not be formed. FM-22 has the smallest pore size of the films commercially available from Fuji. Measurement of the surface pressure characteristics confirmed that DOPC forms a very stable monomolecular layer on the aqueous solution and that its collapsing pressure is about 0.050 N/m. DOPC molecules dissolved in chloroform were spread on the air/water interface of a Langmuir trough. The porous filter paper was inserted vertically into the aqueous subphase, distilled water (pH 6.3), through the monolayer with a surface pressure of 0.020 N/m, and then pulled out at an appropriate rate (approx. 1 cm/min). LB films were deposited on the porous membrane three times via this procedure. The build-up ratio was found to be 1.0 except in the first deposition, where a Z-film with a build-up ratio of approx. 1.5 was deposited.

The apparatus for the electrical measurement was the same as that reported previously [24,25]. The temperature of the aqueous solutions was 20 °C, the resistance of the membrane 5–50 GΩ and the total membrane area 0.2 cm^2. A scanning

electron micrograph showed that the total fraction of the pore area was 40%. The total pore area was thus calculated to be 0.08 cm^2. The resistance of the lipid membrane on the pore was thus 0.4–4 $G\Omega$ cm^2, corresponding to the known resistance of stable BLM [7]. When the LB membrane was broken, the resistance decreased abruptly, of the order of 10^{-5} fold. This suggests that the LB film was formed on the surface of the filter paper and that lipid molecules did not penetrate into the pores to form micro-films [26,27]. It is noteworthy that the pores in FM-22 are not cylindrical but rather conical; i.e., their average diameter is 0.22 μm on one side and approx. 2 μm on the other. Thus, it is supposed that the phospholipid film is stable only on the side with the smaller pore size and that the pores on the opposite side may not be completely covered with the lipid film. Details of the structure of the LB film will be reported in a separate article.

3. Results

Fig. 1 shows the time course of changes in electrical current through the BLM of DOPC under voltage-clamp conditions. At 10 mV the magnitude of the fluctuation was of the order of 1 pA. With increase in the potential, the fluction increased gradually, and at 50 mV became approx. 6

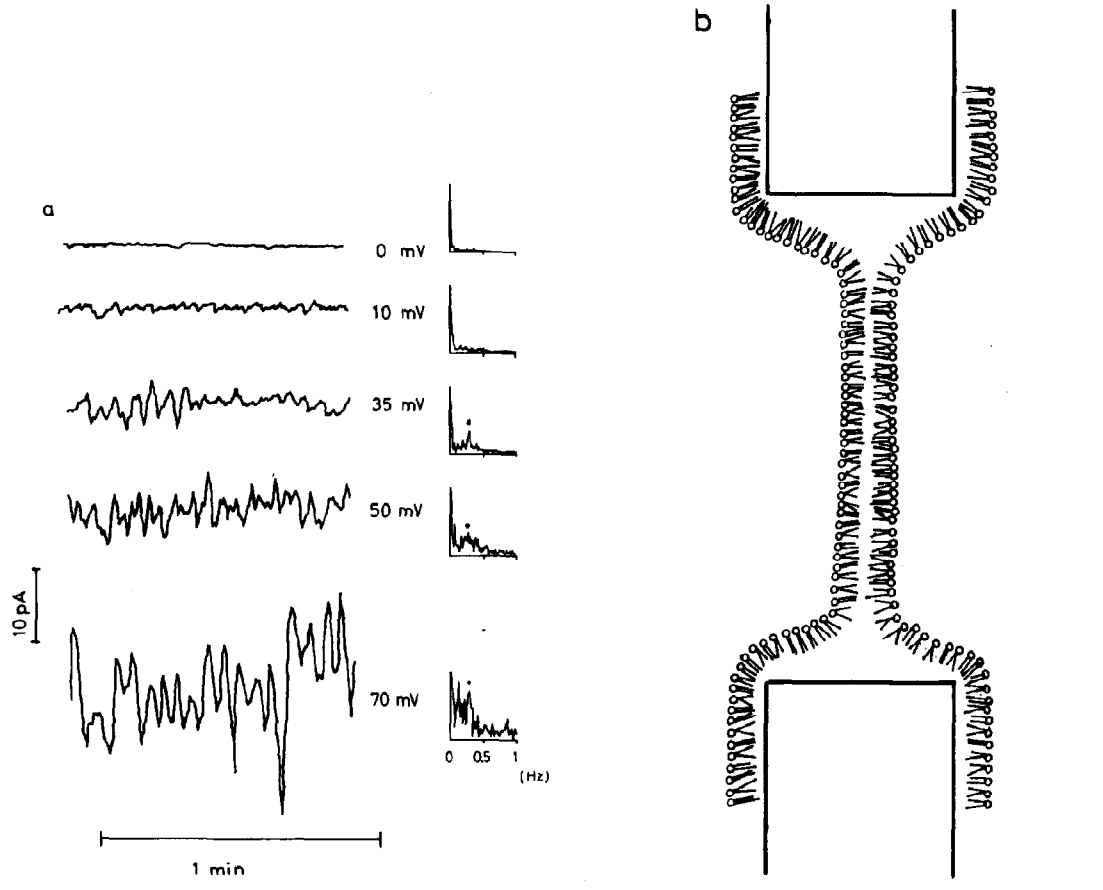

Fig. 1. (a) Fluctuation of the electrical current across a BLM under voltage-clamp conditions, where upward change indicates increase in current. Aqueous phases, 0.5 KCl. Right-hand side; power spectra of the electrical fluctuations. (b) Schematic representation of the lipid bilayer in a BLM.

pA, which corresponds to a fluctuation in conductivity of about 120 pS. This result suggests that the permeability of the membrane to ions through the membrane fluctuates with time and that a simple linear relationship between the electric current and voltage (Ohm's law) does not hold in this membrane. In other words, 'gating' of pores in the membrane occurs due to the application of an external electrical potential. On the right-hand side of fig. 1a, the Fourier-transformed power spectra of the time fluctuation are shown. It is interesting that the peak around 0.3 Hz increases with increase in applied voltage, indicating that the rate of the gating process is most frequent at about 0.3 Hz.

A similar experimental trend was observed with a bilayer membrane clamped on a pipette, as shown in fig. 2. With increasing external voltage, fluctuation of the current tended to increase, and at 50 mV, the magnitude of the fluctuation in current was 0.5–3 pA, corresponding to a change in conductivity of 10–60 pS. Similar values for the fluctuation in membrane conductance have been reported as the single-channel conductance for 'reconstituted' lipid bilayer membranes with incorporated channel protein [10,11,28–33]. For example, Hartshorne et al. [32] reported a fluctuation of 25 pS in a planar lipid membrane into which sodium channels from rat brain had been incorporated, and Suarez-Isla et al. [33] observed a single-channel conductance of 40 ± 5 pS in a membrane containing acetylcholine receptors clamped on the tip of a patch pipette. The Fourier-transformed power spectra of the electrical fluctuation are given on the right-hand side in fig. 2a. Here again the peak near 0.3 Hz increases with increase in applied voltage, similarly to the results shown in fig. 1.

Fig. 3a and b shows the time course of changes in electrical current through the LB membrane

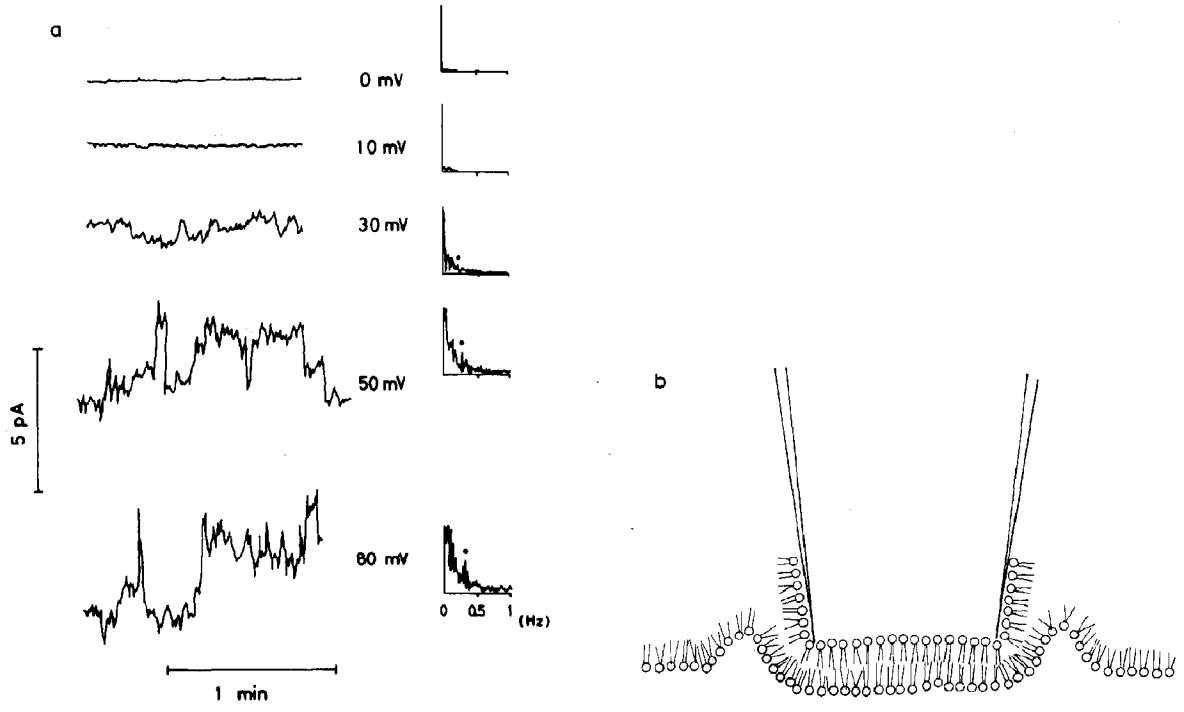

Fig. 2. (a) Fluctuation of the electrical current across a pipette-clamp membrane under voltage-clamp conditions, where upward change indicates increase in current. Aqueous phases, 0.5 M KCl. Right-hand side: power spectra of the electrical fluctuations. (b) Schematic representation of the lipid bilayer in a pipette-clamp membrane.

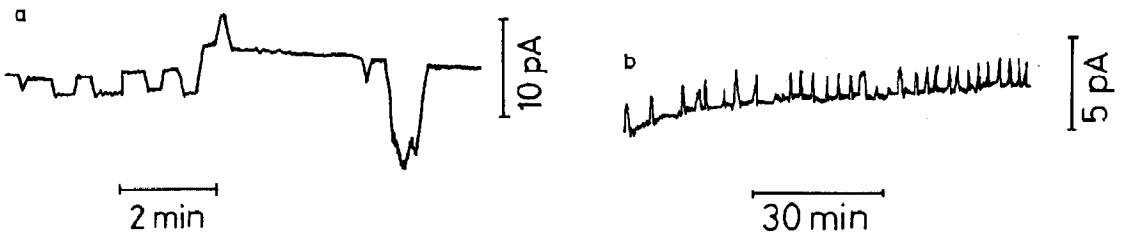

Fig. 3. Fluctuation of the electrical current across an LB membrane under voltage-clamp conditions at 20 mV, bathed in (a) 0.5 M and (b) 1 M KCl solutions where upward change indicates increase in current.

under voltage-clamp conditions at 20 mV in 0.5 and 1 M KCl solutions, respectively. These traces clearly show the periodic gating of the current of 1–2 pA, indicating a conductance change of 50–100 pS. The rate of gating in the LB film is about one order of magnitude smaller than those in the BLM and pipette-clamp membrane. The large difference in gating speed in the LB film may be attributed to the fact that the LB film used in this study in a multilayer, whereas the BLM and pipette-clamp membranes are bilayers.

The above-mentioned results with three different DOPC membranes clearly indicate that the permeability of the membrane to ions changes periodically or fluctuates in the absence of any channel protein. We also found that scarcely any electrical oscillation or fluctuation is observed in the membranes when the concentration of NaCl or KCl is below 0.3 M.

We have measured the electrical fluctuation for the bilayer membranes, BLM and pipette-clamp membrane with saturated phospholipids such as dipalmitoyllecithin and also with soybean lecithin. However, the magnitude of the electrical fluctuations was found to be two orders of magnitude lower than that with the unsaturated lipid, dioleoyllecithin.

4. Discussion

Recently we showed that sustained rhythmic oscillations of electrical potential occurred spontaneously across fine-pore membranes doped with various lipids containing an oleyl chain, viz., glycerol α-monooleate [34], glycerol trioleate [35], or sorbitan monooleate (Span 80) [36], separating equimolar solutions of NaCl and KCl; these oscillations occurred in the absence of any external stimulus such as voltage, electrical current, hydrostatic pressure or osmotic pressure. We found [34–36] that the presence of a *cis* double bond on the hydrophobic moiety, as is in the oleyl chain, is essential for gating or pulsing in these membranes, and that the functional groups in the lipids determine the selectivity for inorganic ions and the sign of the membrane potential. We also observed that a DOPC film deposited on porous paper can exhibit periodic changes in potential and conductance [24,25]. The motive force for the oscillatory phenomenon arises from the difference in chemical potentials between the inorganic ions in the two aqueous solutions: 0.5 M NaCl and 0.5 M KCl were used in the right and left phases, respectively [25,34–36]. Measurement of the current-voltage characteristics indicated that the conductance of an LB membrane of DOPC showed pulse-like changes between two states, approx. 500 and 50 pS, when a voltage of 100 mV was applied across the membrane [24]. From this experiment together with the results in fig. 3, the gating process across the LB membrane may be due to the repeated opening and recovery in a defect within the phospholipid membrane on a pore of the filter paper as illustrated in fig. 4.

In this study we have examined the electrical properties of a thin film of DOPC, and found that an oscillatory change or fluctuation is also generated in these membranes. This result seems especially significant because phospholipids with an unsaturated alkyl chain, including DOPC, are the main constituents of biomembranes. Lipids with a

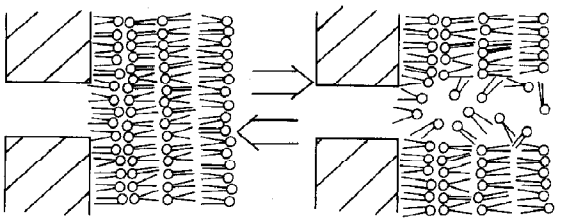

Fig. 4. Schematic representation of the gating process on the LB membrane of DOPC.

cis double bond in the alkyl chain exhibit looser packing than those with a saturated chain. It is thus expected that a kind of defect or dislocation in the assembly of lipid molecules can be formed across a bilayer membrane containing unsaturated lipids. When a perturbation is applied to a membrane across which a difference in chemical potential or external electric field exists, the defect or dislocation is forced to form a pore or ion channel and the pore is closed with recovery of the bilayer structure, as phospholipids can assemble by themselves. We have also examined the effect of temperature, increasing from 20 to 30 °C, on the fluctuation for BLM and found that neither the magnitude nor the rate changes markedly. This result suggests that the fluctuation is not due to the macroscopic phase transition of DOPC molecules. In relation to the above, it should be noted that the phase transition temperature of DOPC was reported as −22 °C [47]. From the foregoing text, the gating of DOPC membrane is most probably due to the ion leakage through a defect in the membrane.

Electrical oscillation or fluctuation was observed only at high concentrations of NaCl and KCl, suggesting the importance of the interaction of Na^+ and K^+ with phospholipid molecules. Concerning this point, it is noteworthy that rather high (0.5–1 M) concentrations of NaCl or KCl have frequently been used in experiments on reconstituted membranes containing channel proteins [28–33,37]. Bilayer lipid membranes have been found to become mechanically unstable and show increased conductance at high salt concentrations [38]. Therefore, it may be possible that gating phenomena are due to time-dependent changes in the state of lipid molecules at high salt concentrations, at least in some experiments on reconstituted membranes.

Antonov et al. [15,16] reported that in planar lipid bilayer membranes (BLM) of synthetic distearoylphosphorylcholine (DSPC), current fluctuations occurred at the phase transition temperature, 59 °C. The appearance of ion channels has been suggested to result from lipid domain interactions. Similar results have been obtained by Boheim et al. [14]. Von Klizing et al. [39] also reported pulsing phenomena in a lecithin BLM although they did not provide experimental details. Furthermore, Sokabe [40] showed that lysotriphosphoinositide can form channels through BLM without protein, and Yafuso et al. [41] found that oxidized cholesterol BLM exhibits multilevel conduction states in the absence of any protein. These studies together with our results [24,25] indicate that lipid molecules may form channels in biomembranes. As lipids are the major components of biomembranes, it is important to investigate their role in the function of channels in biomembranes, since channel proteins are quite hydrophobic and bind lipids [42]. It is possible that channel proteins work cooperatively with surrounding unsaturated lipid molecules in the actual biomembranes.

The above-mentioned function of unsaturated lipids provided the basis for a recent metamorphic model of biomembranes [43]. In this model lipids represent not only relatively inert 'building blocks', as in the widely accepted liquid mosaic model [44], but also play an active part in various functions of membranes. Conceivably, at least some of the gating processes in reconstituted membranes containing so-called channel proteins that have been reported are attributable to the effects of unsaturated lipids, and not to proteins. Unsaturated lipids may constitute a dynamic channel for ions.

We therefore wish to stress the importance of studying unsaturated lipids in relation to the function of biomembranes. The present idea does not contradict the basis of the phenomenological Hodgkin-Huxley model [45,46], but is an attempt to explain the molecular events that induce gating of the membrane.

Acknowledgments

This work was partly supported by a Grant-in-Aid for Scientific Research to K.Y. from the Ministry of Education, Science and Culture of Japan. We thank Mr. T. Omochi for technical assistance.

References

1 P. Mueller and D.O. Rudin, Nature 213 (1967) 603.
2 P. Mueller and D.O. Rudin, Nature 217 (1968) 713.
3 M. Parisi, T.A. Reader and E. De Robertis, J. Gen. Physiol. 60 (1972) 454.
4 V.A. Parsegian, Biophys. J. 45 (1984) 1.
5 P. Mueller, D.O. Rudin, H.T. Tien and W.C. Wescott, Nature 194 (1962) 979.
6 T. Hanai, D.A. Haydon and J. Taylor, Proc. R. Soc. A281 (1964) 377.
7 H.T. Tien, Bilayer lipid membranes. Theory and practice (Marcel Dekker, New York, 1974) p. 1.
8 E. Neher and B. Sakmann, Nature 260 (1976) 799.
9 D.W. Tank, C. Miller and W.W. Webb, Proc. Natl. Acad. Sci. U.S.A. 79 (1982) 7749.
10 R. Coronado and R. Latorre, Nature 298 (1982) 849.
11 R. Coronado and R. Latorre, Biophys. J. 43 (1983) 231.
12 D. Papahadjopoulos, Biochim. Biophys. Acta 241 (1971) 254.
13 A.A. Lev in: Membrane transport processes, vol. 2, eds. D.C. Tosteson, Yu.A. Ovchinnikov and R. Latorre (Raven Press, New York, 1978) p. 269.
14 G. Boheim, W. Hanke and H. Eibl, Proc. Natl. Acad. Sci. U.S.A. 77 (1980) 3403.
15 V.F. Antonov, V.V. Petrov, A.A. Molnar, D.A. Predvoditelen and A.S. Ivanov, Nature 283 (1980) 585.
16 V.F. Antonov, E.V. Shevchenko, E.T. Kozhomkulov, A.A. Mol'nar and E.Yu. Smirnova, Biochem. Biophys. Res. Commun. 133 (1985) 1098.
17 K.B. Blodgett and I. Langmuir, Phys. Rev. 51 (1937) 964.
18 Y.K. Levine, A.I. Bailey and M.H.F. Wilkins, Nature 220 (1968) 577.
19 M. Montal and P. Mueller, Proc. Natl. Acad. Sci. U.S.A. 69 (1972) 3561.
20 M. Seul, P. Eisenberger and H.M. McConnell, Proc. Natl. Acad. Sci. U.S.A. 80 (1983) 5795.
21 H. Terada, H. Nagamune, Y. Osaki and K. Yoshikawa, Biochim. Biophys. Acta 646 (1981) 488.
22 H. Terada, N. Kumazawa, M. Ju-ichi and K. Yoshikawa, Biochim. Biophys. Acta 767 (1984) 192.
23 N. Takeguchi, T. Saitoh, M. Morii, K. Yoshikawa and H. Terada, J. Biol. Chem. 260 (1985) 9158.
24 K. Yoshikawa, T. Omochi, T. Ishii, Y. Kuroda and K. Iriyama, Biochem. Biophys. Res. Commun. 133 (1985) 740.
25 T. Ishii, Y. Kuroda, T. Omochi and K. Yoshikawa, Langmuir 2 (1986) 319.
26 J.M. Mountz and H.T. Tien, Photochem. Photobiol, 28 (1978) 395.
27 M. Thompson, R.B. Lennox and R.A. McClelland, Anal. Chem. 54 (1982) 76.
28 G. Boheim, W. Hanke, F.J. Barrantes, H. Eibl, B. Sakmann, G. Fels and A. Maelicke, Proc. Natl. Acad. Sci. U.S.A. 78 (1981) 3586.
29 B.K. Krueger, J.F. Worley, III and R.J. French, Nature 303 (1983) 172.
30 K. Kaufmann and I. Silman, Biophys. Chem. 18 (1983) 89.
31 J.O. Bullock and F.S. Cohen, Biochim. Biophys. Acta 856 (1986) 101.
32 R.P. Hartshorne, B.U. Keller, J.A. Talvenheimo, W.A. Catterall and M. Montal, Proc. Natl. Acad. Sci. U.S.A. 82 (1985) 240.
33 B.A. Suarez-Isla, K. Wan, J. Lindstrom and M. Montal, Biochemistry 22 (1983) 2319.
34 K. Yoshikawa, K. Sakabe, Y. Matsubara and T. Ota, Biophys. Chem. 20 (1984) 107.
35 T. Ishii, Y. Kuroda, K. Yoshikawa, K. Sakabe, Y. Matsubara and K. Iriyama, Biochem. Biophys. Res. Commun. 123 (1984) 792.
36 K. Yoshikawa, K. Sakabe, Y. Matsubara and T. Ota, Biophys. Chem. 21 (1985) 33.
37 K. Kaufmann and I. Silman, Naturwissenschaften 67 (1980) 608.
38 S. Noguchi and S. Koga, J. Gen. Appl. Microbiol. 15 (1969) 41.
39 L. von Klitzing, M. Daber and H.D. Bergeder, Biophysik 9 (1973) 166.
40 M. Sokabe, in: Transmembrane signaling and sensation, eds. F. Oosawa, T. Yoshioka and H. Hayashi (Jap. Sci. Soc. Press, Tokyo, 1984) p. 119.
41 M. Yafuso, S.J. Kennedy and A.R. Freeman, J. Membrane Biol. 17 (1974) 201.
42 J.-L. Popot and J.-P. Changeux, Physiol. Rev. 64 (1984) 1162.
43 P.R. Cullis, B. De. Kruijff, M.J. Hope, R. Nayar and S.L. Schmid, Can. J. Biochem. 58 (1980) 1091.
44 S.J. Singer and G.L. Nicolson, Science 175 (1972) 720.
45 A.L. Hodgkin and A.F. Huxley, J. Physiol. 116 (1952) 449, 473, 497.
46 A.L. Hodgkin and A.F. Huxley, J. Physiol. 117 (1952) 500.
47 C. Tanford, The hydrophobic effect. Formation of micelles biological membranes, 2nd edn. (Wiley, New York, 1980) p. 132.