Biochimica et Biophysica Acta, 1068 (1991) 245–248
© 1991 Elsevier Science Publishers B.V. All rights reserved 0005-2736/91/$03.50
ADONIS 000527369100352S

BBAMEM 75367

Electromechanical stability of planar lipid membranes from bipolar lipids of the thermoacidophilic archebacterium *Sulfolobus acidocaldarius*

G.B. Melikyan [1], N.S. Matinyan [1], S.L. Kocharov [2], V.B. Arakelian [1],
D.A. Prangishvili [3], K.G. Nadareishvili [3]

[1] *Yerevan Physics Institute, Yerevan, Armenia (U.S.S.R.),* [2] *Research and Engineering Technology Institute of Amino Acids, Yerevan, Armenia (U.S.S.R.) and* [3] *Institute of Molecular Biology and Biological Physics of Georgian, Academic Science, Tbilisi, Georgia (U.S.S.R.)*

(Received 28 January 1991)
(Revised manuscript received 22 May 1991)

Key words: Bipolar lipid; Planar lipid membrane; Electrical breakdown

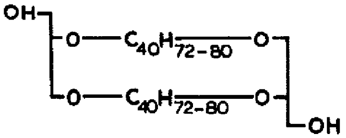
Stable planar membranes have been obtained from the bipolar lipid glycerol dialkyl nonitol tetraether (GDNT) extracted from the thermoacidophilic archebacterium *Sulfolobus acidocaldarius*. The electric capacity C_m, the resistance R_m and tension σ of these membranes were measured. The dependence of the bipolar lipid membranes mean life time τ_l on voltage was investigated. It was shown that the irreversible electric breakdown of membranes from GDNT and usual phospholipids is due to the same mechanism, viz., due to formation of a hydrophilic pore with an overcritical radius. Under electric field the GDNT molecules take U-shape, and the polar headgroups of such molecules cover the pore's interior.

Introduction

Archebacteria are distinguished by an ability to thrive under extreme conditions: high temperature (80–90 °C), low pH (about 2) and in saturated salt solutions [1]. Obviously, under these conditions the cell membrane must have a special construction which protects the cell from a fatal environment. Indeed, unique bipolar lipids were found [2,3] in the membranes of the thermoacidophilic archebacterium *S. solfataricus*.

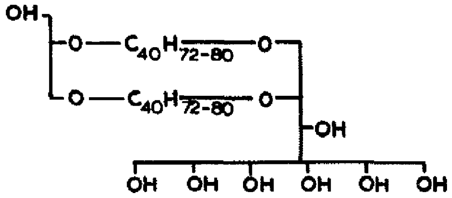
The lipid molecules of thermoacidophils consist of two biphytanyl residues, with 0 to 4 cyclopentanes per chain, ether-linked at both ends to glycerol and nonitol groups. The lipids studied are all derived from two compounds:

(1) glycerol dialkyl glycerol tetraether (GDGT) (see Formula 1)



Formula 1.

(2) glycerol dialkyl nonitol tetraether (GDNT) (see Formula 2)



Formula 2.

where $(-C_{40}H_{72-80}-)$ represents a biphytanyl chain.

Gliozzi et al. [4–6] obtained black planar membranes from the asymmetric lipid GDNT extracted from *S. solfataricus* and investigated their physical and chemical properties. The authors have obtained some indirect evidences for a monolayer organization of such planar membranes (Fig. 1a). They have shown that the thickness of the hydrophobic part of such membranes is virtually independent of the organic solvent present in the membrane-forming mixture [5]. The weak dependence of the capacity of the membranes, formed from GDNT on the voltage applied is also in agreement with their monolayer organization [5]. It was shown [4] that when a constant potential was applied to the membranes, there might arise an asymmetry in the distribution of glycerol and nonitol polar headgroups on the different sides of the membrane [6]. Thus, one

---

Correspondence (present address): G.B. Melikyan, Department of Physiology, Rush Medical College, 1653 West Congress Parkway, Chicago, IL 60612, U.S.A.

can assume that membranes formed from GDNT are monolayers of bipolar lipids. The absence of a fracture plane over the hydrophobic part of membranes in cryofractography [7,8], and the experiments on chemical labeling of lipids in vivo [9] indicate to a monolayer organization of the cell membrane of thermoacidophils.

It can be a priori assumed that membranes formed from such unusual lipids must have properties essentially different from those of phospholipid bilayers. One of such properties, in our opinion, can be their electromechanical stability. The electric breakdown of membranes is due to formation of a hydrophilic pore (the polar headgroups of lipids cover the pore interior) with a overcritical radius [10]. Apparently, formation of these pores supposes joining of the two monolayers of a bilayer through the pore's surface. It may turn out that the breakdown of a lipid monolayer is due to quite a different mechanism.

In the present work we have investigated the properties of membranes formed from GDNT, extracted from *S. acidocaldarius.*

Materials and Methods

Lipids were extracted from the archebacteria *S. acidocaldarius* by the method described in Refs. 11 and 12. The lyophilized biomass was extracted by 2:1 and 1:1 chloroform/methanol mixtures. The lipid extract was treated by mixture of acetyl chloride with dehydrated methanol and was boiled for 6 h in reflux at $t \equiv 65\,^\circ$C. The substance obtained was chromatographed on a Silica-gel (KSK 100–150 mesh) column (40 × 10 mm) and was eluted by chloroform/dehydrated ether (92:2 and 95:5) and chloroform/methanol (9:1) mixtures. The chloroform/methanol mixture eluted lipids mainly consisting of GDNT (55%). The method of preparative thin-layer chromatography (TLC) was used to separate different lipid components. The substances obtained had been checked on TLC microplates. Shiff's reagent was used as the main detector of bipolal lipids. The obtained substance was chromatographically homogeneous.

The planar membranes were obtained by the usual method [13] from GDNT in squalene/chloroform (2:1) and decane/chloroform (2:1) solutions with concentration 25 mg/ml on a hole ($\varnothing \approx 1$ mm) in the partition of a two-chamber teflon cell in the temperature range from 45 to 80 °C. The experiments were conducted in a 0.1 M non-buffered KCl solution (pH 6.0). The area of membranes was determined by a graduated binocular microscope. Electrical measurements were carried out by Ag-AgCl electrodes inserted directly into the solutions on both sides of the membrane.

The capacity was measured by the technique described in Refs. 14 and 15.

The boundary potential difference of the membranes was measured by the potentiodynamic method [16,17].

The method of measuring the membrane life time as a function of the voltage was described in Ref. 10.

The membrane tension σ was determined in terms of the change of their capacity (i.e. area) when a transmembrane hydrostatic pressure difference, ΔP, was created. σ was calculated according to Eqn. 1.

$$\sigma = \frac{\Delta P \cdot r_m \cdot I}{8 \cdot I_0 \cdot \sqrt{I/I_0 - 1}} \qquad (1)$$

where r_m is the radius of membrane, I_0 is the initial value of capacitive current, I is the capacitive current at the pressure difference ΔP [17].

Results

We have obtained quite stable planar membranes formed from solutions of the native mixture of GDNT (not separated over the degree of cyclization of biphy-

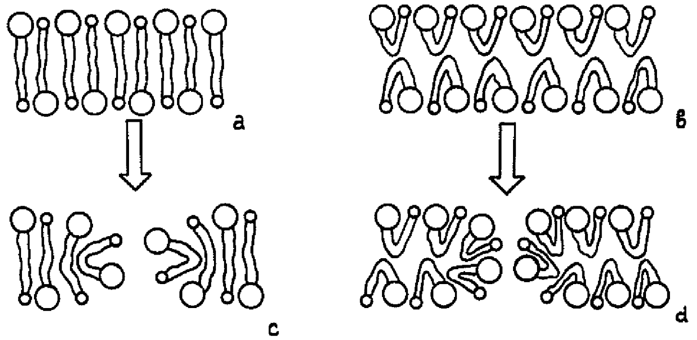


Fig. 1. Schematic diagram of bipolar lipids packing in membrane: (a) bilayer model; (b) monolayer model; (c) and (d) hydrophilic pore formation at electric breakdown in case of bilayer and monolayer packing of lipids, respectively. Large circles denote nonitol polar headgroups and the little circles glycerols.

tanyl chains) in squalene/chloroform (2:1) mixtures in the temperature range from 40 to 80 °C. In contrast to Gliozzi et al. [4–6], we could not form membranes from GDNT in squalene because of the extremely low solubility of that lipid. Black film formation time was several seconds even at relatively low temperatures, about 45 °C. During blackening the membranes were unstable and often ruptured, but after the black film was formed, they became quite stable. Their life time usually was more than two hours. Membranes formed from GDNT in decane/chloroform mixtures were less stable.

The resistance of the membranes formed from GDNT in squalene/chloroform mixtures at 46 °C was $T_m = (1\text{–}3)\cdot 10^7$ Ω cm^2, the specific capacity $C_m = 7.0 \pm 0.6$ μF/m^2, which agrees to that of the membranes formed from GDNT in decane/chloroform (7.0 ± 0.1 μF/m^2). Thus, the latter parameter is practically independent of the solvent's nature.

According to the potentiodynamic capacity curves, the dependence of capacity on voltage is equally weak for the membranes in both decane and squalene at $t = 46\text{–}70$ °C. That is why it is difficult to determine the difference of the boundary potentials, $\Delta\phi$, by the potentiodynamic methods [16]. The tension of membranes formed from GDNT was $\sigma = 4.3 \pm 0.4$ dyne/cm.

Note that in contrast to usual membranes, these films withstand a pH gradient of 3.5–4 units during more than one hour.

TABLE I

Some physico-chemical properties of lipid membranes

Lipid composition of membranes	Specific capacity C_m (μF/m^2)	Tension σ(mN/m)	Line tension of pore γ(10^{-11} N)
GDNT/squalene +chloroform	7.0 ± 0.6	4.3 ± 0.4	1.25
Phosphatidylcholine/decane	3.2 ± 0.2	1.0 ± 0.3	0.86
Phosphatidylethanolamine/squalene	6.6 ± 0.3	2.4 ± 0.2	1.66
Asolectin/squalene	7.8 ± 0.4	2.2 ± 0.2	0.9

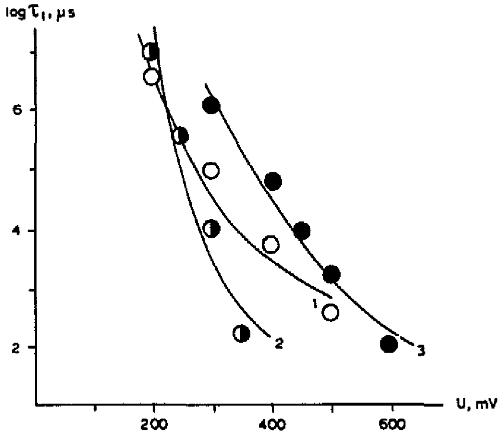


Fig. 2. The mean life time, τ_l of the bilayers as a function of initial voltage, U. ○, membranes formed from GDNT in squalene/chloroform mixture. ◑, membranes formed from phosphatidylcholine in decane. ●, membranes formed from phosphatidylethanolamine in decane. The theoretical curves 1–3 are plotted according to the Eqn. 2.

The mean life time of membranes formed from GDNT in the squalene/chloroform mixtures as a function of voltage is shown in Fig. 2 (curve 1). Note, that this curve is similar to those of the phospholipid bilayers (curves 2 and 3). All of the three theoretical curves in Fig. 2 are plotted according to Eqn. 2 [10]

$$\bar{\tau}_l = A\cdot\exp\left\{\frac{\pi\gamma^2}{KT\left[\sigma + C_m(\epsilon_s/\epsilon_m - 1)\frac{U^2}{2}\right]}\right\} \tag{2}$$

where A is a pre-exponential multiplier, γ is the pore edge line tension, $\epsilon_s = 80$ and $\epsilon_m = 2$ are the dielectric permeabilities of the water solution and the hydrocarbon part of the membrane, respectively; other notations have their usual meaning. Fitting the theoretical curves to experimental points in Fig. 2, one can determine the A and γ (Table I). For the membranes formed from GDNT $\gamma = 1.25 \cdot 10^{-11}$ N. This is the intermediate value between those for phosphatidylcholine and phosphatidylethanolamine bilayers (see Table I) [18].

Discussion

The obtained data on the physical and chemical properties of membranes formed from GDNT in the squalene/chloroform mixture agree the results of Gliozzi et al. [5] for $t = 46$ °C: $R_m = (1\text{–}3)\cdot 10^7$ Ω cm^2, $C_m = 7.7 \pm 0.6$ μF/m^2. In our experiments the weak dependence of specific capacity of the membranes under investigation on the nature of the solvent used was also shown. Like the data of the authors of [4,5], the dependence of the capacity of the membranes formed from GDNT in the squalene/chloroform mixtures on voltage was close to those of usual solvent-free bilayers [17] (no data presented). This complies with the assumption that the solvent is effectively excluded from the bipolar membranes due to the special structure.

The experiments on determination of the electromechanical stability of planar membranes formed from GDNT yielded an unexpected result in terms of the monolayer model. It turned out that the dependence of the mean life time, τ_1, on voltage coincided with those for usual phospholipid bilayers. Moreover, γ, which characterizes the pore edge energy, lies between the values obtained for phospholipid bilayers and is equal to $1.25 \cdot 10^{-11}$ N [17,18]. One can suppose that the irreversible electric breakdown of membranes formed from GDNT (as for usual bilayers) is due to the same mechanism, i.e. due to formation of a hydrophilic pore with an overcritical radius [10]. In terms of the monolayer model (Fig. 1a) the obtained results can be interpreted as follows. Suppose a GDNT molecule can bend, i.e. take U-shape. When applying a definite voltage to the membrane, a hydrophilic pore covered by the bent (U-shaped) molecules is formed. As the polar headgroup of the bipolar lipid molecule is rather large, ($d \cong 10.7$ Å), one may assume that the bending of one molecule is enough to form a unit length of the pore edge. In this case $\gamma \simeq \Delta E/d$. If the pore is covered by not one, but n U-shaped molecules, then the pore edge creation energy increases by a factor of n. It can be shown that $n = h/d$, where h is the membrane thickness. It is easy to estimate the line tension of such a pore, proceeding from the fact, that to bend one molecule two trans-gauche transitions are necessary in each biphytanyl chain (one *trans-gauche* transition brings to a chain bending by 30°). If the *trans-gauche* energy is taken $\Delta E = 0.6$ kcal/mol [19] and $n = 1$, then one can calculate the value of γ equal to $\Delta E/d$. The obtained value, $1.57 \cdot 10^{-11}$ N, is close to the experimental one ($1.25 \cdot 10^{-11}$ N). In our opinion this fact tells in favour of the proposed mechanism of breakdown of monolayer membranes formed from GDNT.

References

1 Langworthy, T.A. (1985) Bacteria 8, 459–497.

2 Langworthy, T.A., Smith, P.F. and Mayberry, W.R. (1974) J. Bacteriol. 119, 106–116.

3 De Rosa, M., Gambacorta, A., Millonig, G. and Bu'Lock, J.D. (1974), 30, 866–868.

4 Gliozzi, A., Rolandi, R., De Rosa, M. and Gambacorta, A. (1982) Biophys. J. 37, 563–566.

5 Gliozzi, A., Paoli, G., Rolandi, R., De Rosa, M. and Gambacorta, A. (1982) Bioelectrochem. Bioenerg. 9, 591–601.

6 Gliozzi, A., Rolandi, R., De Rosa, M. and Gambacorta, A. (1983) J. Membr. Biol. 75, 45–56.

7 Gliozzi, A., Rolandi, R., De Rosa, M., Gambacorta, A. and Nicolaus, B. (1982) Transport in Biomembranes: Model Systems and Reconstitution (Antolini, R., et al., eds.), pp. 39–47, Raven Press, New York.

8 Langworthy, T.A. (1978) Biochemistry and Thermophyly, (Friedman, S.M. ed.), pp. 11–30, Academic Press, New York.

9 De Rosa, M., Gambacorta, A. and Nicolaus, B. (1983) J. Membr. Sci. 16, 287–294.

10 Abidor, I.G., Arakelian, V.B. and Chernomordik, L.V., Chizmadzhev, Yu.A., Pastushenko, V.F. and Tarasevich, M.R. (1979) Bioelectrochem. Bioenerg. 6, 37–52.

11 De Rosa, M., Gambacorta, A., Nicolaus, B., Chappe, B. and Alberch, P. (1983) Biochim. Biophys. Acta 753, 249–256.

12 De Rosa, M., Gambacorta, A., Nicolaus, B., Sodano, S. and Bu'Lock, J.D. (1980) Phytochemistry 19, 833–836.

13 Mueller, P., Rudin, D.O., Tien, H.Ti. and Wescott, W.S. (1964) Recent Progress in Surface Sci., Vol. 1, pp. 379–393, Academic Press, New York.

14 Melikyan, G.B., Abidor, I.G., Chernomordik, L.V. and Chailakhyan, L.M. (1983) Biochim. Biophys. Acta 730, 395–398.

15 Tien, H.Ti. (1974) Bilayer Lipids Membranes (BLM), Theory and Practice. pp. 532 M. Dekker, New York.

16 Abidor, I.G., Aitian, S.Kh., Cherny, V.V., Chernomordik, L.V. and Chizmadzhev, Yu.A. (1979) Dokl. Akad. Nauk SSSR 245, 977–981.

17 Chernomordik, L.V., Melikyan, G.B. Dubrovina, N.I., Abidor, I.G. and Chizmadzhev, Yu.A. (1984) Bioelectrochem. Bioenerg. 12, 155–166.

18 Chernomordik, L.V., Kozlov, M.M., Melikyan, G.B., Abidor, I.G., Markin, V.s. and Chizmadzhev, Yu.A. (1985) Biochim. Biophys. Acta 812, 643–655.

19 Cevc, G. and Marsh, D. (1987) Phospholipid Bilayers. Physical Principle and Models, Vol. 5, p. 271, Wiley Interscience, New York.