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Influence of cholesterol on electroporation of bilayer lipid membranes: chronopotentiometric studies

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Abstract

This paper presents the results of constant-current (chronopotentiometric) measurements of the egg yolk phosphatidylcholine (PC) bilayer membrane without and with cholesterol. The experiments were performed on planar bilayer lipid membrane (BLM) formed by the Mueller–Rudin method. It is demonstrated that the constant-intensity current flow through bilayer membranes generated fluctuating pores in their structure. The presence of cholesterol in the membrane caused an increase in the value of the breakdown potential. It is postulated that greater stability of the bilayer with cholesterol can result from an increased critical pore radius (at which the bilayer would undergo irreversible rupture). This confirms that cholesterol has a stabilizing effect on BLM. Besides, our results suggest that addition of cholesterol causes shift in the distribution of pore conductance towards a smaller value. It is suggested that this can be connected with the phenomenon of domain formation in the membranes containing high concentration of cholesterol. Moreover, it is shown that chronopotentiometry with programmable current intensity is a promising method for observation of the membrane recovery process.

Keywords: Electroporation; Chronopotentiometry; Cholesterol; Bilayer lipid membrane; Cholesterol domain

1. Introduction

Cholesterol research was and still is one of the key investigation areas [1]. Many works are concerned with cholesterol metabolism, biosynthesis, and relationship between cholesterol and arteriosclerosis. Mammalian cells require cholesterol for normal cell function [2–4]. Free cholesterol is an important structural component of the cell plasma membrane, where it regulates lipid bilayer dynamics and structure. For example, cholesterol reduces molecular order in gel phase bilayer and increases the order of molecules in liquid crystal phase bilayers [5]. The interaction of cholesterol with phospholipids may result in an increase in membrane cohesion leading to reduction of its passive permeability to small molecules [2].

Lipid membrane properties (especially membrane electropermeability) are affected by the cholesterol content [6]. The distribution of cholesterol among different membranes is not uniform. Cellular membranes, such as liver plasma membranes, erythrocyte and myelin membranes contain a high level of cholesterol (molar ratio cholesterol/phospholipids from 0.83 to 1.32) [3]. Some natural membranes, in particular in the lens of the eye, can contain very high cholesterol concentration. The cholesterol-to-phospholipid mole ratio of the eye lens fiber cell ranges from 1 to 2 in the cortex of the lens, to as high as 3 to 4 in the lens nucleus [7]. Intracellular membranes, e.g. endoplasmic reticulum, mitochondria, and microsomes, contain a low amount of cholesterol (molar ratio cholesterol/phospholipids from 0.11 to 0.33) [2,3,6].

At high concentration, free cholesterol can self-associate and form domains in the plasma membrane. This phenomenon is intensively studied using many biophysical methods [8,9]. The process of domain formation contributes to both physiological and pathological cellular processes [10]. The formation of amyloid plaques in Alzheimer's disease [11], HIV and other retrovirus assembly [12] are just a few examples where domains have been indicated.

Abbreviations: BLM, bilayer lipid membrane; PC, phosphatidylcholine; Chol, cholesterol; Chol/PC1, membrane containing cholesterol and phosphatidylcholine (molar ratio 1:1); Chol/PC2, membrane containing cholesterol and phosphatidylcholine (molar ratio 2:1)

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The physical interactions between cholesterol and lipids have been the subject of intensive experimental investigations using various techniques: calorimetry [13], magnetic resonance spectroscopy [14], micromechanics [15], neutron and X-ray scattering [16], Raman spectroscopy [17], electron microscopy [18], fluorescence spectroscopy [19], and electrical measurements [20–22]. Despite a great deal of research, the molecular picture of cholesterol–phospholipid interactions and the molecular structure of domains is still incomplete. Mostly, the role of cholesterol in the membrane stability (membrane resistance to rupture) has not been fully understood yet [23,24]. Bilayer stability may be considered in the formation of transmembrane aqueous pores [25,26]. This phenomenon may occur under the influence of the electric field and is known as electroporation. During electroporation, membranes exhibit a sharp transition to a higher conductance, indicating pore formation. A too strong electric field can be the reason for membrane destruction. The experimental verification of pore-dependent stability of membranes is difficult because no popular method enables direct observation of pore evolution and dynamic. Additionally, using the most popular voltage-clamp methods, all population of the pores in the membrane is generated. The current-clamp method is seldom used in planar membrane electroporation. It was supposed that current-clamp conditions should facilitate the formation stable pores in the membrane. Under such conditions, the membrane should not rupture because of the reduction of the transmembrane voltage directly after pore appearance [20–22,27].

Pore formation in the membrane is also of rapidly growing interest due to its potential applications in biology, biotechnology and medicine. The most frequent applications of electroporation are: (a) the transfection of cells, (b) introduction of fluorescent probes, enzymes and antibodies for research purposes, (c) loading cells with molecules for drug delivery purposes, and (d) transporting molecules into and out of tissue for therapeutic purposes [28,29]. One major criterion is that after poration the cells should maintain their natural integrity in subsequent suspension or culture. Conditions are therefore sought which elicit minimum membrane damage (irreversible breakdown) but maximum loading [30].

Recently the chronopotentiometric method for studies of electroporation and resealing of pores in bilayer lipid membranes (BLM) has been developed at our laboratory [31,32]. This method enables the observation of pore time evolution under current-clamp conditions. One fluctuating (open and close cyclically) pore, whose diameter depends on the constant current applied, was generated. This paper presents the application of this method to clarify the cholesterol effect on pore formation, pore time evolution and pore-dependent stability of BLM. The critical pore radius (radius at which the bilayer would rupture) is one of the parameters describing the stability of BLM. It was postulated [23,33] that greater bilayer stability would be connected with the fact that the critical pore radius would increase for cholesterol-containing bilayer. It was also postulated that cholesterol might decrease the ability of pore to extend in the bilayer. This means that the distribution of pore sizes (statistically) with cholesterol present would shift towards smaller pore sizes.

The paper confronts these assumptions with our results, discusses the ability of cholesterol to form domains and the applications of electroporation.

2. Materials and methods

2.1. Chemicals

The following chemicals were used: egg yolk phosphatidylcholine (PC) obtained from Fluka (Buchs, Switzerland), cholesterol (Chol) obtained from Sigma (St. Louis, USA), analytical-grade KCl obtained from POCH (Gliwice, Poland), *n*-decane purchased from Aldrich (Gillinghem-Dorset). The chemicals were used without additional purification. The forming solution for BLM preparation contained PC or PC and Chol dissolved in *n*-decane. The total concentration of the lipids in the forming solution was 20 mg/ml. The electrolytes (0.1 M KCl) were buffered with HEPES (Aldrich) to pH = 7.0. Ultrapure water was prepared in the Milli-Q system (Millipore).

2.2. Measurements

The experiments were performed using planar BLM formed by the Mueller–Rudin method [34]. The following membranes were used:

(a) membrane consisting of phosphatidylcholine,
(b) membrane consisting of Chol and PC at the molar ratio 1:1 (Chol/PC1),
(c) membrane consisting of Chol and PC at the molar ratio 2:1 (Chol/PC2).

The membranes were formed in a 1.0-mm-diameter, round aperture in two Teflon hydrophobic septum-separated cells filled with 10 ml of electrolyte.

The process of membrane formation was monitored by visual observation in transmitted light and by recording the membrane capacitance. The pictures of the membranes made in transmitted light were used for calculating the thickness of the hydrophobic part of the membranes. It was assumed that the membrane was completed when the capacitance drift was lower than 10 pF/min. The membrane resistance was calculated using voltammetric curves recorded in the range of potential from −50 to +50 mV, applying the least square method. Electrical measurements were performed using the four-electrode potentiostat-galvanostat and four-electrode capacitance meter described in previous papers [35,36]. The system uses two pairs of Ag/

AgCl electrodes. The experiments were performed at a temperature of 22 ± 1 °C.

2.3. Calculation of pore conductance and diameter

The pore conductance G_p was calculated according to the method described previously [31]. G_p was calculated from chronopotentiometric curves. This method allows to take into account the resistance of membranes without pores, and changes in the membrane capacitance caused by membrane potential. Pore conductance, presented in Fig. 2, was calculated according to this method by the computer program for analysis of chronopotentiometric curves, developed at our laboratory.

The pore diameter was calculated basing on simplified assumptions: (a) the shape of the pore is cylindrical; (b) the pore was filled with the same electrolyte as bulk electrolyte; (c) the change in the temperature inside the pore, resulting from the current flow, did not significantly change the pore conductance.

The pore diameter d was calculated from:

$$d = 2\sqrt{\frac{G_p L}{\kappa\pi}} \quad (1)$$

where G_p is pore conductance; κ is specific conductance of electrolyte.

In order to calculate the membrane thickness, the specific capacitance of each membrane was measured. Unfortunately, this method allowed to measure the thickness of the hydrophobic part of the membrane only. The results indicate that this thickness changed from about 5.0 nm to about 3.9 nm for PC- and cholesterol-containing membranes, respectively. According to the literature data [4,41], thickness of the hydrophilic parts of the membrane is about 1 nm. While calculating the pore diameter, the thickness of each membrane (hydrophobic and hydrophilic parts) was assumed 7 nm for PC- and 5.9 nm for cholesterol-containing membranes.

The data were processed and analyzed with the computer program Excel (Microsoft) or Prism (GraphPad Software, Inc.).

3. Results and discussion

3.1. Chronopotentiometric characteristics of BLM

The chronopotentiometric characteristics (constant-current measurements) were registered for the membrane without and with addition of cholesterol. Fig. 1 shows typical chronopotentiometric curves registered with different constant-currents flowing through bilayer membranes: (a) 0.005, (b) 0.2 and (c) 2.0 nA. The shape of these curves strongly depends on the current intensity. At low current intensity (order of 0.005 nA), the membrane voltage increased within a few seconds, reaching a constant value (Fig. 1, curve a) described by the Ohm's law. Higher current intensity (order of 0.2–0.5 nA) caused a faster increase in membrane voltage, which after reaching a certain critical value decreased suddenly (Fig. 1, curve b). The membrane voltage, however, did not reach the 0 mV value, indicating that the membrane was not destroyed. Instead, the membrane voltage set at a specific level and the voltage fluctuated around this level. The third region of current intensities (several nA) represents the currents causing membrane destruction (irreversible breakdown): a fast voltage increase is followed by its sudden drop to zero (Fig. 1, curve c). The chronopotentiometric curves perfectly matched those observed earlier [31,32,37,38]. The highest value of the potential on curve b (Fig. 1) was interpreted as the potential at which the reversible breakdown would start (breakdown potential), e.g. the potential for pore formation. Irregular opening and closing of one pore under these conditions was assumed [31]. Fluctuations of the potential registered for this type of chronopotentiometric curves were interpreted as the effect of changes in the pore size.

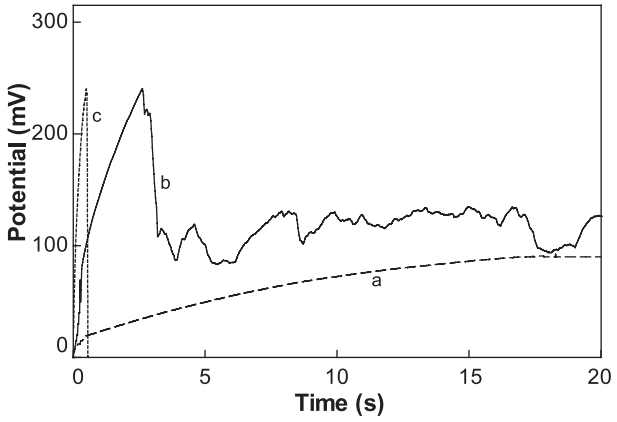

Fig. 1. Typical chronopotentiometric curves for BLM registered under different constant currents: (a) 0.005, (b) 0.2 and (c) 2.0 nA. Forming solution: Chol/PC1 in *n*-decane. Electrolyte: 0.1 M KCl, HEPES pH = 7.0.

3.2. Influence of cholesterol on breakdown potential

The important feature of our results is the difference in the breakdown potential between membranes with or without cholesterol. Cholesterol increased the critical potential from about 255 ± 20 mV for PC membranes to about 280 ± 25 mV for Chol/PC1 ones and to about 360 ± 30 mV for Chol/PC2 membranes. This reflects the stabilizing effect of cholesterol on lipid bilayer membranes. The examples of chronopotentiometric curves obtained at a current of 0.2 nA for membrane of different composition are presented in Fig. 2.

The studies described in literature revealed an unclear picture of the cholesterol influence on the membrane breakdown voltage. In the work with giant unilamellar vesicles examined by the aspiration technique, it was

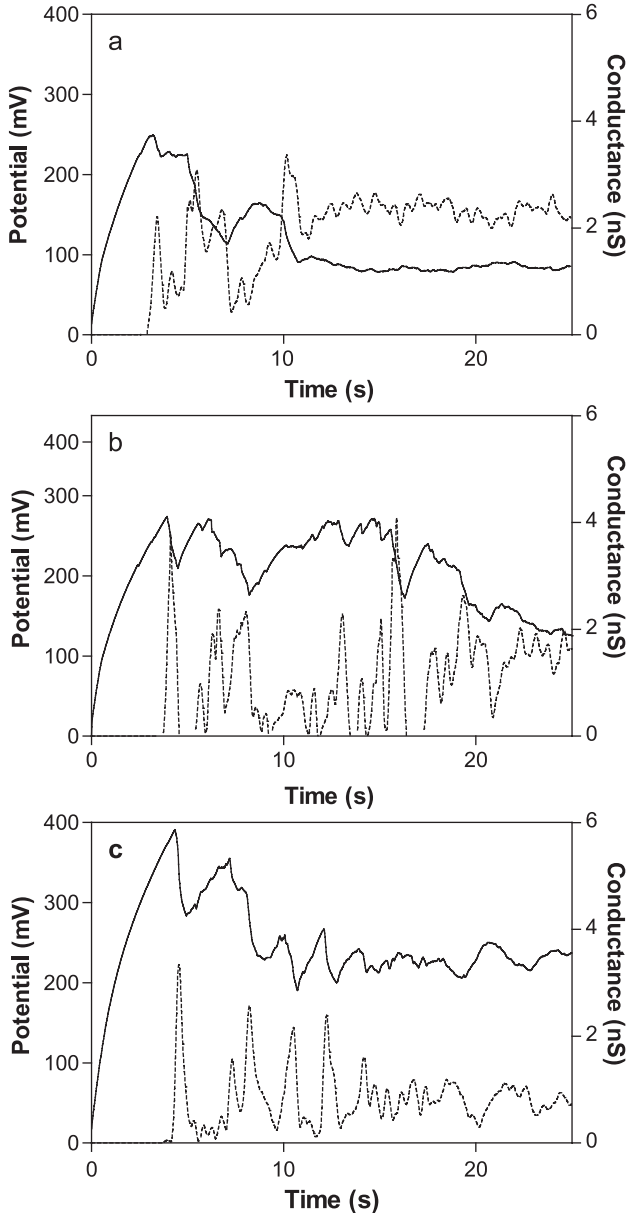

Fig. 2. Chronopotentiometric characteristics (solid line, left axis) of membranes consisting of: (a) PC, (b) Chol/PC1, (c) Chol/PC2. Electrolyte, 0.1 M KCl, HEPES pH = 7.0. After BLM formation, constant intensity current (0.2 nA) was applied to the membranes. The conductance of a single pore was calculated from the proper chronopotentiometric curve (broken line, right axis).

observed that the breakdown voltage was very high (more than 1 V) [23,24]. When cholesterol was present (50 mol%), a considerable increase in the breakdown voltage was observed. At lower concentration of cholesterol (up to 29 mol%), under voltage-clamp [6] and current-clamp conditions [20,21], no change in the breakdown voltage was detected. Our results concern 50 mol% (Chol/PC1) and 67 mol% (Chol/PC2) concentration of cholesterol and partly confirm those obtained by the Needham's team. The relatively high cholesterol content in the membranes made the breakdown voltage higher. It can be concluded, following the results obtained by Raffy and Teissie [6], that: "cholesterol has a concentration-dependent effect on membrane organization".

According to the electroporation theory [39], BLM is a system in a metastable state, containing a fluctuating population of pores (or defects). The work needed to form a pore is given by a balance between the energy necessary to create the edge of the pore (represented by γ, the line tension) and the energy released by the pore surface (represented by Γ, the surface energy of the bilayer). The energy barrier to pore formation is characterized by a difference in the ability of lipid molecules to pack at the edge region compared to the planar lipid bilayer. Adding lisophosphatidylcholine (it has a conical shape) to the membrane promotes a grater packing ability and decreases the line tension. Adding cholesterol increases the line tension [23]. This suggests that cholesterol-containing BLM should be more resistant to structure "opening" and (aqueous) pore formation.

Moreover, other factors influencing pore formation should be taken into account. One of them might be a delicate balance between the forces presented between the polar heads, which include electrostatic repulsive and attractive interactions. It has been suggested that "the stronger lateral repulsion of some lipids makes it easier for pores to be formed" [21]. This may indicate that cholesterol reduces the head group repulsive interactions, which results in more difficult formation of pores in Chol/PC1 and Chol/PC2 membranes than in PC ones. Also the electrostatic interaction between zwitterionic head groups of lipid heads might be considered as the factor influencing pore formation. Cholesterol incorporation into PC can increase the distance between PC molecules [40] because their heads can lie flatter on the bilayer surface. Hence, the electrostatic interaction between the positive charge of the PC head and the negative charge of the adjacent PC might be facilitated. In turn, this interaction should hamper pore formation.

3.3. Influence of cholesterol on the pore conductance and diameter

The chronopotentiometric method was applied in order to observe the dynamic behaviors of membranes. Fig. 2 shows an examples of typical chronopotentiometric curves for membrane formed without (Fig. 2a) or with incorporated cholesterol (Fig. 2b and c). The current intensity was 0.2 nA. The shape of the curves representing pore formation and its fluctuations was similar: a fast voltage increase until reaching a certain, characteristic critical value (breakdown voltage), followed by its sudden decrease and oscillations around the characteristic value. Similar fluctuations have been observed and described by other authors [20–22,27].

Potential fluctuations are attributed to changes in membrane conductance induced by pore diameter fluctuations [31]. Our investigations indicate that pore conductance was

Table 1
Conductance and diameter of pores induced in BLM under current-clamp conditions

Current intensity, *i* (nA)	0.2	0.4	0.6	1.0	1.5	2.0
PC						
Level of conductance oscillation, *G* (nS)	2.10 ± 0.30	2.94 ± 0.67	4.35 ± 1.10	8.10 ± 0.95	irreversible	irreversible
Pore diameter, *d* (nm)	3.8	4.5	5.5	7.5	breakdown	breakdown
Chol/PC1						
Level of conductance oscillation, *G* (nS)	1.90 ± 0.34	2.53 ± 0.60	3.75 ± 0.75	6.93 ± 0.83	9.4 ± 0.99	13.10 ± 1.20
Pore diameter, *d* (nm)	3.3	3.8	4.7	6.4	7.4	8.7
Chol/PC2						
Level of conductance oscillation, *G* (nS)	1.10 ± 0.30	2.35 ± 0.66	_	6.08 ± 1.10	7.98 ± 1.20	irreversible
Pore diameter, *d* (nm)	2.5	3.7	_	6.0	6.8	breakdown

Forming solution: phosphatidylcholine in *n*-decane (PC) or mixture of cholesterol and phosphatidylcholine in *n*-decane. Molar ratio cholesterol to phosphatidylcholine was 1:1 (Chol/PC1) or 2:1 (Chol/PC2). Electrolyte: 0.1 M KCl, HEPES pH = 7.0. Each value was calculated as an arithmetic mean and standard deviation for at least eight membranes.

affected by the cholesterol content of membranes (Fig. 2, broken lines). The average level of pore conductance fluctuations decreased with an increase in the cholesterol content: 2.1 ± 0.3 nS for PC, 1.9 ± 0.3 nS for Chol/PC1, and 1.1 ± 0.3 nS for Chol/PC2. The values of pore diameter, calculated according to Eq. (1) (see Section 2.3), are: 3.8, 3.3, and 2.5 nm for PC, Chol/PC1 and Chol/PC2 membranes, respectively.

Moreover, these experiments show that the conductance of fluctuating pores generated in Chol/PC1 and Cho/PC2 membranes occasionally reached the value of 0 nS (Fig. 2b and c). This means that the pores could be temporarily sealed during electroporation. In the case of PC membrane, this state was not reached very often (Fig. 2a).

Conductance depended on the current flow through the membrane: higher current intensity caused an increase in pore conductance (Table 1). The mean diameter of pores generated in Chol/PC1 under the highest current intensity

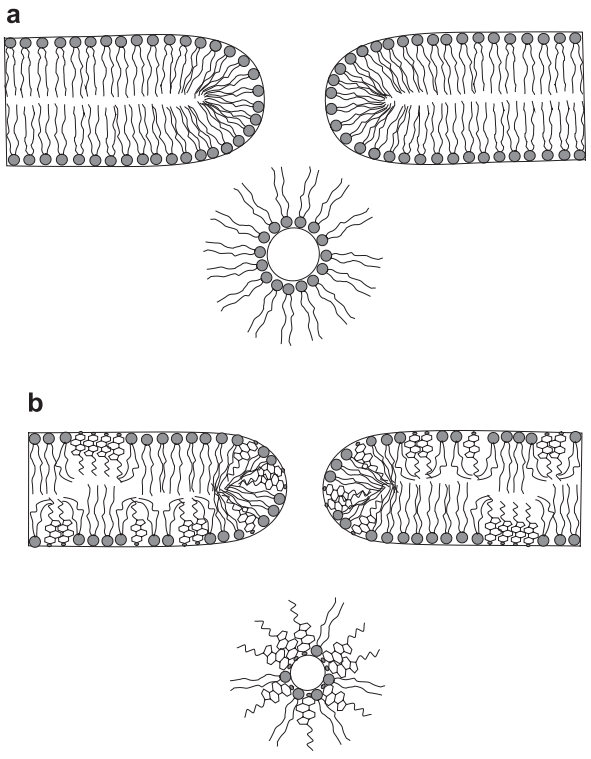

Fig. 3. Influence of cholesterol on pore diameter. The picture shows longitudinal and cross section of the pore axis. Pores were generated in membrane consisting of: (a) PC, or (b) mixture of PC and Chol.

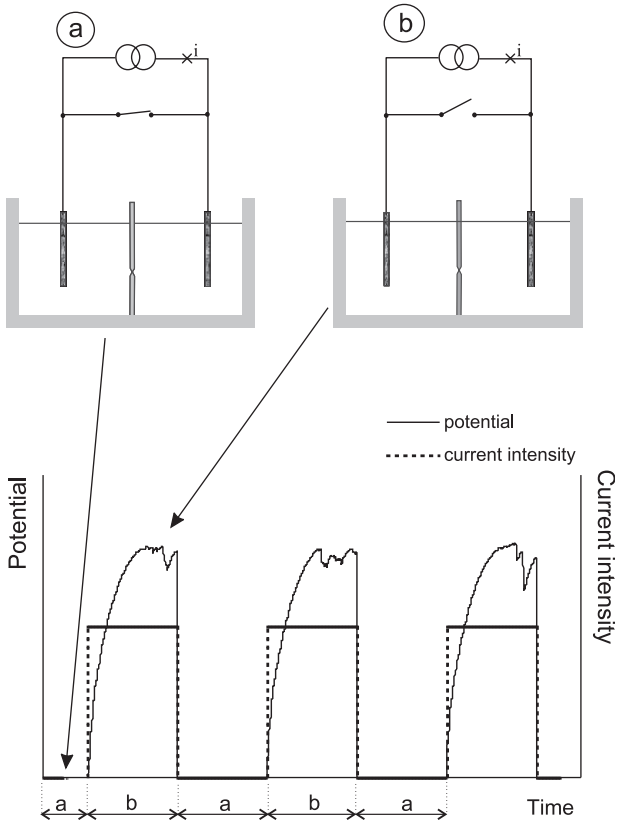

Fig. 4. An example of the chronopotentiometric curves registered under programmable current. The switch causes a shortening of the electrodes and as a result the membrane potential is forced equal to zero (a). When the current electrodes are not short-circuited, the flow of constant-current through the membrane is forced and typical chronopotentiometric curves are registered (b).

(i = 2.0 nA) was about 8.7 nm. Such a high value of current is the reason for irreversible breakdown of PC membranes. In this case, curves similar to that showed in Fig. 1 (curve c) were observed. The pores generated in PC membranes had the maximum diameter of about 7.5 nm (under i = 1 nA) and could be destroyed under much higher current intensity. Conductance and diameter of pore given at high current intensities are relatively high. Reasons of such value can be explained by possibility of formation of more than one pore or formation of one large pore. We suppose that the second reason is more probable. Conditions of pore formation using the current-clamp method are more "gentle" and pore may have higher stability by comparison with impulse methods. The transmembrane potential increases slowly. The creation of the first pore causes fast decrease of the transmembrane potential. In turn, this decreases the probability of next pore creation. The reasons for such interpretation have already been discussed in details earlier [31]. However, only permeability measurements with molecules of different diameter could clarify this point.

Our experiments suggest that bilayer membrane containing cholesterol can be forced to open more widely by external factors (like electric current in the present experiments) without its destruction. The critical pore radius (at which the bilayer would rupture) was increased by cholesterol inclusion. This was reflected by greater bilayer stability. Similar conclusions can be found in literature [33]. Other authors found that the membrane becomes more elastic and more cohesive with cholesterol included [24]. Our results confirm these assumptions.

A significant decrease in conductance was observed particularly for Chol/PC2 membranes (molar ratio Chol/PC of about 2), although at higher currents they were more vulnerable to irreversible breakdown. In this sort of membranes, cholesterol concentration corresponds with its concentration in the cortex of the eye lens. It was hypothesized

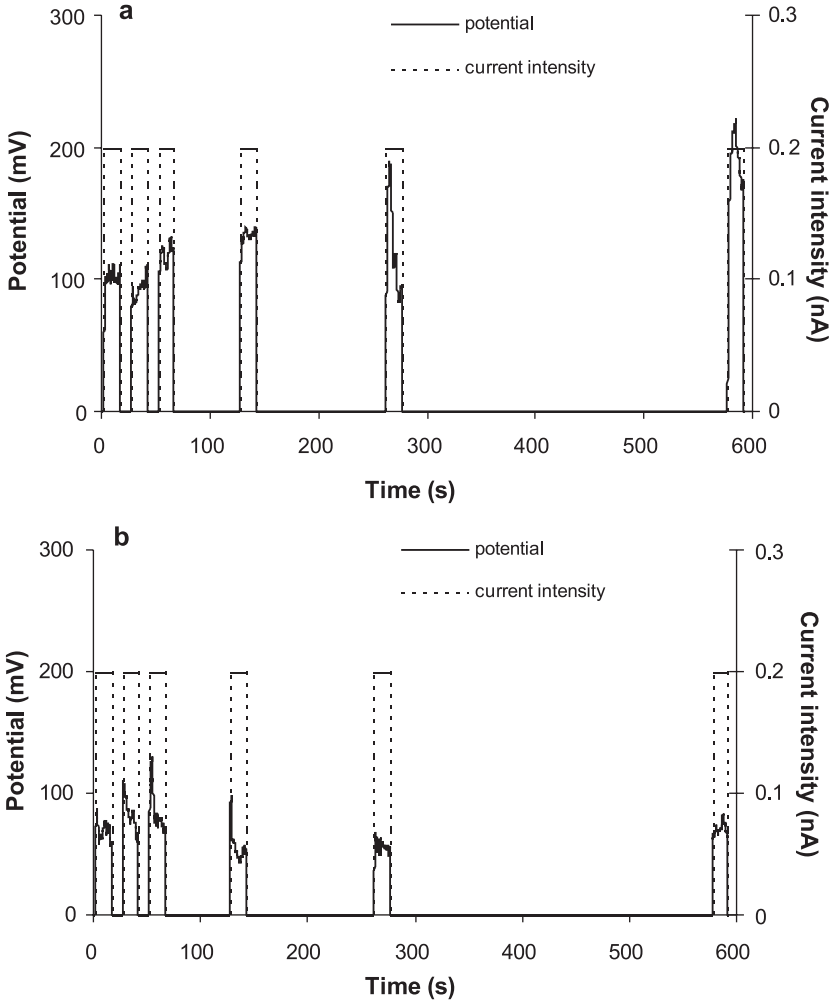

Fig. 5. Chronopotentiometric characteristics of Chol/PC1 membranes registered under programmable current with changeable interruptions for a short circuit of electrodes for an increasing period of time. Changes in the potential—solid line, left axis; current intensity—broken line, right axis. Forming solution: mixture of cholesterol and phosphatidylcholine (molar ratio 1:1) in *n*-decane (Chol/PC1). Electrolyte: 0.1 M KCl, HEPES pH = 7.0. Before recording both membranes were electroporated under current-clamp conditions (i = 0.2 nA). Electroporation was prolonged for 20 and 100 s for the a and b membranes, respectively.

that such a high level of membrane cholesterol content would result in the appearance of a mosaic of phosphatidylcholine and cholesterol patches (domains or rafts) [7,10]. How may this affect the pore shape and membrane stability?

The presumptions described in literature [23,33] suggest that cholesterol, which has a small polar head and a large hydrophobic part, would not pack well into the highly curved pore region. It was hypothesized that this may be the reason for pore diameter reduction in cholesterol-containing membranes. The obtained results confirm this suggestion. The interaction of cholesterol molecules with lipids in the BLM shifts the distribution of pore diameters towards smaller values (Table 1, Fig. 3). Under the influence of cholesterol, membranes become thinner and pores become smaller.

3.4. Studies of pore resealing using programmable chronopotentiometry

For a living organism, it is important to regenerate a membrane, and return it to initial continuous structure after the external stress disappears. This stress might be, for example, electroporative opening of the membrane structure during medical or biotechnological manipulations. In this work, programmable chronopotentiometry was applied as a tool for studying pore resealing. During the experiments the current was consecutively switched on and off, and the time of current switching on and off was regulated (Fig. 4). The current electrodes were short-circuited with a view to forcing potential equal to zero. This method allows, among other things, to observe gradual (time-dependent) pore resealing [32]. Surprisingly, no significant dependence of the time needed to pore resealing on the cholesterol present in the membranes was observed.

Fig. 5 shows examples of two typical chronopotentiometric curves for BLM recorded with programmable intensity of current. Before this recording in both instances pores had already been generated. Electroporation was prolonged for 20 and 100 s for membranes a and b, respectively. Fig. 5 (part a) presents an example of curves that may suggest gradual pore resealing when the time of electrode short-circuiting is prolonged. The potential on the last chronopotentiometric curve reached approximately the value of the original breakdown voltage. In other words, the membrane recovered its initial continuous structure.

Fig. 5 (part b) represents an example of curves showing not complete recovering of membrane continuous structure. Breakdown potential did not reach the initial value. The pore did not seal. This may suggest that the relatively long time of electroporation (100 s) promotes the creation of pores that do not close immediately in the lipid bilayer. This is important as regards the current-clamp method applications in the future. Prolongation of electroporation can provide impediment of pore resealing and recovery of the continuous structure of cell membranes.

4. Concluding remarks

The molecular phenomena responsible for electroporation are still poorly understood, which makes its wider application hardly possible. It can be concluded that the chronopotentiometric method of investigation of membrane electroporation can be successfully used in electroporation research. The present results and those published earlier [31,32,37,38] can complement several aspects of electroporation. The method for recording of the transmembrane voltage as a function of time (under current-clamp conditions) and the method for calculation of pore conductance changes as a function of time have been already developed. A promising method for membrane recovery observation has been presented. It was found that changes in the membrane composition (inclusion of cholesterol) affect its stability and pore diameter. This seems to be a promising tool facilitating the investigations of membrane organization at the molecular level.

Our experiments show that membranes with cholesterol were more resistant to structure ‘‘opening’’ (pore formation) than those composed of phosphatidylcholine only. However, once the pore was opened, it kept the open state in the form of fluctuating pore. The average conductance and diameter of Chol/PC1 (or Chol/PC2) pores were smaller than that generated in PC membrane. It seems to be connected with the phenomenon of domain formation in membranes containing high concentration of cholesterol. Also, the maximum pore diameters in Chol/PC1 and Chol/PC2 membranes were much larger, larger than the membrane thickness. PC membrane with such pores would be destroyed. Under an external electric field, the pores formed in Chol/PC1 and Chol/PC2 could be temporarily closed. However, this state was not easily reached in PC membranes. The disappearance of the external field diminished the differences mentioned above: both types of pores resealed similarly.

References

[1] D.E. Vance, H. Van den Bosch, Cholesterol in the year 2000, Biochim. Biophys. Acta 1529 (2000) 1–8.
[2] P.L. Yeagle, Cholesterol and the cell membrane, Biochim. Biophys. Acta 822 (1985) 267–287.
[3] D. Chapman, M.T.C. Kramers, C.J. Restall, Cholesterol and biomembrane structures, in: H. Danielsson, J. Sjovall (Eds.), Sterols and Bile Acids, Elsevier Science, Amsterdam, 1985, pp. 151–174.
[4] R.B. Gennis, Biomembranes, Molecular Structure and Function, Springer-Verlag, New York, 1989, pp. 1–61.
[5] A. Genz, F. Holzwarth, T. Tsong, The influence of cholesterol on the main phase transition of unilamellar dipalmitoylphosphatidylcholine vesicles. A differential scanning calorimetry and iodine laser T-jump study, Biophys. J. 50 (1986) 1043–1051.
[6] S. Raffy, J. Teissié, Control of lipid membrane stability by cholesterol content, Biophys. J. 76 (1999) 2072–2080.
[7] L.K. Li, L. So, A. Spector, Membrane cholesterol and phospholipid in consecutive concentric sections of human lenses, J. Lipid Res. 26 (1985) 600–609.
[8] L. Addadi, M. Geva, H.S. Kruth, Structural information about orga-

nized cholesterol domains from specific antibody recognition, Biochim. Biophys. Acta 1610 (2003) 208–216.
[9] J.R. Silvius, Role of cholesterol in lipid raft formation: lessons from lipid model systems, Biochim. Biophys. Acta 1610 (2003) 174–183.
[10] R.P. Mason, T.N. Tulenko, R.F. Jacob, Direct evidence for cholesterol crystalline domains in biological membranes: role in human pathobiology, Biochim. Biophys. Acta 1610 (2003) 198–207.
[11] W.G. Wood, G.P. Eckert, U. Igbavboa, W.E. Müller, Amyloid beta-protein interactions with membranes and cholesterol: causes or casualties of Alzheimer's disease, Biochim. Biophys. Acta 1610 (2003) 281–290.
[12] A. Ono, E.O. Freed, Plasma membrane rafts play a critical role in HIV-1 assembly and release, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 13925–13930.
[13] S. Imaizumi, I. Hatta, Binary mixtures of phospholipids and cholesterol studied by dynamic heat capacity measurements, J. Phys. Soc. Jpn. 53 (1984) 4476–4487.
[14] F.T. Presti, R.J. Pace, S.I. Chan, Cholesterol-phospholipid interaction in membranes, Biochemistry 21 (1982) 3831–3835.
[15] D. Needham, T.J. McIntosh, E. Evans, Thermomechanical and transition properties of dimyristoylphosphatidylcholine/cholesterol bilayers, Biochemistry 27 (1988) 4668–4673.
[16] S.W. Hui, N.-B. He, Molecular organisation in cholesterol-lecithin bilayers by X-ray and electron diffraction measurements, Biochemistry 22 (1983) 1159–1164.
[17] T. O'Leary, I.W. Levin, Raman spectroscopy of selectively deuterated dimyristoylphosphatidylcholine: studies on dimyristoylphosphatidylcholine–cholesterol bilayers, Biochim. Biophys. Acta 854 (1986) 321–324.
[18] B.R. Copeland, H.M. McConnell, The rippled structure in bilayer membranes of phosphatidylcholine and binary mixtures of phosphatidylcholine and cholesterol, Biochim. Biophys. Acta 599 (1980) 95–109.
[19] A. Chabanel, M. Flamm, K.L.P. Sung, M.M. Lee, D. Schachter, S. Chien, Influence of cholesterol content on red cell membrane viscoelasticity and fluidity, Biophys. J. 44 (1983) 171–176.
[20] M. Robello, A. Gliozzi, Conductance transition induced by an electric field in lipid bilayers, Biochim. Biophys. Acta 928 (1989) 173–176.
[21] I. Genco, A. Gliozzi, A. Relini, M. Robello, E. Scalas, Electroporation in symmetric and asymmetric membranes, Biochim. Biophys. Acta 1149 (1993) 10–18.
[22] E. Scalas, A. Ridi, M. Robell, A. Gliozzi, Flicker noise in bilayer lipid membranes, Europhys. Lett. 43 (1998) 101–105.
[23] D.V. Zhelev, D. Needham, Tension-stabilized pores in giant vesicles: determination of pore size and pore line tension, Biochim. Biophys. Acta 1147 (1993) 89–104.
[24] D. Needham, R.M. Hochmuth, Electro-mechanical permeabilization of lipid vesicles. Role of membrane tension and compressibility, Biophys. J. 55 (1989) 1001–1009.
[25] D.S. Dimitrov, R.K. Jain, Membrane stability, Biochim. Biophys. Acta 779 (1984) 437–468.
[26] W. Meier, A. Graff, A. Diederich, M. Winterhalter, Stabilization of planar lipid membranes: a stratified layer approach, Phys. Chem. Chem. Phys. 2 (2000) 4559–4562.
[27] A. Ridi, E. Scalas, A Gliozzi, Noise measurements in bilayer lipid membranes during electroporation, Eur. Phys. J., E Soft Matter 2 (2000) 161–168.
[28] T.Y. Tsong, Electroporation of cell membranes, Biophys. J. 60 (1991) 297–306.
[29] S.B. Dev, D.P. Rabussay, G. Widera, G.A. Hofmann, Medical applications of electroporation, IEEE Trans. Plasma Sci. 28 (2000) 206–223.
[30] J.C. Weaver, Molecular basis for cell membrane electroporation, Ann. N.Y. Acad. Sci. 720 (1994) 141–152.
[31] S. Kalinowski, G. Ibron, K. Bryl, Z. Figaszewski, Chronopotentiometric studies of electroporation of bilayer lipid membranes, Biochim. Biophys. Acta 1369 (1998) 204–212.
[32] S. Koronkiewicz, S. Kalinowski, K. Bryl, Programmable chronopotentiometry as a tool for the study of electroporation and resealing of pores in bilayer lipid membranes, Biochim. Biophys. Acta 1561 (2002) 222–229.
[33] Ch. Karolis, H.G.L. Coster, T.C. Chilcott, K.D. Barrow, Differential effects of cholesterol and oxidised-cholesterol in egg lecithin bilayers, Biochim. Biophys. Acta 1368 (1998) 247–255.
[34] P. Mueller, D.O. Rudin, H.T. Tien, W.C. Wescott, Methods for the formation of single bimolecular lipid membranes in aqueous solutions, J. Phys. Chem. 67 (1963) 534–535.
[35] S. Kalinowski, Z. Figaszewski, A four-electrode system for measurements of bilayer lipid membrane capacitance, Meas. Sci. Technol. 6 (1995) 1043–1049.
[36] S. Kalinowski, Z. Figaszewski, A four-electrode potentiostat-galvanostat for studies of bilayer lipid membranes, Meas. Sci. Technol. 6 (1995) 1050–1055.
[37] S. Koronkiewicz, K. Bryl, Cholesterol-induced variation in fluctuation of the pores in bilayer lipid membrane, Cell. Mol. Biol. Lett. 4 (1999) 567–582.
[38] S. Koronkiewicz, S. Kalinowski, K. Bryl, Changes of structural and dynamic properties of model lipid membranes induced by α-tocopherol: implication to the membrane stabilization under external electric field, Biochim. Biophys. Acta 1510 (2001) 300–306.
[39] J.C. Weaver, Yu.A. Chizmadzhev, Theory of electroporation: a review, Bioelectrochem. Bioenerg. 41 (1996) 135–160.
[40] H.L. Scott, S. Kalaskar, Lipid chains and cholesterol in model membranes: a Monte Carlo study, Biochemistry 28 (1989) 3687–3691.
[41] L. Stryer, L. Biochemia, Wydawnictwo Naukowe, PWN, Warszawa, 1986.