Biophysical Chemistry 142 (2009) 27–33

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

Effect of acetylsalicylic acid on the current–voltage characteristics of planar lipid membranes

Cezary Watala [a,*], Aleksandr Drapeza [b], Valery Loban [b], Monika Asztemborska [c], Dzmitry Shcharbin [d]

[a] Department of Haemostasis and Haemostatic Disorders, Medical University of Lodz, 113 Zeromski str., 90-549 Lodz, Poland
[b] Department of Biophysics, Belarusian Stete University, 4 Prospect Nezavisimosti str., 220080 Minsk, Belarus
[c] Institute of Physical Chemistry, Polish Academy of Sciences, 44/52 Kasprzaka str., 01-224 Warsaw, Poland
[d] Department of General Biophysics, University of Lodz, 12/16 Banacha str., 90-237 Lodz, Poland

ARTICLE INFO

Article history:
Received 16 December 2008
Received in revised form 27 February 2009
Accepted 1 March 2009
Available online 12 March 2009

Keywords:
Acetylsalicylic acid (ASA)
Planar lipid membranes
Cholesterol
Transmembrane transport
Passive diffusion

ABSTRACT

Monitoring of influence of acetylsalicylic acid (ASA) on lipid bilayer conductance may contribute to better understanding of molecular mechanisms underlying passage of ASA into cells. This paper presents effects of increasing sweeping potential on stability of egg yolk phosphatidylcholine planar bilayer lipid membranes (BLM) without or with cholesterol incubated in the presence of ASA. We demonstrated that current flow through bilayer membranes generated fluctuating pores in their structure. Presence of cholesterol in the membrane caused an increase in the value of the breakdown potential, thus confirming that cholesterol had a stabilizing effect on BLM. Otherwise, ASA significantly reduced these values regardless of cholesterol concentration. Overall, by destabilizing the lipid bilayer, ASA contributed to the formation of metastable single pores, which facilitated ASA diffusion through a bilayer. Our data point out that ASA transport across the lipid bilayer takes place predominantly via the process of passive diffusion. In conclusion, the effects of ASA on lipid bilayer stability may contribute to drug transport through membrane lipid bilayers.

1. Introduction

Acetylsalicylic acid (ASA, aspirin), the most commonly used antiplatelet drug, has been used for years for prevention of primary and secondary cardiovascular complications. It inhibits the synthesis of thromboxane A_2 in blood platelets by irreversible acetylation of cyclooxygenase, the cellular enzyme catalyzing the formation of an unstable endoperoxide intermediate prostaglandin H_2. In blood platelets, it acetylates the serine-530 residue close to the active site of cyclooxygenase (COX) [1], which is thought to constitute the molecular mechanism underlying the non-enzymatic inhibition of the cyclization of arachidonic acid [2,3]. However, to inhibit the activity of COX, ASA has to penetrate the platelet membrane lipid bilayer, getting into the cell through the mechanism yet not fully elucidated.

The optimization of a successful and effective ASA-dependent therapy has become a challenge largely due to the so-called "aspirin-resistance," to a major extent encountered in special groups of patients at risk for cerebro- and cardiovascular complications [4]. Recently, we have demonstrated an association between reduced platelet sensitivity to ASA (Aspirin®) and a higher plasma cholesterol concentration [5]. Accordingly, it has been hypothesized that elevated cholesterol causes alterations in the platelet membrane lipid profile [6], and hence, it may significantly affect platelet function by changing the dynamic properties of cell membrane [7]. However, the question of whether and how ASA molecules may be freely diffused or be transported across the membrane lipid bilayer has not been resolved so far.

In the present study, we aimed at verifying the hypothesis that ASA, due to its interaction with lipid membranes, is able to alter the current–voltage characteristics of a lipid bilayer, which may further affect the incidence of the pore formation, and consequently facilitate the transmembrane passage of the drug. The possible effect of bilayer lipid composition on the rate of such diffusion has also been studied. In order to resolve the question, we monitored (a) the current–voltage characteristics as the function of lipid bilayer stability, and (b) pH changes, as due to the penetration of weak organic acid (acetylsalicylate) through the bilayer of planar lipid membranes composed of phosphatidylcholine and containing the increasing fractions of cholesterol.

2. Materials and methods

2.1. Chemicals

L-α-phosphatidylcholine (*from egg yolk*) and 3-2*sn*-phosphatidylethanolamine (*from bovine brain*) were purchased from Fluka GmbH (Buchs, Switzerland). All other chemicals, including cholesterol,

* Corresponding author. Department of Haemostasis and Haemostatic Disorders, Medical University of Lodz, Medical University Hospital No. 2, 113 Zeromskiego street, 90-549 Lodz, Poland. Tel.: +48 42 6393471; fax: +48 42 6787567.
E-mail addresses: cwatala@csk.umed.lodz.pl (C. Watala), Drapeza@bsu.by (A. Drapeza), monika@ichf.edu.pl (M. Asztemborska), dg.shcharbin@mail.ru (D. Shcharbin).

0301-4622/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2009.03.003

acetylsalicylic acid (ASA), and salicylic acid (SA), were from Sigma (St. Louis, MO, USA). Water used for solution preparation and glassware washing was passed through an Easy Pure UF water purification unit (Thermolyne Barnstead, IA, USA).

2.2. Formation of planar lipid membranes

Planar lipid membranes (black lipid membranes, BLMs) were formed at 22 °C in a Teflon chamber with two compartments (*cis* and *trans*), separated by a diaphragm with the orifice for bilayer formation (1.5 mm in diameter), using the technique previously described by Mueller et al. [8]. Briefly, the bilayer was obtained by bubbling the lipid solution toward the orifice, and the potential across was maintained at −60 mV. Applying very short impulses of a given voltage accelerated the formation of the membranes.

The lipid bilayers used as model membranes in this study were composed of lipids naturally present in biomembranes: phosphatidylcholine (100% phosphatidylcholine considered as a control) and cholesterol, mixed at varying proportions (90–70/10–30%), with small portions (up to 5%) of phosphatidylethanolamine added to some samples in order to verify its effect on bilayer stability, formation time, and lifespan. Firstly, we prepared a chloroform mixture of egg yolk phosphatidylcholine with 0, 10, 20, or 30 mol% cholesterol, evaporated off chloroform under nitrogen, and the lipid film (total lipids 1 mg) was dissolved in nonane (C_9H_{20}) to give the solution of 25 mg/mL. Both compartments of the Teflon chamber were filled with electrolyte (PBS, pH 7.4), and lipid bilayers were formed on the orifice of the chamber's diaphragm by applying a drop of previously prepared lipid solution. Membrane formation was monitored using a video camera and by recording an increased capacitance between compartment electrodes. The *V–A* characteristics were monitored during 30 min following BLM stabilization at RT. The values of the 'breakdown voltage' (the moment of BLM rupture) and the 'pre-rupture voltage' (the moment of formation of metastable single pores, *msp*), as well as the time interval between the application of a given voltage and the onset of the rupture, were evaluated based on current–voltage characteristics using the relevant plots [9,10] (Fig. 1).

2.3. System for the study of current–voltage characteristics of planar lipid membranes

A customized multifunctional BLM system, in which two chambers of a Teflon cell, each containing a platinized electrode, are separated by a septum with the orifice of 1.5 mm, was used to study the current–voltage (*V–A*) characteristics up to the picoampere level, as well as to monitor the kinetics of electric conductivity of planar BLMs [9,10]. The device allows measuring of the penetration of acid/alkaline ions (like SA or ASA) through a lipid bilayer, which is stabilized by the constant electric field of −30 mV/−20 mV. Since it is possible to modulate both voltage and current (amperage), the device also ensures the possibility of analyzing the resistance of the lipid bilayer, which appears linear as long as the bilayer remains intact, while the formation of holes and pores in the bilayer results in the appearance of non-linear regions. In addition, the fluctuations in lipid composition of a bilayer may affect *V–A* characteristics, i.e., the formation of holes or pores is strongly influenced by varying cholesterol concentration [9,10]. The formation of the BLM over the orifice was monitored on the plot of the current versus a sweeping membrane potential, as well as by means of a microvideo system, which enabled observation of the membrane formation in a real-time mode on a PC monitor and saving of the video files. For each series of measurements, the calibration curve has been recorded, which further enabled estimation of the bilayer resistance, R_m.

The data analysis of all the acquired signals — current–voltage characteristics, pH and video signals — was performed by means of the in-house software [9,10]. In the used BLM system, there were two principal components of current flowing through the lipid bilayer: the charging current, I_C, and the ohmic current, I_R. These can be determined as follows:

$$I_C = \frac{dU}{dt} = C_m A \tag{1}$$

$$I_R = \frac{U}{R_m} \tag{2}$$

$$I = I_R + I_C = \frac{U}{R_m} + C_m A_t \tag{3}$$

where C_m and R_m represent, respectively, membrane capacitance and membrane resistance, A_t is the scan rate in mV s^{-1}, and the capacitance charging current, I_c, is constant [11–13]. As shown in Eq. (3), increasing the scanning voltage results in an increased current through the resistor. When scan rate A_t is constant, with fixed values of C_m and R_m, the current I is linearly related to the sweeping potential U. Hence, the slope reflects R_m, while C_m may be determined by measuring of the $I = f(U)$ response [11–13].

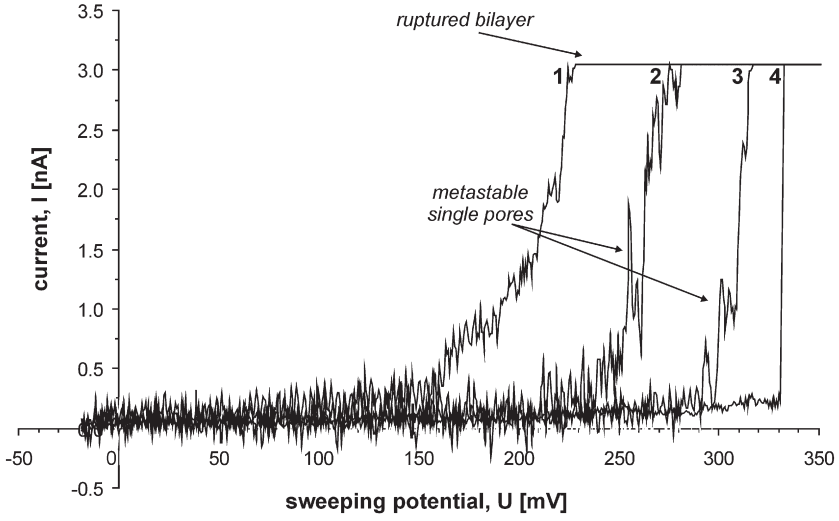

Fig. 1. Current–voltage characteristics of planar lipid membranes with varying lipid composition. The dependence of current (I) on the sweeping potential (U) for BLMs composed of egg yolk phosphatidylcholine (PCh) and the increasing portions of cholesterol (CH): (1) 100% PCh; (2) 90% PCh, 10% CH; (3) 80% PCh, 20% CH; (4) 70% PCh, 30% CH, in PBS, pH 7.4, 20 °C. The formation of metastable single pores ('spikes') and bilayer rupture shown by arrows. The range of the sweeping potential was from −60 mV to 650 mV. The recording time was 20 s. For experimental details, see Sections 2.2 and 2.3.