PLANAR PHOSPHOLIPID MEMBRANE CHIPS FOR THERMODYNAMICS STUDIES OF CERAMIDE ION CHANNELS

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ABSTRACT

A thermodynamic study based on a microfluidic planar phospholipid membrane (PPM) system is presented in this work. The open well polycarbonate microchip is integrated with a temperature controller and temperature sensor. Ceramide channel is formed in the PPM and channel gating activities are recorded in response to varied temperature. The result confirms the direct correlation between temperature change and physiochemical events at the membrane. More accurate control and measurements can be realized in proposed micro fabricated version. The PPM microchip with temperature variable is particularly useful to gain thermodynamic information for better understanding of structure and function of ion channels.

KEYWORDS: Lipid Membrane, Thermodynamics, Ion channels, Ceramide

INTRODUCTION

Microfabricated planar phospholipid membrane (PPM) systems have been widely investigated as powerful tools for the study of ion channel structure and function. We recently reported a robust microfluidic PPM system [1] capable of monitoring kinetic interactions between ceramide ion channels and modulating chemicals perfused through the surrounding aqueous buffer. Here we extend our initial studies through the integration of on-chip temperature control, enabling the investigation of thermodynamic properties of ion channels within the microfluidic system.

Ceramide (N-acylated spingosine), a sphingolipid enriched within the mitochondrion membrane, is known to regulate apoptosis through the formation of stable transmembrane channels. The mechanism of channel formation is believed to include the hiding of hydrophobic tails into the lipid membrane by molecular rotation, together with the formation of hydrogen bonds between ceramide molecules (Fig 1). The organized circular arrangement of ceramide molecules forms a pore in the phospholipid membrane and the curvature of the channel opening, dictated by ceramide rotation, is optimized to minimize exposure of hydrophobic tails to water (Fig.1a). Thus the assembly and disassembly of monomers involve both entropy and enthalpy. While there is some evidence for this mechanism [2-3], a quantitative thermodynamics study of channel growth and disassembly would significantly improve our understanding of lipid channels and their role in apoptosis. However, macro scale electrophysiology systems have large thermal masses and slow time constants and thus are not suitable tools to this end. In contrast, microfluidics offers a unique approach to the investigation of channel thermodynamics.

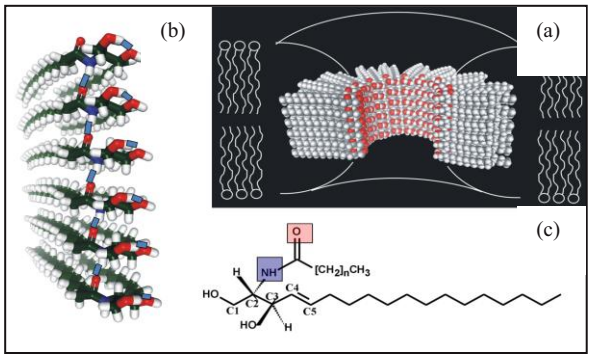

Figure 1: (a)Ceramide channel model. (b) Inter-molecular hydrogen bonds link ceramide into a column. (3) C2-ceramide structure with electron donor in blue box and acceptor in pink box.

EXPERIMENTAL

As shown in Fig. 2, a glass brush is used to gather lipid solution and wiped across an aperture in a thin film polyvinylidene chloride (PVDC), and a lipid membrane can form. The composition of lipids depends on what type of ion channels are studied. In this study, ceramide is mixed with phospholipid in 1:50 molar fraction in hexanol/hexadecane mixture (v/v 10:1). Ceramide channel begins to form typically minutes after the membrane stabilizes. Micro channels are fabricated at bottom and upper polycarbonate (PC) chips, and PC-PVDC-PC stack can be bonded through thermal bonding. Buffer salt solution is filled by syringe pumps and Ag/AgCl electrodes are attached to the reservoir by nanoports (Upchurch, WA). The electrical signal across the membrane and ceramide channel is collected and analyzed in pClamp software (Molecular Devices, CA). Measurements of temperature-dependent channel formation and stability have been performed using a thermoplastic PPM chip containing a small thermocouple and Peltier element integrated into a milled recess beneath the channel formation site.

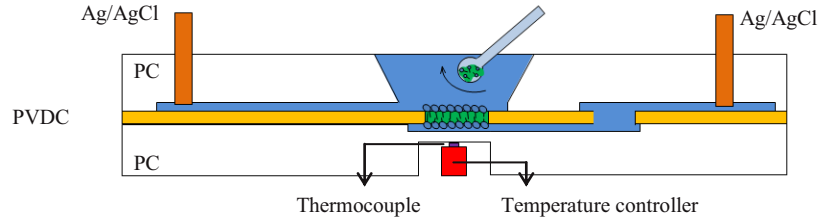

Figure 2: Schematic view of a PPM chip with bulk thermal controller and sensor.

RESULTS AND DISCUSSION

As depicted in Fig.3 and Fig.4, initial data reveal a direct correlation between physiochemical events at the membrane and temperature change. As expected, there is an inverse relationship between ceramide channel conductance and temperature, consistent with the prediction that hydrogen bonds are weakened at higher temperatures, allowing ceramide molecules to dissociate from the channel structure and thus lower the pore diameter and conductance of the ceramide channel. At the same time, more frequent formation and disassembly events were observed as the temperature increased, possibly due to higher diffusion rate of ceramide "rafts" at the membrane and higher thermal energy to overcome rotation barriers at the initial step of formation.

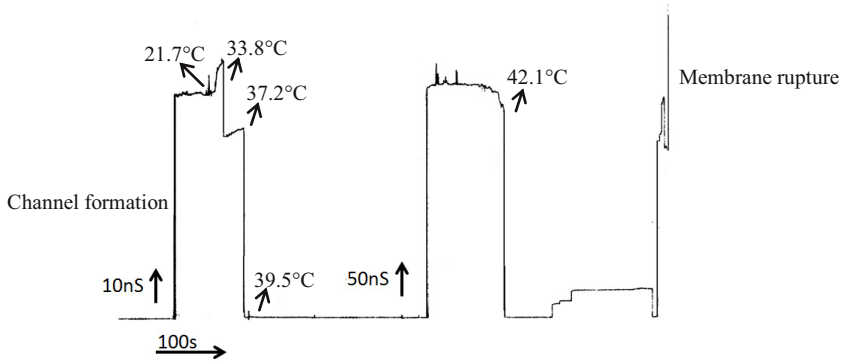

Figure 3: Trans-membrane conductance measurements during heating of the membrane site from room temperature to 42.1°C. The temperature increase is observed to lead to a characteristic pattern of channel disassembly. At higher temperatures, channel lifetime was observed to rapidly degrade.

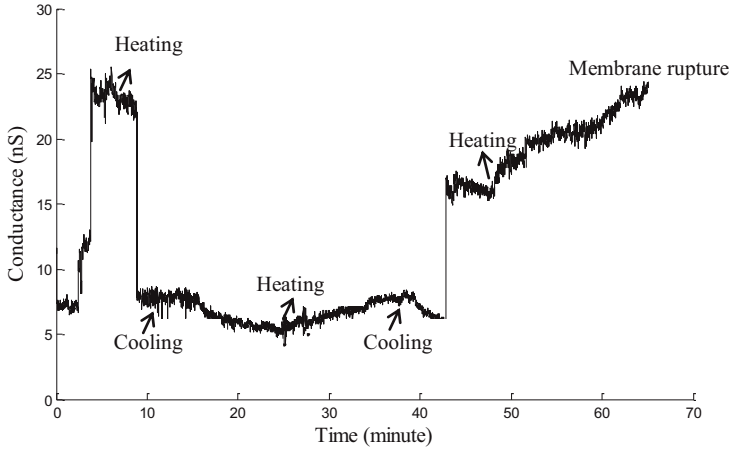

Figure 4: Trans-membrane conductance measurements during repetitive heating and cooling of the membrane site. The heating process raised membrane temperature from 25°C to 33°C and cooling cooled it down back to 25°C. The graduate conductance change is due to conductivity change of salt solution conductivity. Sudden shrinkage is observed at high temperature while sudden enlargement is observed at lower temperature.

To extend these initial measurements, a new microfluidic platform enabling more rapid and precise temperature modulation has been developed by integrating a thin film resistive temperature detector (RTD) adjacent to the phospholipid membrane site. The resistive element was fabricated by shadow masking a 1 μm aluminum pattern on a polyvinylidene chloride (PVDC) film, with contact lines extending along the sloped open well to the upper chip surface, enabling external 4-wire resistive measurements of the RTD for monitoring of the local temperature at the PPM site (Fig.5). Parylene conformal coating

is performed before and after metal deposition to reduce residual stress in the aluminum film and prevent aging of the metal. An image of a typical fabricated chip is shown in Fig 6. Using this platform, statistical studies of ceramide channel lifetimes as a function of temperature will be reported toward the elucidation of channel equilibrium thermodynamics, with the goal of gaining insight into ceramide's role in cell apoptosis.

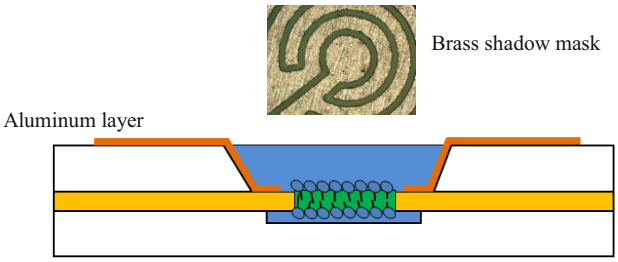

Figure 5. Schematic view of a thin film RTD integrated in microfluidic PPM chip. A 320μm thick brass sheet is pattern by computer numerically controlled machine to work as shadow mask in e-beam evaporation process. 1μm aluminum is deposited and 2μm parylene is coated before and after metal deposition to improve the quality of the metal resistor. Shadow mask for the spiral RTD electrode is shown.

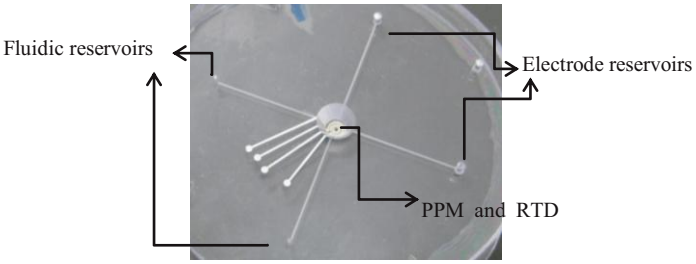

Figure 6. A plastic PPM chip with aluminum RTD element patterned adjacent to PPM site. Stainless steel needles are connected to fluidic reservoirs and wire Ag/AgCl electrodes are held to electrodes reservoirs by nanoports and adhesive paste (not shown).

CONCLUSION

An *in vitro* ion channel thermodynamics study system is fabricated by integrating thermal controller and sensor into a PPM micro chip. Smaller thermal mass offered in this system enables us to study responses of ion channel to rapidly modulated temperature. We demonstrate that the activities of ceramide channel differ significantly in room temperature and temperature higher than 35°C. The ability to study temperature dependent ion channel activities will provide unique insight for ion channel conformation change and their roles in cell signaling. The proposed chip design with integrated thin film temperature sensor will improve the accuracy of temperature, and enable a quantitative study of ceramide channel thermodynamic.

ACKNOWLEDGEMENTS

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