4-21-2023

Changes in Lipid Order Induced by Hypoosmotic Stress and Channels Insertion

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Abstract

In this work, we report on the effects of hypoosmotic stress, cholesterol depletion, and pore-forming toxin on physical properties of membranes, such as lipid order and fluidity. To achieve our goals, we employed Red Blood Cells as model membranes, and Laurdan as a solvato-chromic indicator of the membrane state. The generalized polarization function of Laurdan's fluorescence was measured in different environmental conditions, and the experimental data were interpreted in terms of changes in lipid order and fluidity as previously reported for lipid membranes undergoing temperature-induced phase transitions. Our experiments indicate that hypoosmotic stress leads to a reduced lipid order and increased fluidity of the membrane, with a sharp transition between the two states. Controlled depletion of cholesterol in the membranes by exposure to Methyl-beta-cyclodextrin also indicates the role played by cholesterol in the ordering of the lipids, permeability, and general stability. Exposure of membranes to the pore-forming toxins Streptolysin O and lysenin also leads to decreased lipid order and increased fluidity, which we explained by the hydrophobic mismatch between the membrane's core and inserted proteins.

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RONALD E. MCNAIR POST-BACCALAUREATE ACHIEVEMENT PROGRAM

ABSTRACT

In this work, we report on the effects of hypoosmotic stress and pore-forming toxin on physical properties of membranes, such as lipid order and fluidity. To achieve our goals, we employed Red Blood Cells as model membranes, and Laurdan as a solvato-chromic indicator of the membrane state. The generalized polarization function of Laurdan's fluorescence was measured in different environmental conditions, and the experimental data were interpreted in terms of changes in lipid order and fluidity as previously reported for lipid membranes undergoing temperature-induced phase transitions. Our test investigations indicate that controlled depletion of cholesterol in the membranes by exposure to Methyl-β-cyclodextrin indicates the role played by cholesterol in the ordering of the lipids, permeability, and general stability. Hypoosmotic stress leads to a reduced lipid order and increased fluidity of the membrane, with a sharp transition between the two states. Exposure of membranes to the pore-forming toxin lysenin also leads to decreased lipid order and increased fluidity, which we explained by the hydrophobic mismatch between the membrane's core and inserted proteins.

MOTIVATION

Mechanical properties originating in lipid ordering in membranes gain more and more attention as modulators of fundamental cellular functions, which has large implications for a better understanding of basic biological processes in health and disease [1]. In this work, we demonstrate the use of solvato-chromic dyes as indicators of lipid order in target membranes.

MATERIALS AND METHODS

RBC Preparation and Characterization:

Sheep RBC were centrifuged at 3,500 RPM and washed four times with PBS (pH 7.2). After the last wash, an RBC density of 3E9 cells/mL was determined with a bench cytometer. 1 mL of the RBC solution was exposed to Laurdan in EtOH (5 μM final concentration in the RBC mixture) for two hours. After exposure, the cells were washed three times with PBS. Next, the RBCs (20 μL RBC stock to 3 mL solution) were suspended in PBS-only (for Methyl- β -Cyclodextrin MBCD and Pore-Forming Toxin exposure) or mixtures of PBS and water (for hypoosmotic shock). RBCs were treated with MBCD (final concentration ranging from 0 μM to 1000 μM) for one hour at room temperature. Exposure to hypoosmotic shock was carried out at room temperature for 20 minutes, and exposure to Lysenin (Lys) was performed for 40 minutes also at room temperature. All the exposures were carried out under continuous rotation of the reaction tubes in a bench rotator.

Fluorescence:

The fluorescence of the sample was measured by employing a n Horiba Fluoromax 4 fluorometer (Jobin Yvon). We utilized the single point measurement protocol for simultaneous detection of fluorescence at 440 nm and 490 nm upon excitation at 350 nm. The maximum measurement error for each individual point was set to 1%; none of the samples presented a measurement error surpassing 0.8%. The emission intensities at 440 nm and 490 nm were used to estimate the Generalized Polarization function (GP) [2] with the formula:

$$GP = \frac{I_{440} - I_{490}}{I_{440} + I_{490}}$$

RESULTS AND DISCUSSION

MBCD addition leads to a gradual decrease of generalized polarization (GP), in a concentration dependent manner (Figure 1). This is indicative of the known fact that cholesterol extraction reduces the lipid order and increase the fluidity of the target membrane [3].

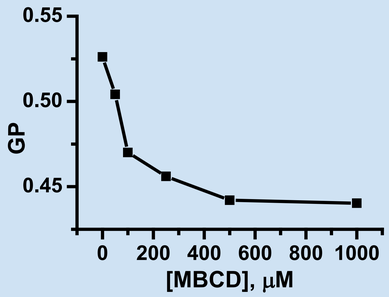

Figure 1. Cholesterol extraction by exposure to MBCD reduces the GP in a concentration-dependent manner and indicates an increased lipid disorder and membrane fluidity.

Our next fluorescence spectroscopy explorations aimed at investigating the suitability of Laurdan to indicate solvation of membranes and changes in lipid order upon controlled hypoosmotic pressure. One may observe that osmotic pressure leads to a relatively sharp transition of GP (Figure 2), very similar to what is observed around the melting point for artificial membrane systems that do not contain cholesterol [3]. We hypothesize that the sharp transition occurs due to the short-range interactions between the hydrophobic tails of the lipids.

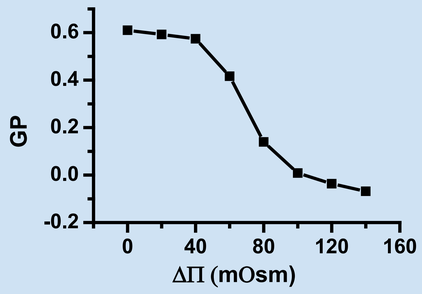

Figure 2. Hypoosmotic pressure adjust the lipid order and fluidity, as indicated by the gradual yet sharp decrease of the GP. The sharp transition resembles the effect of temperature on cholesterol-free artificial membranes.

We employed Laurdan's ratiometric measurements to determine the GP function for RBCs exposed to increasing amounts of lysenin (Figure 3). Lysenin addition led to a gradual decrease in the GP values, which we interpreted based on the Laurdan's ability to indicate lipid order, fluidity, and water exposure. The GP decreased with lysenin's concentration, indicating that toxin addition improved the accessibility of water into the membrane in a concentration dependent manner. This effect is assumed to originate in a hydrophobic mismatch [4] between the protein and the membrane (Figure 4).

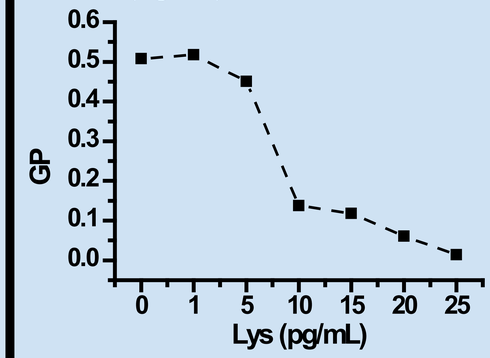

Figure 3. Addition of Lysenin also diminished the lipid order and increased the target membrane's fluidity in a concentration dependent manner.

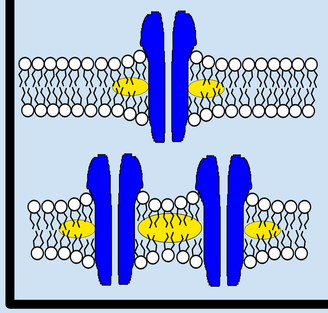

Figure 4. Hydrophobic mismatch leads to lipid destabilization. At low Lys concentrations, the destabilization occurs only for a short range around the inserted protein (top panel). At higher Lyse concentration, a more significant destabilization and lipid disordering occurs between the inserted proteins (bottom panel). The highlighted regions indicate destabilization.

CONCLUSION

Our experiments demonstrate that hypoosmotic shocks and protein insertion may lead to lipid disordering in target membranes. The sharp transition observed under hypoosmotic pressure may be explained by the short-range intercations between lipids. Lipid disordering by inserted proteins may be explained by considering a hydrophobic mismatch between the protein and the hydrophobic core of the membrane.

References: 1. Los, D., Murata, N., DOI: 10.1016/j.bbamem.2004.08.002; 2. Hamada N., Longo M., DOI: 10/1016/j.bbamem.2022.183887; 3. Stott B., et al, DOI: 10.11954/jlr.M700479-JLR200; 4. Phillips R, et al, DOI: 10.1038/nature08147

Acknowledgement: This work was supported by the National Science Foundation CAREER Award 155416, the TRIO-McNair Program, the Department of Physics, and the Biomolecular Sciences Graduate Program at Boise State University.