Lipid Order in Red Blood Cell Membranes Exposed to Hypo-osmotic Stress and Self-Inserting Pore-Forming Proteins

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Background

The bilayer lipid membrane that serves as the scaffold for all cell membranes is recognized to be key to life for all organisms as it allows for intercellular communication and transport. One characteristic of the bilayer lipid membrane is the compactness of the phospholipids in the layers: this is referred to as the lipid order. The lipid order in the membrane influences the passive and active transport of ions' and molecules' motility, signaling, and receptor functionality. A high lipid order is densely packed. Lipid order research has clarified temperature-induced phase transitions, but other means of changing lipid order have not been sufficiently explored. This can advance our current knowledge as environmental factors affecting lipid order may change cell functionality and relate specific biomechanical membranal properties to diseases. Exploitation of lipid order transition methods could result in a more efficient drug uptake design and further define the process of cancer cells traveling through the bloodstream and spreading. In this experiment, we sought to quantify the changes in lipid order resulting from inserted pore-forming toxins and hypo-osmotic stress.

Materials and Methods

The red blood cells prepared for this experiment were centrifuged multiple times, washed using 1X PBS, and resuspended in PBS. For each experiment, 2 mL of red blood cells were stained with fluorescent marker [Laurdan or diphenylhexatriene (DPH)], 5 μM final concentration. To ensure the fluorescent markers were evenly distributed in the solution, stained red blood cells were vortexed immediately prior to fluorescence measurements.

For hypo-osmotic experiments, stained, red blood cells were placed in solutions of PBS: water in concentrations ranging from 0% water to 80% water and rotated for 20 minutes before fluorescence measurements.

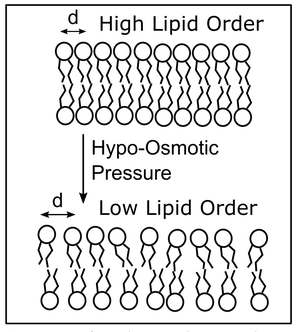

Figure 1: The figure to the left is a model representing the changes in lipid order induced by hypo-osmotic pressure. As pressure increases, the distance between adjacent lipids increases and lipid order decreases. The figure is not to scale.

For pore-forming toxin experiments, stained red blood cells were added to serially diluted solutions of a pore-forming toxin (lysenin) and rotated for 1 hour at room temperature before fluorescence measurements were taken.

Two fluorescence measurements were used to quantify our data: general polarization and anisotropy. To obtain the information needed to calculate general polarization, we used a spectrofluorometer to excite Laurdan at 410 nm with two resulting emission wavelengths at 440 nm and 490 nm. From there, we used the formula

$$GP = \frac{I_{440}-I_{490}}{I_{440}+I_{440}} \quad (1)$$

to calculate general polarization. To obtain the information needed to calculate anisotropy, the spectrofluorometer was equipped with a polarizer which could be turned horizontally or vertically to obtain measurements from DPH parallel with the emission wavelength. With the obtained intensity measurements, we used the formula

$$p = \frac{I_{\parallel}-I_{\perp}}{I_{\parallel}+2I_{\perp}} \quad (2)$$

to calculate anisotropy.

Results and Discussion

1. General Polarization vs Hypo-osmotic Pressure

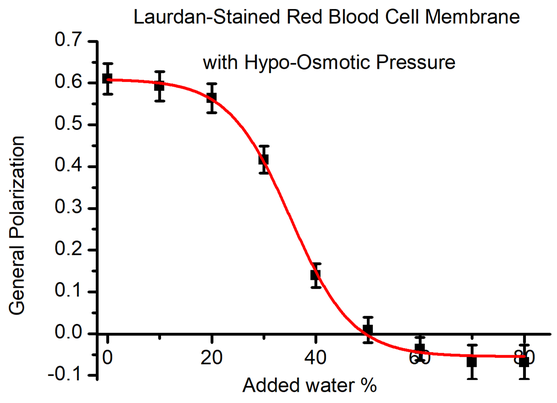

Figure 2: Graph of general polarization (calculated from spectrofluorometer measurements and Equ. 1) vs hypo-osmotic pressure (controlled by the concentration of water in the environmental solution. As the surrounding concentration of water increases, the equilibrium shifts to force water entry into the red blood cell membrane. As the intramembrane concentration of water increases, the Laurdan, which is an electric dipole, undergoes dipolar relaxation, decreasing general polarization.

2. Anisotropy vs Hypo-osmotic Pressure

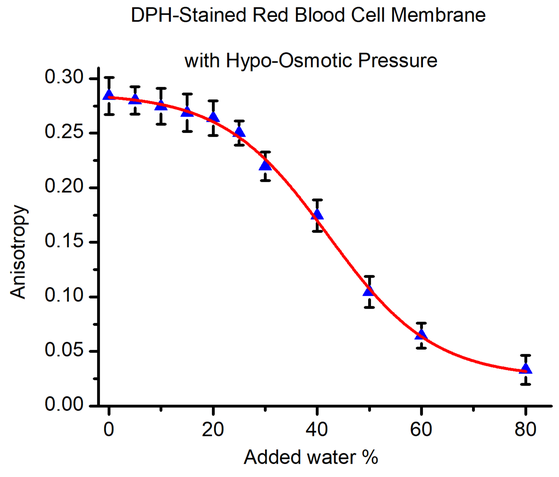

Figure 3: Graph of anisotropy (calculated from spectrofluorometer measurements and Equ. 2) vs hypo-osmotic pressure (controlled by the concentration of water in the environmental solution). Anisotropy measures intensity of the dye in directions parallel to the excitation wavelength. Using polarized excitation beams, we obtained data measuring the fluorescence of lipids constituting the membrane in specific directions, which is influenced by lipid order. The lower calculated anisotropy is indicative of higher rotational diffusion (decreased lipid order) within the membrane.

3. General Polarization vs Lysenin Concentration

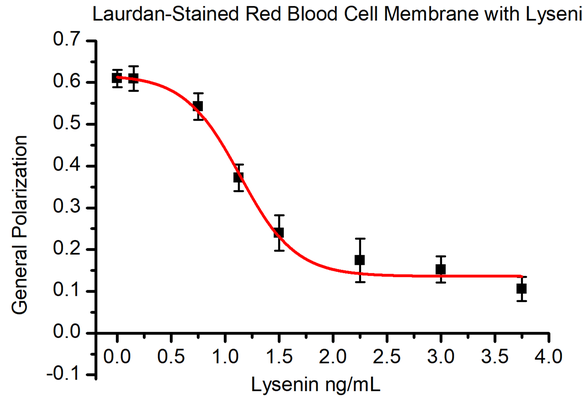

Figure 4: Graph of general polarization (calculated from spectrofluorometer measurements and Equ. 1) vs concentration of lysenin in the environmental solution. As more proteins are inserted into the membrane, the lipid order decreases and the average calculated general polarization decreases.

4. Anisotropy vs Lysenin Concentration

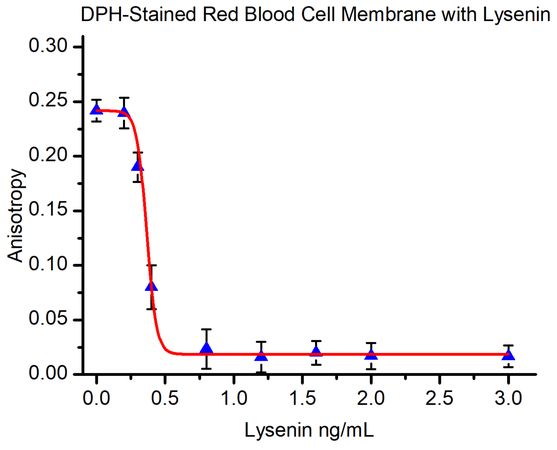

Figure 5: Graph of anisotropy (calculated from spectrofluorometer measurements and Equ. 2) vs concentration of lysenin in the environmental solution. As proteins self-insert into the membrane, we see the rotational diffusion within the membrane increases, indicative of a decreased lipid order within the membrane.

5. Proposed Model of Inserted Protein's Effect on Lipid Order

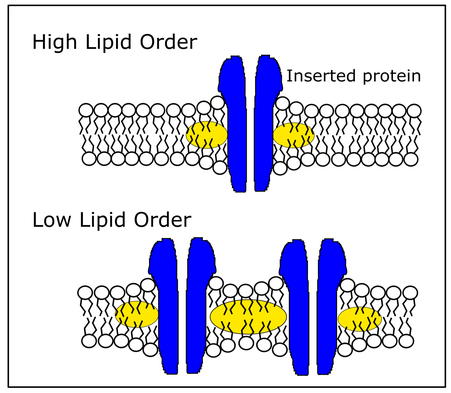

Figure 6: Proposed model of self-inserting pore-forming proteins into a red blood cell membrane. As the thickness of the cell membrane varies to accommodate transmembrane proteins, the distance between the monolayers increases, resulting in a weakened attraction between nonpolar phospholipid tails. This weakened attraction, combined with the variable thickness of the membrane, constitutes a lower lipid order.

Conclusions

The experimental data produced in our experiments is indicative of a changing lipid order due to hypo-osmotic pressure and self-inserting pore-forming proteins similarly observed in temperature-induced phase transitions. The sigmoidal curve produced by general polarization and anisotropy of the membrane as a function of hypo-osmotic stress and pore-forming proteins indicates a strong correlation between lipid order and the extent of the hypo-osmotic pressure and concentration of self-inserting pore-forming proteins. Because of the importance of lipid order in cell signaling and cell transport, including drug delivery systems, our findings can be used to artificially alter lipid order and increase the likelihood of cell transport for drugs and bioactive molecules.

Acknowledgements

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References & Additional Information

Whiting, Rose, Sevio Stanton, Maryna Kucheriava, Aviana R. Smith, Matt Pitts, Daniel Robertson, Jacob Kammer, Zhiyu Li, and Daniel Fologea. 2023. "Hypo-Osmotic Stress and Pore-Forming Toxins Adjust the Lipid Order in Sheep Red Blood Cell Membranes" *Membranes* 13, no. 7: 620. https://doi.org/10.3390/membranes13070620

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