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Characterization of giant unilamellar vesicles composed of natural lipids extracted from rat kidney brush border and basolateral cell membranes: A two photon excitation microscopic study.




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**Characterization of giant unilamellar vesicles composed of natural lipids extracted from rat kidney brush border and basolateral cell membranes: a two photon excitation microscopic study.**

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Abstract

In polarized epithelial cells there is a marked difference in the lipid fluidity of apical and basolateral membranes, due to major differences in cholesterol and sphingomyelin composition. The purpose of this study was to further characterize differences in lipid dynamics of these cell membranes. We prepared giant unilamellar vesicles (GUVs) by the electroformation procedure, using natural lipids extracted from rat kidney Brush Border (BBM) and Basolateral (BLM) membranes. We studied the temperature response of LAURDAN labeled individual GUVs using the sectioning capability of the two-photon excitation microscope. Fluorescence intensity images of LAURDAN labeled GUVs were used to calculate the spatial distribution of the LAURDAN Generalized Polarization function (GP) that allows discrimination between different lipid phases. At all temperatures, the center of the GP histograms is higher in the BBM with respect to BLM preparations. As the temperature was decreased, we detected formation of lipid domains on the surface of the BBM and BLM GWVs at -45°C and -37°C, respectively. The lipid domains persisted on the vesicle surface throughout the temperature range 37- 25°C. The domains drifted upon the vesicle surface. From analysis of the LAURDAN intensity images, we concluded that the GUVs exhibit ordered and less ordered phases. Our results are consistent with the formation of "lipid rafts" on these natural lipid extract membranes. Supported by grants from the National Institutes ofHealth (RRO3 155 and FFCC), JDFI and VA.