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Characterization of giant unilamellar vesicles of integral membrane extracts from rat kidney brushborder and basolateral membranes using two photon fluorescence microscopy.

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Abstract

Polarized epithelial cells exhibit differences in structure and composition in certain regions of the cell membrane. In this study, we characterize differences between brushborder (BBM) and basolateral (BLM) membranes from rat kidney cells in terms of membrane fluidity and topology. Previous studies using steady-state fluorescence spectroscopy suggested that these membranes exhibit coexistence of lipid domains at physiological temperatures. Giant unilamellar vesicles (GUVs) were formed from integral (containing both lipid and protein components) BBM and BLM extracts. Images of the Laurdan labeled GUVs were obtained through the sectioning capability of twophoton fluorescence microscopy. Laurdan Generalized Polarization function (GP) was calculated at different temperatures to study the membrane phase-state in these preparations. A novel result for both preparations was the direct observation of circular micron-size lipid domains on the GUV surface, supporting the hypothesis of “lipid raft” formation. GP values were consistently higher for the BBM as compared to the BLM. Furthermore, GP values for the integral BBM and BLM preparations were higher than those found for BBM and BLM natural lipid extracts, suggesting an important role for membrane proteins in bilayer packing. Supported by grants from the NIH (RR03155 and FFCC), VA, JDFI and AHA.