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Membrane dynamics in giant unilamellar vesicles (GUVs) of rat kidney brushborder and basolateral integral membrane extracts.

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Abstract

The plasma membrane in rat kidney proximal tubular cells exhibits polarity in terms of composition, structure and function. It is divided into two distinct regions: the brushborder (BBM) and basolateral (BLM) membranes. We aim to study the complex structure of these biological membranes by applying techniques used in characterizing artificial membrane systems. This involves the direct visualization of GUVs formed from integral BBM and BLM extracts (containing both lipid and protein components) through electroformation. GUVs labeled with the fluorescent probe LAURDAN were imaged using two-photon scanning microscopy. Fluorescence intensity images reveal the formation of micron-sized nonfluorescent domains at physiological temperatures (42-20°C). The absence of intensity in the domains is presumably due to the exclusion of LAURDAN from these regions. These domains differ greatly in shape from those observed in GUVs formed from natural lipid extracts. This implies that membrane proteins also play an important role in the organization of natural membranes. The presence of proteins in the GUVs was confirmed through primary and secondary antibody detection of several membrane proteins (NaPi-II cotransporter, b-actin and Na+/K+ ATPase). Supported by NIH (RR03155 and FFCC), VA, JDFI, Fundación Antorchas (Argentina) and AHA.