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Two-photon fluorescence microscopy of laurdan gp domains in biological membranes using polarized laser excitation.

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Abstract

Two-photon microscopy images of membranes of whole red blood cells, renal tubular cells (ORcells) and purified rat renal brush border and basolateral membranes labeled with LAURDAN show a fine texture of coexisting regions of different generalized polarization (GP) values. By measuring the relative water content of the membrane, LAURDAN GP function also monitors the lipid molecular dynamics or "fluidity". We have used polarized laser excitation. In images with well defined membrane orientation, such as the erythrocyte cell sections, the association of high GP with high polarization photoselection allows the attribution of coexisting GP values to lipid domains of different dynamical properties. Due to the presence of strong fluorescence from the cytoplasmic membranes, the interpretation of the membrane GP texture in images of renal tubular cells is more complex. After masking the cytoplasmic membranes, analysis of the images of red blood cells and of renal tubular cells lead to the conclusion that the coexisting regions of different GP values, i.e., of different "fluidity", have dimensions comparable to the microscope resolution in red blood cells and smaller than the microscope resolution (about 200 nm) in renal tubular cells. For their complex and mostly unknown morphology, the presence of GP domains in rat renal brush border and basolateral membranes is of difficult interpretation. Nevertheless, by plotting selected GP values, different membrane structures become apparent. (Supported by grants from NIH, VA, and CNR).