The Localization of Ganglioside GM1 in Phase Separated Model Membranes.

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Atomic force microscopy (AFM) and near-field scanning optical microscopy (NSOM) have been used to study the distribution of ganglioside GM1, and its fluorescent analog BODIPY-GM1, in model membranes that mimic the composition of lipid rafts. Langmuir Blodgett technique was employed to prepare phase separated, supported monolayers of 2:2:1 sphingomyelin (SM) / dioleoylphosphatidylcholine (DOPC) / cholesterol (CHL). Atomic force microscopy following the addition of 0.5% and 5% GM1 to these monlayers revealed ganglioside enriched clusters that were localized in the ordered SM/CHL phase, however ganglioside clusters could not be detected by AFM following the addition of 0.5% or 5% BODIPY-GM1. Near-field scanning optical microscopy of the BODIPY-GM1 monolayers revealed that the dye-labeled ganglioside was not in the ordered SM/CHL phase, but in the fluid DOPC phase. The BODIPY fluorophore emits maximally at 520 nm as a monomer but when clustered, can form an eximer that exhibits a red shifted emission at 620 nm. Closer inspection of the fluid DOPC phase by NSOM revealed that monomeric BODIPY-GM1 was located homogenously throughout, with small clusters of BODIPY-GM1 that showed excimer emission associated with the margins of SM/CHL domains. Vesicle fusion was used to study the localization of GM1 in supported bilayers of 2:2:1 SM/DOPC/CHL. Atomic force microscopy following the addition of 0.5% GM1 to these bilayers and incubation with cholera toxin B subunit resulted in the detection of the GM1 in the SM/CHL rich domains via the specific binding of the protein. Supported bilayers with 0.5% BODIPY-GM1 do not bind cholera toxin B subunit indicating that it is in the fluid phase. Visualization of BODIPY-GM1 in these supported bilayers by NSOM and possible fluorescence resonance energy transfer between BODIPY-GM1 and dye-labeled cholera toxin will be discussed.