Characterization of glycolipids of sand dollar (*Mellita quinquiesperforata*): identification of novel glycosphingolipid containing sialic acid and sulfate group

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**Introduction:** Glycosphingolipids (GSLs) are ubiquitous membrane components present mostly in the outer leaflet of the plasma membrane and organized in microdomains, these glycoconjugates have key roles in a variety of biological systems. Here we describe the expression of GSLs of sand dollar (*Mellita quinquiesperforata*) as a part of a study on the structural characterization and organization of membrane microdomains in invertebrates.

**Objectives:** The aim of this study was to analyze the GSL profile of sand dollar by high performance thin layer chromatography (HPTLC) and to perform structural studies of GSLs by mass spectrometry (ESI-MS).

**Methods:** Total GSLs of sand dollar were extracted with isopropyl-alcohol/hexane/water and chloroform/methanol. Neutral and acidic GSLs were separated by DEAE-Sephadex chromatography. GSLs were quantified by HPTLC densitometry and further purified by HPLC. Structural studies of isolated GSLs were performed by mass spectrometry in a Varian 310-TQ-MS. The carbohydrate moieties were analyzed by HPTLC after trifluoroacetic acid hydrolysis.

**Results:** Thirteen GSL bands were visualized by HPTLC (205.6 µg GSLs/mg tissue). Structural analysis by ESI-MS of the acidic GSL fraction eluted with 0.6M of sodium acetate from DEAE column, revealed a sulfated-GM4, with isoform peaks at 1187, 1189, 1203 and 1205\textit{m/z}. CID-MS of these sulfated-GM4 showed peaks at 817\textit{m/z} (loss of sialic acid and sulfate) and 654\textit{m/z} (loss of a hexose). The ceramide moieties of these hybrid GSLs is composed of t18:0 phytosphingosine, the fatty acid identified were h22:2 and h22:1. Also it was detected the presence of N-Acetylneuraminic acid for peaks 1187/1189\textit{m/z} and N-Glycolylneuraminic acid for peaks 1203/1205\textit{m/z}.

**Discussion and Conclusions:** The data presented indicate that sand dollar expresses a glycosphingolipid with sialic acid and sulfate group. This unusual compound has a glucose residue to which is linked a sialic acid and also a sulfate group. It is also noteworthy that this equinoid expresses both N-Acetylneuraminic and N-Glycolylneuraminic transferases as well as sulfotransferases.

Keywords: Glycosphingolipids, sand dollar, sulfated GM4, gangliosides, *Mellita quinquiesperforata*.

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