DETERMINATION OF STRUCTURAL-FUNCTIONAL INTERACTIONS OF GANGLIOSIDES WITH PEPTIDES AND PROTEINS BY MICROFLUIDICS –MASS SPECTROMETRY

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Abstract

Gangliosides (GGs) mediate vital biological processes through non-covalent intermolecular interactions. To understand the structure-function relationship at the molecular level for each GG structural entity involved in a physiological / pathological process and to improve the therapeutic significance, it is necessary to determine their interactions in detail using the most accurate methods of analysis. To address the issues of high biological relevance of GGs, mass spectrometry (MS) has lately become a method of choice due to its capability to detect minor species in complex mixtures with an unsurpassed sensitivity.

The noncovalent interaction between the Amyloid beta (Aβ) protein and a native complex mixture of gangliosides extracted and purified from normal adult human brain was studied using an analytical platform encompassing fully automated chip-nanoelectrospray ionization (nanoESI) on a NanoMate robot coupled to a high-capacity ion trap (HCT) mass spectrometer (MS). The interaction assay involved the incubation at 37 °C under constant steering of Aβ and gangliosides dissolved in 10 mM ammonium acetate buffer, pH 6.0, up to a concentration of 1 pmol μL^{-1} and 10 pmol μL^{-1}, respectively. Aliquots of the reaction products were collected directly after 1, 5, 10, 15, 30, 60 and 180 min of incubation in the 96-well plate of the NanoMate robot and immediately submitted to MS analysis.

Chip-nanoESI QTOF MS and CID MS/MS revealed the formation of the Aβ-GT1 (d18: 1/18:0) non-covalent complex formed between the protein and the dihydroxylated sphingoid base of GT1, detected as $[M + 4H]^{4+}$ at *m/z* 1615.181 and the Aβ-GT1 complex (t18:1/18:0) of the protein with a trisialylated trihydroxylated ceramide species, detected at *m/z* 1618.902. CID MS/MS top-down fragmentation analysis at low energy demonstrated that the Aβ protein binds to a GT1b isomer type structure (with a monosaccharide Neu5Ac to external galactose and a disialo element Neu5Ac-Neu5Ac to internal galactose). Thus, by chip-MS and tandem MS experiments it was possible to deduce the structure of this non-covalent complex as: Aβ-GT1b (d18:1/18:0). Similar results (GT1b isomer) were obtained also for the complex formed with the GG having a trihydroxylated ceramide, hence resulting a complex with Aβ-GT1b (t18:1/18:0) composition.

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