Scien. Tech. Bull-Chem. Food Sci. Eng., Vol. 17 (XVIII), 2020, 15-19

HIGH RESOLUTION MASS SPECTROMETRY WITH CHIP-BASED IONIZATION FOR THE ASSESSMENT OF NONCOVALENT INTERACTIONS OF PROTEINS WITH NORMAL BRAIN AND BRAIN TUMOR GANGLIOSIDES

Raluca ICĂ[1,2], Mirela SÂRBU[1] and Alina D. ZAMFIR[1,3]

[1]National Institute for R&D in Electrochemistry and Condensed Matter, Timisoara, Romania
[2]Faculty of Physics, West University of Timisoara
[3]"Aurel Vlaicu" University of Arad, Romania
Corresponding author email: alina.zamfir@uav.ro

Abstract: *The noncovalent interactions between the amyloid beta (Aβ) and α-synuclein (Syn) proteins with native gangliosides extracted and purified from human brain and human glioma were studied using an analytical platform encompassing fully automated chip-nanoelectrospray (nanoESI) on a NanoMate robot coupled to a quadrupole time-of-flight (QTOF) mass spectrometer (MS). The novel interaction assays developed here for this purpose involved several stages: i) the incubation at 37°C under constant steering of the protein and gangliosides in 10 mM ammonium acetate buffer, pH 6.0, up to a concentration of 1 pmol μL^{-1} and 10 pmol μL^{-1}, respectively; ii) collection of aliquots directly into the 96-well plate of the robot after 1, 5, 10, 15, 30, 60 and 180 min of incubation and iii) immediate submission of the reaction products to MS screening and structural analysis. Chip-nanoESI QTOF MS and CID MS/MS revealed the formation of the Aβ-GT1(d18:1/18:0) and Aβ-GT1 (t18:1/18:0) complexes as well as the preferential biding of Syn to GD1, GT1, GQ1 and GO1 species. CID MS/MS top-down fragmentation analysis demonstrated that the Aβ protein binds to a GT1b isomer type structure, characterized by Neu5Ac linkage to the external galactose and a disialo element Neu5Ac-Neu5Ac bound to the inner galactose of the molecule. 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 were obtained also for the Aβ complex formed with the gangliosides having trihydroxylated ceramide as well as the complexes of Syn with G1 class of gangliosides.*

Keywords: Gangliosides, Noncovalent interactions/complexes, Chip-nanoelectrospray mass spectrometry.

INTRODUCTION

Gangliosides (GGs) represent a particular class of glycosphingolipids with a complex structure consisting of a ceramide (Cer) moiety, of variable composition with respect to the types of sphingoid base and fatty acid residues, glycosidically linked to an oligosaccharide chain containing one or more sialic acid units. Gangliosides are present in all mammals, both in tissues and body fluids, systematic quantitative analyses have demonstrated that the highest concentration is found in the central nervous system: brain, spinal cord and cerebrospinal fluid. In brain tissue, for instance, gangliosides represent 6% of the total mass of lipids [1]. 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. 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 [2,3].

The development of MS techniques which, due to superior sensitivity, selectivity, resolution, reproducibility and analysis speed represent nowadays the state-of-the-art in bioanalytics, has led to important findings in glycolipidomics, a vast number of glycolipid

isomers, isobars, and conformers were not only discovered *de novo*, but could also be associated to severe disorders and malignant transformations [4,5] and studied as high-affinity ligands for certain proteins [3,6]. In this context, we report here upon the development of a strategy for studying the noncovalent protein-ganglioside interactions. The interaction was monitored using fully automated chip-nanoelectrospray ionization (nanoESI) on a NanoMate robot in laboratory coupled to a quadrupole time-of-flight (QTOF) mass spectrometer.

MATERIALS AND METHODS

I) INTERACTION PRECURSORS

A) Amyloid beta protein sequence from Merck KGaA (Darmstadt, Germany): *(*Aβ 1-40) Amyloid β Protein Fragment, (molecular weight, MW: 4329.82 Da) derived from the amyloid-β protein (Aβ), *DAEFR HDSGY EVHHQ KLVFF AEDVG SNKGA IIGLM VGGVV* in interaction with a native GG mixture extracted from normal adult (45 y.o.) human brain.

B) Human recombinant α-synuclein (Syn 1-140, MW: 14459.70 Da) from Merck KGaA (Darmstadt, Germany) having the following amino acid sequence: *MDVFM KGLSK AKEGV VAAAE KTKQG VAEAA GKTKE GVLYVG SKTKE GVVHG VATVA EKTKE QVTNV GGAVV TGVTA VAQK T VEGAG SIAAA TGFVK KDQLG KNEEG APQEG ILEDM PVDPD NEAYE MPSEE GYQDY EPEA* in interaction with a native GG mixture extracted and purified from human glioma.

II) CHIP-BASED MASS SPECTROMETRY

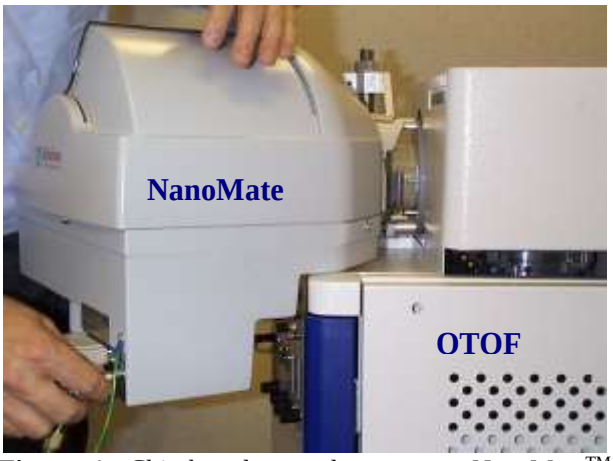

Figure 1. Chip-based nanoelectrospray, NanoMate™ robot incorporating the 400-nozzle ESI Chip technology (Advion BioSciences, Ithaca, USA) in laboratory coupled to a QTOF MS (Waters, Manchester, UK). The electrospray process was initiated at 1.3 kV applied on the pipette tip and 0.40 psi nitrogen back pressure

III) BINDING ASSAY AND CHIP-MS ANALYSIS OF THE REACTION PRODUCTS

In order to achieve the interaction, a series of testing and optimization experiments using different solvent systems for identifying an appropriate buffer system were carried out. Optimal results were obtained using the ammonium acetate/acetic acid buffer system. A schematic of the workflow is presented in Figure 2.

Briefly, the interaction assay involved the incubation at 37 °C under constant stirring of the protein and gangliosides dissolved in 10 mM ammonium acetate buffer, pH 6.0, to a concentration of 1 pmol μL^{-1} and 10 pmol μL^{-1}, respectively. Aliquots of the reaction products were collected after 1, 5, 10, 15, 30, 60 and 180 min in the 96-well plate of the NanoMate robot and immediately submitted to QTOF MS and MS/MS by CID in positive ion mode.

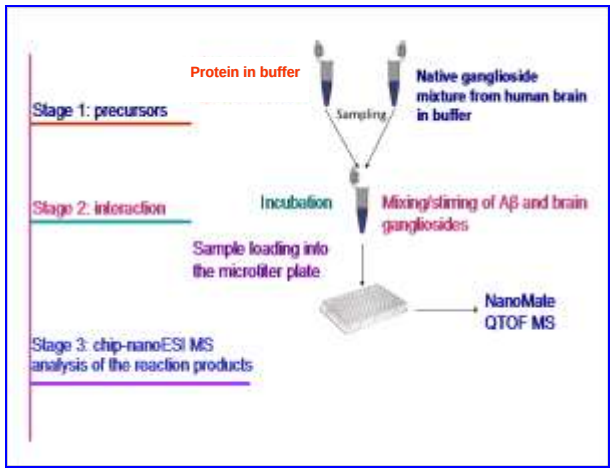

Figure 2. Schematic of the workflow for studying the noncovalent protein-ganglioside interactions by NanoMate-QTOF MS

RESULTS AND DISCUSSIONS

Screening of the Aβ 1-40 (Figure 3) in buffer revelaled two signals, which, according to mass calculation correspond to the triply protonated Aβ 1-40 (measured MW: 4330.563 Da, mass accuracy: 171 ppm) and to Aβ 1-41 having the amino acid sequence *DAEFR HDSGY EVHHQ KLVFF AEDVG SNKGA IIGLM VGGVVI*, which was also detected in the sample as a triply deprotonated molecule at *m/z* 1482.615 (measured MW: 4445.445 Da).

NanoMate-QTOF MS analysis of the reaction products revealed the formation of four noncovalent complexes of Aβ (1-40) with GT1(d16:1/18:0), GT1(d18:1/18:0), GT1(t18:1/18:0) and GQ1(d18:1/18:0), all detected as tetraprotonated $[M+4H]^{4+}$ molecules (Figure 4). Interestingly, only complexes with highly sialylated, tri- and tetrasialo GG species were found. These findings do not exclude the possible formation of complexes with GM and GD structures as well, but highlights the possible role of sialylation in protein-ganglioside interactions, which claims for further studies in this direction.

By applying the same protocol for the study of the noncovalent interaction between α-synuclein and GGs extracted from human glioma tumor it could be deduced that α-synuclein formed non-covalent complexes with the species of G1 class, *i.e.* those species that exhibit the longest glycan chain. Furthermore, a high affinity of α-synuclein protein was observed for polysialylated structures.

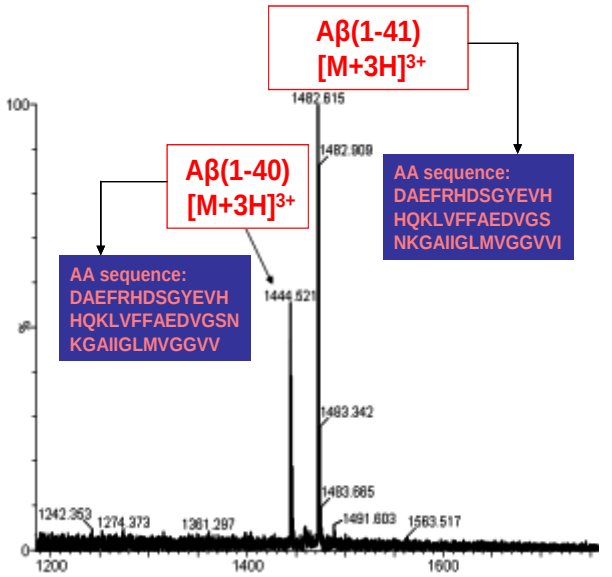

Figure 3. NanoMate-QTOF MS in positive ion mode of the Aβ protein in buffer. Concentration: 1 pmol μL^{-1}; acquisition time 2 min. nanoESI: 1.2 kV; cone voltage: 50 V; nitrogen at 0.40 psi. Buffer system: 10 mM ammonium acetate/ acetic acid pH 6.0.

The complexes formed and detected are with GGs containing several Neu5Ac units in the sugar chain, namely GD1, GT1, GQ1 and GO1.

It is noteworthy to mention that some of these species, *i.e.* GO1, a ganglioside species of high sialylation degree that interacted with α-synuclein, could not be detected in glioma by direct nanoESI chip MS screening of the native ganglioside extract, neither in buffer nor in water/methanol, mainly due to the reduced expression of such structures in the tissue; on the other hand, the unusually high complexity of the mixtures, as those extracted from human brain and brain tumors, represents also a factor leading to the difficulty in detection and identification of low abundant species by simple sample profiling by MS.

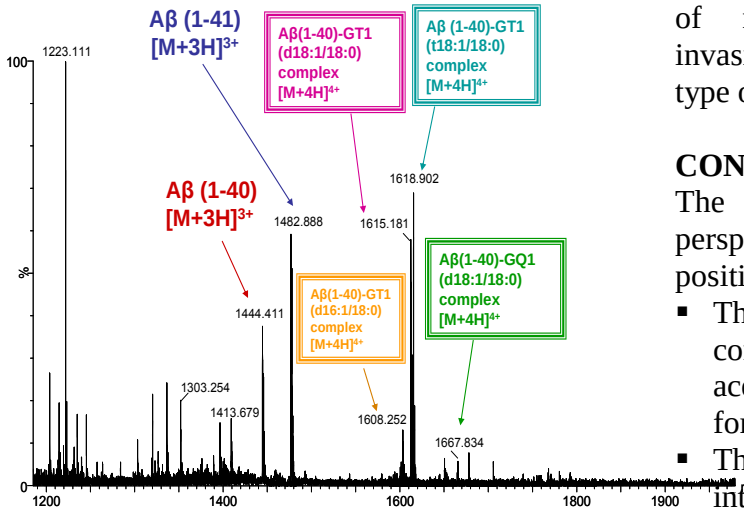

Figure 4. NanoMate-QTOF MS in positive ion mode of the reaction products resulted after 30 min incubation in buffer at 37 ºC of Aβ protein and human brain GG mixture. Acquisition time: 5 min. nanoESI: 1.4 kV; cone voltage: 50 V; nitrogen at 0.40 psi. Buffer system: 10 mM ammonium acetate/acid acetic pH 6.0.

From the biological point of view, these findings reveal that the native GG mixture from glioma contains minor species, previously unidentified and which formed non-covalent complexes with α-synuclein and thus could be identified. Therefore, based on the affinity of proteins for certain species, the method can be used to *de novo* identify components having a low expression in a certain human matrix.

Although α-synuclein protein is most intensively studied in relation to neurodegenerative diseases, recent studies correlate glioblastoma with the overexpression of α-synuclein protein [7]. On the other hand, it was clearly demonstrated a reduction in α-synuclein toxicity in cases treated with GGs and in particular the ability of GM1 monosialo tetraose from G1 class to protect against Syn toxicity *in vivo* [8]. Related to these previous reports, our results indicate for the first time that: a) α-synuclein has affinity for the entire G1 class of gangliosides, not just for GM1 (monosialylated) and b) the entire G1 class should be tested against the toxicity of α -synuclein in gliomas, possibly with the effects of reducing proliferation/infiltration and invasiveness that characterize the cells of this type of cancers.

CONCLUSIONS

The most important conclusions and future persppectives of the work in the field may be positively summarized as follows:

- The results have demonstrated the compatibility of 10 mM ammonium acetate/acid acetic pH 6.0 as buffer system for interaction and spraying solvent;
- The data collected by noncovalent interaction assays followed by chip-based nanoESI MS suggest for the first time that, exposed to a highly complex mixture of gangliosides under conditions facilitating the interaction, these proteins preferentially bind the G1 ganglioside class, having a particular affinity for polysialylated species GT1 and GQ1;
- Minor species, which might play an important biological role, could be detected via their complex formed with Aβ or Syn proteins;
- The platform appears as a method of choice not only for the assessment of the noncovalent interactions, but also for the detection and identification of species of low expression in complex mixtures. Certainly, for this purpose, further method refinements are required and planned for the near future.

ACKNOWLEDGEMENTS

This work was supported by the Romanian National Authority for Scientific Research, UEFISCDI through projects PN-III-P1-1.2-PCCDI-2017-0046 and 541PED/2020 granted to ADZ, and postdoctoral PN-III-P1-1.1-PD-2019-0226 project granted to MS.

REFERENCES

[1] Groux-Degroote, S., Guérardel, Y., Delannoy, P., 2017. Gangliosides: structures,

biosynthesis, analysis, and roles in cancer. ChemBioChem. 18, 1146–1154.
[2] Zamfir, A.D., 2017. Microfluidics-mass spectrometry of protein-carbohydrate interactions: applications to the development of therapeutics and biomarker discovery. Methods Mol. Biol. 109-128.
[3] Han, L., Xue, X., Roy, R., Kitova, E.N., Zheng, R.B., St-Pierre, Y., Lowary, T.L., Klassen, J.S., 2020. Neoglycolipids as glycosphingolipid zurrogates for protein binding studies using nanodiscs and native mass spectrometry. Anal. Chem. 92, 14189-14196.
[4] Wang, W.,X., Whitehead, S.N., 2020. Imaging mass spectrometry allows for neuroanatomic-specific detection of gangliosides in the healthy and diseased brain. Analyst 145, 2473-2481.
[5] Ica, R., Simulescu, A., Sarbu, M., Munteanu, C.V.A., Vukelić, Ž., Zamfir, A.D., 2020. High resolution mass spectrometry provides novel insights into the ganglioside pattern of brain cavernous hemangioma. Anal. Biochem. 609:113976. doi: 10.1016/ j.ab.2020.113976 (Epub ahead of print).
[6] Bolla, J.R., Agasid, M.T., Mehmood, S., Robinson, C.V., 2019. Membrane protein-lipid interactions probed using mass spectrometry. Annu. Rev. Biochem. 88, 85-111.
[7] Duan, J., Ying, Z., Su, Y., Lin, F., Deng, Y., 2017. α-Synuclein binds to cytoplasmic vesicles in U251 glioblastoma cells. Neurosci. Lett. 642, 148-152.
[8] Schneider, J., Aras, R., Williams, C., Koprich, J., Brotchie, J., Singh, V., 2019. GM1 ganglioside modifies α-synuclein toxicity and is neuroprotective in a rat α-synuclein model of Parkinson's disease. Sci Rep. 9, 8362-8369.