HIGH PERFORMANCE MASS SPECTROMETRY FOR ADVANCED INTERACTOMICS STUDIES

Adrian C. Robu[1,2], Laurentiu Popescu[1,2], Daniela G. Seidler[3], Alina D. Zamfir[1,4]

[1]Mass Spectrometry Laboratory, National Institute for Research and Development in Electrochemistry and Condensed Matter, Plautius Andronescu Str. 1, RO-300224, Timisoara, Romania
[2]Faculty of Physics, West University of Timisoara, Blvd. Vasile Parvan 4, RO-300223, Timisoara, Romania
[3]Institute for Physiological Chemistry and Pathobiochemistry, University of Münster, Waldeyer Str. 15, D-49149, Münster, Germany
[4]Department of Chemical and Biological Sciences, "Aurel Vlaicu" University of Arad, Revolutiei Blvd. 77, RO-310130, Arad, Romania
e-mail: alina.zamfir@uav.ro

Abstract

Fibroblast growth factor-2 (FGF-2) is a glycosaminoglycan (GAG) binding protein, involved in different biological processes, such as angiogenesis, bone signaling, embryonic development, morphogenesis or cartilage metabolism. GAGs, one of its binding partners, are long-unbranched polysaccharides exhibiting a repeating disaccharide unit. Moreover, preceding studies have shown that GAGs play an important role in tissue development, cellular behavior or extracellular matrix (ECM) organization. The FGF-GAG noncovalent interactions are of high importance in the biological and biomedical fields of research, as a result of their influence in the tissue regeneration and cell proliferation processes. Here, we have employed one of the most advanced mass spectrometric (MS) techniques consisting of fully automated chip-nanoelectrospray (nanoESI), coupled to a quadrupole time-of-flight (QTOF) MS for studying the FGF-GAG noncovalent complexes.

The experiments were conducted in 10 mM ammonium acetate/formic acid, pH 6.8, by incubating FGF-2 and CS disaccharides dissolved in buffer; aliquots were collected after 5, 10, 30, 60 and 90 minutes and further submitted to chip-based MS analysis. For the first time, a CS disaccharide was involved in a binding assay with FGF-2. The detected complexes in the screening experiments were further characterized by top-down fragmentation in tandem MS (MS/MS) using collision induced-dissociation (CID) at low ion acceleration energies. CID MS/MS provided data showing for the first time that the binding process occurs via SO_3 located at C4 in the GalNAc moiety.

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