Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Research 343 (2008) 2172–2183
Carbohydrate RESEARCH

MALDI-QTOFMS/MS identification of glycoforms from the urine of a CDG patient

Sergey Y. Vakhrushev,[a] Marten F. Snel,[b] James Langridge[b] and Jasna Peter-Katalinić[a,*]

[a]*Institute for Medical Physics and Biophysics, Biomedical Analysis, University of Muenster, D-48149 Muenster, Germany*
[b]*Waters Corporation, Atlas Park, Simonsway, Manchester M22 5PP, UK*

Received 21 September 2007; received in revised form 9 November 2007; accepted 12 November 2007
Available online 22 November 2007

Presented at Eurocarb 14th Lübeck, Germany, September 2007

Abstract—Identification of single glycoconjugate components in a complex mixture from the urine of a patient suffering from a congenital disorder of glycosylation was probed by MALDIMS analysis on a hybrid quadrupole time-of-flight instrument. In negative ion mode, complex maps containing more than 50 ionic species were obtained and a number of molecular ions directly assigned using a previously developed computer-assisted algorithm. To confirm the data and determine the carbohydrate sequence, single molecular ions were selected and submitted to fragmentation experiments. Interpretation of fragmentation spectra was also assisted by the software using alignment with spectra generated in silico. According to fragmentation data, the majority of glycoconjugate ionic species could be assigned to free oligosaccharides along with ten species tentatively assigned to glycopeptides. Following this approach for glycan identification by a combination of MALDI-QTOFMS and MS/MS experiments, computer-assisted assignment and fragment analysis, data for a potential glycan data base are produced. Of high benefit for this approach are two main factors: low sample consumption due to the high sensitivity of ion formation, and generation of only singly charged species in MALDIMS allowing interpretation without any deconvolution. In this experimental set-up, sequencing of single components from the MALDI maps by low energy CID followed by computer-assisted assignment and data base search is proposed as a most efficient strategy for the rapid identification of complex carbohy-drate structures in clinical glycomics.

Keywords: Glycomics; Glycoconjugates; MALDI-QTOFMS; Congenital disorders of glycosylation; Computer-assisted calculations

1. Introduction

Structural and functional analysis of glycosylation in human urine has been established to monitor changes in glycosylation status in healthy persons and diseased patients and to indicate possible aberrant structures as possible biomarkers for congenital diseases. The analytical task to carry such investigation is to establish analytical strategies which include as complete as possible structural identification of any glycoforms present. Among a number of strategies for the structural analysis of glycoconjugates in pre-separated fractions or in complex mixtures obtained from native sources, mass spectrometry represents one of the most efficient methods for mapping and sequencing at high sensitivity and accuracy of identification.[1–6]

Congenital disorders of glycosylation (CDG) are defined as inherited metabolic diseases caused by low or missing activity of enzymes, transporters or other functional proteins responsible for glycosylation processing pathways of glycoconjugates.[7,8] The observation of the decreased serum thyroxin-binding globulin and increased arylsulfatase A activity in two patients with familial psychomotor retardation reported by Jaeken et al.[9] was the basis of the CDG discovery. In 1984, Jaeken et al.[10] determined sialic acid deficiency in serum and cerebrospinal fluid transferrin from identical twin sisters suffering from demyelinating disease. That was the first demonstration of a new syndrome caused by defective

* Corresponding author. Tel.: +49 251 83 52308; e-mail: jkp@uni-muenster.de

0008-6215/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2007.11.014

protein glycosylation. To date most of the presently known CDG cases are related to abnormalities in the N-glycosylation pathway of glycoproteins with only few O-glycosylation diseases identified.[11] CDGs belong to rare diseases: around 500 cases of all types of CDG have been discovered so far.[12] The types of CDG diseases have been classified into two main groups, according to the glycosylation pathway damage.[13]

Presently, serum transferrin IEF is the most widely used method for CDG diagnosis. Our attention, however, was turned towards glycopatterns in urine as an alternative source of glycoconjugate biomarkers and therefore potentially useful diagnostic tool to be correlated with a clinical picture of human disorders.[14–21]

In comparison to the healthy control, the urine of CDG patients was shown to contain O-linked glycans and glycosylated amino acids at concentrations two-to-three orders of magnitude higher.[17] In urine analysis of CDG patients by MS, we applied several approaches such as high resolution/high-mass accuracy FT-ICR MS,[18] capillary-based[19] and chip-based nano-ESI-QTOF CID fragmentation analysis,[20] where we were able to identify from 40% to 75% of components using manual and computer-assisted data interpretation strategy. However, it is known that in ionization process due to still largely unknown factors, different components express different ionization properties, which causes an incomplete overlap of in-toto detected components.[22] In this study, a MALDI mapping by MS and sequencing by MS/MS for high coverage of glycoform identification in the urine of a CDG patient using the QTOF mass analyzer is presented, involving high sensitivity and low sample consumption. An additional helpful feature of the MALDI ionization is the production of predominantly singly charged ionic species, which do not require deconvolution, generating simpler spectra and enhancing data interpretation.

MS fragmentation of complex glycans was already introduced to analytical glycobiology 20 years ago,[23] but an assignment of a carbohydrate structure from a single experiment still represents a challenging task. Basic structural predictions can be proposed for diverse N- and O-glycan involving already known biosynthetic pathways, such as N-glycans which include variations of the common $Man_3GlcNAc_2$ pentasaccharide core, or O-glycans of the GalNAc core type.[24] A number of techniques has been applied for the structural elucidation of components in carbohydrate mixtures, basically attempting to find diagnostic fragment ions responsible for single structures.[25–35] Five series of fragment ions are generally expected: the most prone to occur is the cleavage of one glycosidic bond, resulting in two types of fragments with the reducing or the non-reducing end.[36] Additional cleavage of a second glycosidic bond might result into the third series, called internal fragments. The last two series are produced by the double cleavage of the glycosidic ring (cross-ring) and contain either the reducing or the non-reducing end of the precursor ion. The combinations between these series, such as triple glycosidic or glycosidic and cross-ring dissociation have often been observed.[1,3,14,18–20,28–30,35,37] The first three series represent major tools for structural elucidation, while according to the cross-ring fragmentation linkage branching patterns can be established. Distinct fragmentation patterns under controlled collision energy and gas conditions along with the ability of automatic switching between MS and MS/MS mode by electrospray (ESI) QTOF instrument represent a powerful option for high-throughput analysis of complex carbohydrate mixtures. Cleavage ions obtained by tandem mass spectrometry are described according to the fragmentation nomenclature introduced by Domon and Costello (Fig. 1).[38]

Letters A_i, B_i, C_i are used for the assignment of the fragmentation with the charge at the non-reducing end, where index '*i*' determines the number of the glycosidic bond calculated from the non-reducing side. Those fragmentations, which carry the charge at the reducing end, have been denoted as X_j, Y_j and Z_j, counting the order number from that side. Cross-ring fragmentation has been denoted by letters A_i and X_j, where the exact position of the sugar ring cleavages is determined by superscript at

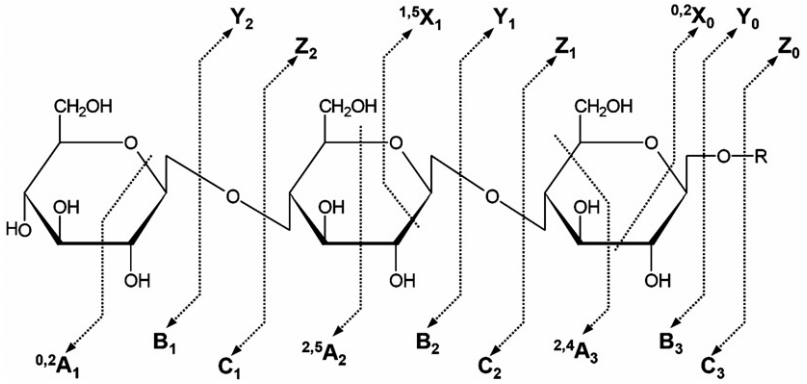

Figure 1. Nomenclature of oligosaccharide fragmentation pattern introduced by Domon and Costello.[38]