Proceedings of the World Congress on New Technologies (NewTech 2015)
Barcelona, Spain – July 15 - 17, 2015
Paper No. 115

N-Glycome Profiling of Prostate Specific Antigen: A Combination of Impedimetric Biosensor Approach with Maldi-TOF/TOF Analysis

Dominika Pihíková, Štefan Belický, Ján Tkáč
Department of Glycobiotechnology, Institute of Chemistry, Slovak Academy of Sciences,
Dúbravská cesta 9, 845 38 Bratislava, Slovakia
dominika.pihikova@savba.sk; jan.tkac@savba.sk

Zuzana Pakanová, Marek Nemčovič, Ján Mucha
Institute of Chemistry, Center of Excellence in Glycomics, Slovak Academy of Sciences,
Dúbravska cesta 9, 845 38 Bratislava, Slovakia
zuzana.pakanova@savba.sk

Extended Abstract

Glycosylation of proteins represents one of the most important co- and post-translational modification. Changes in glycan moieties have been associated with physiological state and a variety of diseases, especially with cancer development in a human body (Varki et al., 2009; Tkac et al., 2014). Altered glycoforms of proteins are becoming a suitable target of novel biomarker discovery and for early-stage cancer diagnosis (Pihíková et al., 2015; Damborský et al., 2014). In the present study, the fabrication of ultrasensitive lectin-based biosensors with nano-scale pattering protocol was performed. Moreover, advanced mass spectrometric techniques were utilized for *N*-glycome profiling of prostate specific antigen (PSA).

In the first section of this study, electrochemical impedance spectroscopy (EIS) analysis with investigation of changes in charge transfer resistance (R_{et}) was provided. Briefly, polycrystalline gold electrode was modified by self-assembled monolayer (SAM), specifically with a mixed solution of 11-mercaptoundecanoic acid and 6-mercapto-1-hexanol. Subsequently, an anti-PSA antibody was covalently immobilized to activated surface *via* EDC/NHS chemistry with further incubation of a blocking agent to minimize non-specific interactions. Afterwards, incubation with a prostate specific antigen (PSA) was performed with final incubation of a glyco-recognition element (a variety of lectins).

Moreover, the released *N*-glycans and glycopeptides from PSA were analysed by MALDI-TOF/TOF-MS and nanoLC-MALDI-TOF/TOF-MS analysis, respectively. The part of reduced and alkylated tryptic digest was enzymatically modified with PNGase-F. PNGase-F released *N*-glycans were several times purified and desalted *prior* to MALDI analysis and subsequently attempted for *N*-glycome profiling. In parallel, a second part of reduced and alkylated tryptic glycopeptides was purified with hydrophilic interaction liquid chromatography (HILIC SPE) and C18 RP micropipette tips. Afterwards, purified glycopeptides were subjected to nanoLC-MS/MS analysis and database searching.

In this study we optimized and compared *N*-glycan profile present on the surface of a prostate specific antigen. These preliminary results are essential for further development of novel glycoprofiling methods for biomarker detection or for potential discovery of novel glyco-biomarkers applicable in diagnostics. Thus, a possibility to clearly distinguish between benign prostate hypertrophy and prostate cancer may be achieved using this sensitive and patient-friendly way.

This work was funded by the National Priorities Research Program (Qatar National Research Fund), number of project NPRP 6-381-1-078. This contribution is the result of the project implementation:

Centre of excellence for Glycomics, ITMS 26240120031, supported by the Research & Development Operational Programme funded by the ERDF.

Damborský, P., Madaboosi, N., Chu, V., Conde, J.P., & Katrlík, J. (2014). Surface Plasmon Resonance Application in Prostate Cancer Biomarker Research. *Chem. Pap.*, 69, 143-149.

Pihíková, D., Kasák, P., & Tkac, J. (2015). Glycoprofiling of Cancer Biomarkers: Label-Free Electrochemical Lectin-Based Biosensors. *Open Chem.*, 13, 636-655.

Tkac, J., Bertok, T., Nahalka, J., & Gemeiner, P. (2014). Perspectives in Glycomics and Lectin Engineering. *Methods Mol. Biol.*, 1200, 421-445.

Varki, A., Cummings, R.D., Esko, J.D., Freeze, H.H., Stanley, P., & Bertozzi, C.R. (2009*). Essentials of Glycobiology 2nd Edition.* Cold Spring Harbor Laboratory Press.