Development of separation methods for the characterization of a glycoprotein at the intact level: application to the human chorionic gonadotropin hormone

Abstract:
Glycosylation is the most common form of post-translational modifications (PTMs) of human proteins, since more than 70% are glycosylated. It regulates numerous biological properties including their stability, half-life, and activity. Nevertheless, proteins can also exhibit other types of PTMs that lead to a very large number of isoforms, varying in mass, properties and concentration in the biological samples. Therefore, the characterization of a glycoprotein is highly challenging and requires the use of powerful separation techniques and sensitive and informative detection modes.
The human chorionic gonadotropin (hCG) is the hormone specific to human pregnancy. It is essential for the development of placenta and fetus. It is based on two heavily glycosylated subunits, hCGα and hCGβ, having 8 glycosylation sites (4 N- and 4 O-glycosylation sites). Some recent studies demonstrated that here is a correlation between the hCG glycosylation state and the fetus implantation. This is why the characterization of the hCG glycoformes is needed. Therefore, new LC/CE-MS methods were developed for the characterisation of hCG at the intact level using two hCG-based drugs having different glycosylation profiles. While the CZE-MS (TQ) method showed its potential for glycosylation fingerprinting, the complementarity of LC-(qTOF) MS methods in RP and HILIC modes allowed the identification of the glycoforms of the hCGα subunit. To limit the identification errors due to the overlapping of isotopic distribution patterns, the profile of each isoform was resolved by FT-ICR MS. For this purpose, a nanoLC separation in RP mode was developed, thus improving the sensitivity of the method by a factor 500 compared to the conventional format. This method allowed the confirmation of the identification of hCGα glycoforms. Then, it was possible to obtain different glycosylation patterns of the hCGβ by promoting its ionization after hCG reduction. Then, a PNGase treatment was carried out to remove the N-glycans in order to obtain the O-glycoprofiles of hCGβ isoforms.