More than amino acids: protein modifications under scrutiny

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The emergence of biopharmaceuticals, *i.e.* therapeutic proteins, has revolutionized modern medicine. Because of their size and complexity, protein therapeutics are produced in cellular expression systems, followed by extensive downstream processing. Modifications of the protein are inherent, *e.g.* glycosylation, glycation and oxidation, resulting in multiple protein variants referred to as proteoforms. These post-translational modifications (PTMs) may impact the safety and efficacy of a drug compound and therefore necessitate rigorous control during process development and drug production. Conventionally, this involves peptide analysis using generic high-performance liquid chromatography (HPLC) and high-resolution mass spectrometry (MS) methods. In contrast to bottom-up approaches, intact protein characterization conserves the context of PTMs and facilitates the resolution of co-existing proteoforms. We implemented native MS as a tool to reveal distinct glycosylation patterns of intact Etanercept (Enbrel^{TM}), a highly *N*- and *O*-glycosylated recombinant Fc-fusion protein applied in the therapy of arthritic diseases. Indeed, highly complex mass spectra unraveled more than 80 different isoforms of the 130 kDa protein. Assignment of specific glycoforms was achieved upon enzymatic digestion of the molecule applying a set of glycosidases and proteases, followed by data integration using advanced computational tools. An analogous approach was successfully applied to human chorionic gonadotropin (hCG), a hormone used in pregnancy tests and approved as fertility drug. Moreover, we assessed glycation of therapeutic monoclonal antibodies and developed a new algorithm to eliminate bias arising in the quantification of glycosylation variants. In summary, comprehensive information combined with minimal sample preparation renders the described analytical approaches highly attractive for industrial applications.