Characterization of the Glycan moiety of the salivary glycosylated basic Proline Rich Protein IB8a CON 1+

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Previous studies demonstrated that several glycosylated proteins are present in human saliva. They are mucins, amylase, IgA and glycosylated proline-rich proteins (gPRPs or PRGs) [1]. Salivary gPRPs, belonging to the family of the basic proline-rich proteins (bPRP), are secreted only by parotid glands, and they were not detected in granules and saliva from the other major salivary glands[2]. The bPRPs complex arises from the expression of 4 loci (named PRB1-4) clustered on chromosome 12p13.2, each locus including at least three alleles [3, 4]. Some of the known alleles express proteins showing a single or multiple glycosylation sites, primarily based on the consensus sequence Asn-X-Ser. In the present study we focused on the characterization of the salivary bPRP IB8a CON 1+, which is known to be glycosylated [5]. To this purpose we collected parotid saliva samples from adult healthy volunteers by a Lashley's cup between 2 pm and 4 pm. Salivary specimens were immediately mixed 1:1 (v/v) with 0.2% aqueous trifluoroacetic acid in an ice bath. The solution was then centrifuged and the acidic supernatant utilized to purify by gel-filtration chromatography the glycosylated forms of IB8a CON 1+. RP-HPLC-ESI-MS analysis allowed establishing that they corresponded to six glycoforms differing for the degree of fucosylation. The mixture of glycoforms was submitted to complete deglycosylation with PNGase F. The decrease of Mav value following deglycosylation allowed to establish the composition of the glycan moiety ($Fuc_5Gal_2Man_3GlcNAc_4$, more complex oligosaccharide). Comparison of the tryptic digestion mixtures obtained from glycosylated and deglycosylated IB8a CON 1+ located the glycosylation site at the level of the unique Asn-98 present in the tryptic fragment 63-99. Tandem-MS experiments were carried out by an HPLC-LTQ Orbitrap apparatus on the tryptic glycopeptides. The results were in agreement with two possible glycan moieties, one corresponding to a structure characterized by other authors in a glycomic study on parotid saliva [6]. The physiological role of gPRPs is mostly unknown so a better comprehension of their glycan moieties would be of the utmost importance in order to decode their functions.

References

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