MASS SPECTROMETRIC CHARACTERIZATION OF THE GLYCOSYLATION SITES PRESENT IN ANTIOPHIDIC PROTEINS, DM43 AND DM64, FROM AN ENRICHED *DIDELPHIS MARSUPIALIS* SERUM FRACTION

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The research on glycoproteomes represents an important field in functional proteomics research. Affinity chromatography and mass spectrometry are powerful techniques used for gaining valuable information on protein glycosylation which can have strong influence on biological activities, protein stability etc. The opossum *Didelphis marsupialis* (gambá) resistance to *Bothrops* snake venoms is attributed to the presence of anti-hemorrhagic (DM43) and antimyonecrotic (DM64) acidic serum glycoproteins showing 21% and 15% (m/m) carbohydrate content, respectively. The aim of this work was to characterize, directly from the serum, the N-glycosylation sites present in these inhibitors. Our experimental pipeline was composed by an initial glycoprotein enrichment with a Wheat Germ Agglutinin lectin column followed by one-dimensional electrophoretic separation, enzymatic *in gel* digestion with trypsin, enzymatic tryptic peptides digestion with peptide-N-Glycosidase F and, finally, mass spectrometric analysis by MALDI-TOF/TOF. We confirmed three glycosylation sites in DM43 (Asn ^{156}, Asn^{160} and Asn^{175}), previously suggested by Edman degradation. Additionally, two putative glycosylation sites in DM64 (Asn^{179} and Asn^{183}) were experimentally confirmed. Our data support the feasibility and efficacy of our experimental approach which is being currently applied to the characterization of other opossum serum glycoproteins.

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