

The identification of xenoantigenic nonhuman carbohydrates in membrane proteins from porcine kidney by using mass spectrometry and exoglycosidase

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Abstract

The total N-glycan structures of membrane proteins obtained from porcine kidney was clarified by using mass spectrometric approaches. The immunogenic nonhuman carbohydrate sequences were identified and characterized using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and electrospray ionization-quadrupole time-of-flight mass spectrometry (ESI-QTOF-MS). The MALDI profile, investigated by incubation with exoglycosidases in sequence, showed a series of about 40 carbohydrates that were identified as high mannose glycans ($\text{Man}_{3-9}\text{GlcNAc}_2$) and complex bi- and tetra-antennary glycans with and without core fucose. The antennae of many of the complex glycans were terminated with -galactose residues ($\text{Gal}\alpha\text{-3Gal}\beta\text{-4GlcNAc-R}$), with the numbers of these residues ranging from one up to the number of antennae. Negative ion ESI-MS/MS spectra confirmed the location of the -galactose residues on the ends of the antennae. This total glycan profile of the membrane proteins from porcine kidney will thus provide important information for the study of molecular interactions between antigenic carbohydrates and proteins in xenotransplantation.