

The sperm agglutination antigen-1 (SAGA-1) glycoforms of CD52 are O-glycosylated

Abstract

CD52 is composed of a 12 amino acid peptide with N-linked glycans bound to the single potential glycosylation site at position 3, and a glycosylphosphatidylinositol-anchor attached at the C-terminus. Some glycoforms of this molecule expressed in the male reproductive tract are recognized by complement-dependent sperm-immobilizing antibodies in infertile patients making this antigen an important target for immunocontraception and fertility studies. Although the amount of posttranslational modification is already remarkable for such a small polypeptide, O-glycosylation of CD52 has additionally been implicated by several studies, but never rigorously characterized. In this report, we show clear evidence for the presence of O-glycans in CD52 preparations immunopurified using the murine S19 monoclonal antibody generated against sperm agglutination antigen-1 (SAGA-1), a male reproductive tract specific form of CD52. The O-glycans have been characterized by MALDI-TOF and tandem mass spectrometry after reductive elimination and permethylation. The data indicate that the major SAGA-1 O-glycans are core 1 and 2 mucin-type structures, with and without sialic acid (NeuAc0-2Hex1-3HexNAc1-2HexNAcitol). Minor fucosylated O-glycans are also present including some structures with putative Ley epitopes (NeuAc0-1 Fuc1-3Hex1-2 HexNAc0-1HexNAcitol). Analysis of O-glycopeptides by tandem mass spectrometry provided an additional level of support for the O-glycosylation of SAGA-1. Elucidation of the O-glycosylation of SAGA-1 adds to the complexity of this molecule and may help to explain its biological activity. © The Author 2007. Published by Oxford University Press. All rights reserved.