One-step immunopurification and lectinochemical characterization of the Duffy atypical chemokine receptor from human erythrocytes.

Autorzy

Magdalena Grodecka

Olivier Bertrand

Ewa Karolak

Marek Lisowski

Kazimiera Waśniowska

Rok wydania

2012

Czasopismo

Glycoconjugate Journal

Numer woluminu

29

Strony

93-105

DOI

10.1007/s10719-011-9367-9

Kolekcja

Naukowa

Język

Angielski

Typ publikacji

Artykuł

Streszczenie

Duffy antigen/receptor for chemokines (DARC) is a glycosylated seven-transmembrane protein acting as a blood group antigen, a chemokine binding protein and a receptor for Plasmodium vivax malaria parasite. It is present on erythrocytes and endothelial cells of postcapillary venules. The N-terminal extracellular domain of the Duffy glycoprotein carries Fy(a)/Fy(b) blood group antigens and Fy6 linear epitope recognized by monoclonal antibodies. Previously, we have shown that recombinant Duffy protein expressed in K562 cells has three N-linked oligosaccharide chains, which are mainly of complex-type. Here we report a one-step purification method of Duffy protein from human erythrocytes. DARC was extracted from erythrocyte membranes in the presence of 1% n-dodecyl-β-D-maltoside (DDM) and 0.05% cholesteryl hemisuccinate (CHS) and purified by affinity chromatography using immobilized anti-Fy6 2C3 mouse monoclonal antibody. Duffy glycoprotein was eluted from the column with synthetic DFEDVWN peptide containing epitope for 2C3 monoclonal antibody. In this single-step immunoaffinity purification method we obtained highly purified DARC, which migrates in SDS-polyacrylamide gel as a major diffuse band corresponding to a molecular mass of 40-47 kDa. In ELISA purified Duffy glycoprotein binds anti-Duffy antibodies recognizing epitopes located on distinct regions of the molecule. Results of circular dichroism measurement indicate that purified DARC has a high content of α-helical secondary structure typical for chemokine receptors. Analysis of DARC glycans performed by means of lectin blotting and glycosidase digestion suggests that native Duffy N-glycans are mostly triantennary complex-type, terminated with α2-3- and α2-6-linked sialic acid residues with bisecting GlcNAc and α1-6-linked fucose at the core.