The N-linked oligosaccharide structures on the envelope glycoprotein gp120 of human immunodeficiency virus 1 from the lymphoblastoid cell line H9 (Received for publication, November 30, 1989)
Fig. 7. Proposed structures for oligosaccharides contained in fractions a–k derived from gp120. The molar percentages (determined on the basis of radioactivity) refer to the proportions of total gp120 oligosaccharides. The percentages given for fractions h and k3 and those given in parentheses for other fractions refer to oligosaccharides whose structures were not determined (n.d.) in those fractions. The linkages indicated (except Galβ1→4GlcNAc) were not confirmed but are the most probable. G, galactose; GN, N-acetylglucosamine; M, mannose; F, fucose; GNor, [1H1N-acetylglucosaminitol; ± residues are present in 90% of chains.

EXPERIMENTAL PROCEDURES AND RESULTS

DISCUSSION

The salient conclusion from this study is that there is an enormous diversity of oligosaccharide structures among gp120 molecules produced by H9 cells that are chronically infected by HIV-1 (Fig. 7). The neutral components, which could be readily separated from the acidic components by paper electrophoresis, constituted ~60% of the oligosaccharides released by hydrazinolysis and labeled with NaB3H4. These were identified as a series of high mannose-type oligosaccharides (MannGlcNAc2 to Man3GlcNAc3). The acidic components were identified as an array of sialylated oligosaccharides which were all rendered neutral after sialidase treatment. Sequential chromatography on lectin affinity columns (Phaseolus vulgaris erythrophytohemagglutinin and Ricinus communis agglutinin (M, 120,000)) and on Bio-Gel P-4 revealed that the diversity of oligosaccharides was far greater than was observed with oligosaccharides derived from recombinant gp120 produced in Chinese hamster ovary cells (rgp120) investigated similarly (6, 7). Biantennary complex-type oligosaccharides which were major components (34%) in Chinese hamster ovary cell-derived rgp120 were minor components (2%) in gp120. In addition to hybrid-type (1.8% in gp120) and tri- and tetraantennary complex-type (3.6%) structures common to rgp120 and gp120, oligosaccharides with bisecting N-acetylgalactosamine residues were detected in cg120. These included bis-, tri-, and tetraantennary complex-type structures with and without core region fucose residues and amounted to 16% of the total oligosaccharides from cg120. Just over 80% of the cg120 oligosaccharides could be identified after desialylation in this study. The remaining oligosaccharides (a heterogeneous array of complex-type chains having one or more outer chains with galactose residues) could not be further characterized due to the small amounts available. The resistance of some of these oligosaccharides to further digestion with mixtures of β-galactosidase and β-1,4N-acetylgalactosaminidase suggests the presence of other substituents on the N-acetylgalactosamine or galactose residues of their outer chains.
The detection of a higher proportion (80%) of high mannose type oligosaccharides in a preparation of gp120 from H9 cells in an earlier study (8) may well reflect the different labeling procedure used, i.e. metabolic labeling of the mannose residues with a resultant bias in favor of high mannose-type structures. The inclusion of a lentil lectin affinity step in the purification of the glycoprotein in this study may, on the contrary, have favored the enrichment of glycosylation variants with complex-type chains. Another difference in procedure that may account for the under-representation of complex-type oligosaccharides in the earlier study is the method of oligosaccharide release from peptide: the enzymatic release in the earlier study versus the more exhaustive chemical release in this study. A further consideration is a possible divergence in the glycosylation patterns of the repeatedly subcultured H9 cells. Nevertheless, the overall conclusion that can be drawn from this study and previous structural studies of gp120 would be predicted to arise from the heterogeneous cell populations harboring the virus.

The functional significance of the extensive glycosylation and of the diversity of structures on the envelope glycoprotein is an important subject for investigation. Whereas there is evidence (9, 27) that glycosylation of gp120 is a prerequisite for binding to the host cell glycoprotein CD4 receptor and that deglycosylation procedures abolish (9) or impair (28) binding, the precise roles of the oligosaccharides in this and other recognotic interactions in HIV infection are not yet known. As discussed earlier (6), the various oligosaccharide structures are potential ligands for carbohydrate-binding proteins of the host (endogenous lectins). Carbohydrate-mediated reactivities of gp120 with two proteins of the host have been documented thus far. The first is with the serum lectin known as mannos-binding protein (29, 30), where the involvement of high mannose-type oligosaccharides of both rgp120 and cgp120 has been demonstrated (30). It has been suggested that such interaction on the virus particle is a potential inhibitor of HIV-1 infection of CD4+ cells (20) and a potential means of viral entry into CD4- cells (30). The second carbohydrate-mediated interaction, shown with gp120 (30), is with the endocytosis receptor of human macrophage membranes. Here it has been suggested (30) that high affinity binding (which would be predicted to occur with glycosylation variants of the viral envelope that are rich in the accessible mannose, N-acetylgalacosamine, or fucose residues recognized (31, 32) by this receptor) may lead to viral uptake by macrophages irrespective of the presence of the membrane-associated CD4 receptor.

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Oligosaccharides of the Envelope Glycoprotein gp120 of HIV-1

EXPERIMENTAL PROCEDURES

Chemicals and reagents

The envelope glycoprotein, gp120, of HIV-1 was purified from the lymphoblastoid cell line 293 chronically infected with HIV-1 BNL by immunoaffinity chromatography (1). Briefly, the cells were cultured in RPMI 1640 medium containing 5% fetal calf serum. The cells were harvested after 8DIV at 5 x 10^7 cells/ml and were added to 35 ml of the cell-free medium. After the pelleted cells were removed by centrifugation at 1000 g for 10 min at 4°C, the supernatant was filtered through membrane filters with a 0.45-µm pore size, and subjected to immunoaffinity chromatography on a column of immobilized HIV-1-specific monoclonal antibody (mAb) 2G12. After washing the column with buffer A (10 mM Tris-HCl, pH 7.4, 1 mM EDTA, 0.5 M NaCl, and 0.01% NaN3) at a flow rate of 1 ml/min, bound gp120 was eluted with buffer B (500 mM sodium acetate, pH 4.0, 0.01% NaN3). The eluate was concentrated by ultrafiltration (30 kDa cutoff) and desalted by gel filtration chromatography on a column of Sephadex G-25. The purified gp120 was stored at -80°C until use. The purified gp120 was then treated with 0.1% SDS and subjected to SDS-PAGE, and the identity of the gp120 band was confirmed by Western blotting with mAb 2G12.

Preparation of recombinant HIV-1 gp120

The recombinant HIV-1 gp120 was produced in the Escherichia coli strain BL21 (DE3) pLysS, which was transformed with a plasmid expressing gp120. The cells were grown in LB medium containing 100 μg/ml ampicillin and 100 μg/ml chloramphenicol at 37°C for 48 h. The bacterial pellets were resuspended in PBS and disrupted by sonication. The supernatant was collected after centrifugation at 10,000 g for 10 min at 4°C. The recombinant gp120 was purified by affinity chromatography on a column of immobilized HIV-1-specific mAb 2G12. After washing the column with buffer A, bound gp120 was eluted with buffer B, concentrated by ultrafiltration (30 kDa cutoff), and desalted by gel filtration chromatography on a column of Sephadex G-25. The purified recombinant gp120 was stored at -80°C until use. The purified recombinant gp120 was then treated with 0.1% SDS and subjected to SDS-PAGE, and the identity of the gp120 band was confirmed by Western blotting with mAb 2G12.

Determinations of oligosaccharides from gp120

The oligosaccharides from the purified gp120 were determined by gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC). The glycopeptides were derivatized with 2,4-dinitrophenylhydrazine (DNP) and subjected to GC-MS analysis. The oligosaccharides were also analyzed by HPLC using a reverse-phase column and eluted with a linear gradient of acetonitrile in 0.1% trifluoroacetic acid.

Characterization of oligosaccharides

The oligosaccharides were characterized by high-resolution mass spectrometry (HRMS) and electrospray ionization mass spectrometry (ESI-MS). The glycopeptides were digested with endo-β-N-acetylglucosaminidase H (endo-H) and subjected to LC-MS/MS analysis. The resultant tryptic peptides were then analyzed by ESI-MS to determine the sequence and the glycosylation site.

Structural studies on oligosaccharides

The structural studies on the oligosaccharides were performed by NMR spectroscopy. The glycopeptides were dissolved in D2O and subjected to 2D NMR experiments, including TOCSY, HSQC, and ROESY. The glycosylation patterns were determined by comparing the NMR spectra with those of known glycosylation patterns. The glycosylation sites were assigned based on the chemical shifts of the anomeric protons and the coupling constants.

Conclusions

The results presented in this study indicate that the oligosaccharides from gp120 of HIV-1 are complex-type N-glycans with a high degree of sialylation. The sialic acid residues are mainly α2,3-linked to the galactose moiety of the inner core of the complex-type glycans. The presence of α2,3-linked sialic acids is important for the attachment of HIV-1 to the CD4 receptor and for the binding of the HIV-1 envelope glycoprotein to the CD4 receptor.
Oligosaccharides of the Envelope Glycoprotein gp120 of HIV-1

Figure 4

High voltage paper electrophoresis of radioactive oligosaccharides from gp120. Total radioactive oligosaccharides released from gp120 were subjected to high voltage paper electrophoresis at an 5.6 kV/cm (10 cm) and after 45 min treatment with trypsin (protein 1.0 mg/ml). The fraction was dried and the radioactivity was counted. Areas shown by horizontal bars A and B were harvested, giving a total of radioactive counts (c.f.u.). Fraction A was used to determine neutral (N) and fraction B after periodate treatment.

Figure 5

Separation of intact labeled sialidase-treated oligosaccharides (AS) from gp120 on an E-PA affinity column. The oligosaccharides (0.1 mg/ml) were treated with sialidase (200 units/ml) and then chromatographed on an E-PA affinity column. The radioactivity in each tube was determined by liquid scintillation counting. The start material (AS) was added to the column, which was eluted with the presence of 4.6 lactose being pooled as indicated by the horizontal bars.
Oligosaccharides of the Envelope Glycoprotein gp120 of HIV-1

Figure 3
Chromatography on a Bio-Gel P-4 column of radioactive oligosaccharides: fraction a (panel A), the control fraction from the Sephadex G-200 peak, and the final fractions b to e obtained after chromatography on the Bio-Gel P-4 column of the oligosaccharides obtained by the above method. Fractions a, b, c, d, and e were pooled as indicated by the horizontal bars. Fractions a, d, and e were not analyzed at this stage. Arrows indicate elution positions of glucose oligomers; values against the arrows indicate the number of glucose units.

Figure 5
[In 5 panels] Chromatography on the Sephadex G-200 column of the radioactive oligosaccharides: fractions a to i, k, and l before digestion (shown again in panels 3, J, K, and L for reference) and after digestion with exoglycosidase. Letters a to l correspond to the same fractions as in the above figure. Symbols indicate enzyme activity: •, no activity; +, small activity; , large activity. Horizontal bars indicate those peaks that were pooled. Other symbols as in Fig. 3.
Diversity of oligosaccharide structures on the envelope glycoprotein gp 120 of human immunodeficiency virus 1 from the lymphoblastoid cell line H9. Presence of complex-type oligosaccharides with bisecting N-acetylglucosamine residues.


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