Diversity of Oligosaccharide Structures on the Envelope Glycoprotein gp120 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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The N-linked oligosaccharide structures on the envelope glycoprotein gp120 of human immunodeficiency virus 1 derived from chronically infected lymphoblastoid (H9) cells have been investigated by enzymatic microsequencing after release from protein by hydrazinolysis, labeling with NaB³H₄, and chromatography on adsorbent columns of Phaseolus vulgaris erythrophytohemagglutinin and Ricinus communis agglutinin (Mₚ, 20,000) and on Bio-Gel P-4. A substantially greater diversity of oligosaccharide structures was detected than among those released by hydrazinolysis from recombinant gp120 produced in Chinese hamster ovary cells and investigated by similar procedures (Mizuochi, T., Spellman, M. W., Larkin, M., Solomon, J., Basa, L. J., and Feizi, T. (1988) Biochem J. 254, 599-603) and among those released by endoglycosidases from virus-derived gp120 isolated from infected H9 cells after metabolic labeling with D-[2-³H]mannose or D-[6-³H]glucosamine (Geyer, H., Holschbach, L., Hunsmann, G., and Schneider, J. (1988) J. Biol. Chem. 263, 11760-11767). In this study, 16% of the oligosaccharides were identified as complex-type bi-, tri-, and tetraantennary siao-oligosaccharides with bisecting N-acetylglucosamine residues. Such structures were lacking on recombinant gp120 and could not be detected on the metabolically labeled, virus-derived glycoprotein. As in the earlier investigations, complex-type chains lacking bisecting N-acetylglucosamine residues, hybrid-type chains, and a series of high mannose-type structures with 5-9 mannose residues were identified. In addition, an array of complex-type chains having one or more outer chains with β-galactosyl residues were detected in this study, but with additional substitutions that require further investigation. The number of potential N-glycosylation sites on gp120 is on the order of 20, but the oligosaccharide structures are far more numerous. Thus, the salient conclusion from this and earlier investigations is that alternative structures occur on at least some of the glycosylation sites and that numerous glycosylation variants of this glycoprotein are produced even within a single cell line. Since the glycosylation is the product of host cell glycosyltransferases, an even greater number of glycosylation variants of gp120 are predicted to arise from the heterogeneous cell populations harboring the virus in vivo infection.

The envelope glycoprotein gp120 of human immunodeficiency virus 1 (HIV-1) is the surface component with a key role in viral adhesion and the initiation of infection through interaction with the CD4 glycoprotein of T lymphocytes (1-4). gp120 is richly glycosylated, with approximately half the molecular mass consisting of carbohydrate distributed on some 20 N-glycosylation sites (5). Detailed structural characterization by methylation analyses and enzymic microsequencing of NaB³H₄-labeled oligosaccharides after release by hydrazinolysis from recombinant gp120 produced in Chinese hamster ovary cells (rgp120) has been reported (6, 7). A diversity of structures were identified including high mannose-type (Man₅ to Man₉ structures amounting to ~33%) and hybrid-type (4%) chains as well as four categories of complex-type chains (mono-, bi-, tri-, and tetraantennary) with or without N-acetyllactosamine repeats and with or without core region fucose residues among which digalactosyl biantennary structures predominated (34%). Altogether, 29 structures were identified after desialylation. The actual number of oligosaccharides is much greater (estimated in excess of 100) since before desialylation, there was evidence that among the hybrid- and complex-type chains, all but 6% contained sialic acid at C-3 in terminal galactose residues, and partially sialylated forms of the bi- and multiantennary chains were present. In another study (8), the oligosaccharides of

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1 The abbreviations used are: HIV-1, human immunodeficiency virus 1; HTLV, human T cell leukemia virus; cgpl20, envelope glycoprotein gp120 isolated in this study from H9 cells chronically infected with HIV-1; rrgp120, recombinant envelope glycoprotein gp120 produced in Chinese hamster ovary cells; E-PHA, Phaseolus vulgaris erythrophytohemagglutinin; PBS, 137 mM NaCl containing 2.7 mM KCl, 1.4 mM KH₂PO₄, 4.86 mM Na₂HPO₄, 0.68 mM CaCl₂-2H₂O, and 0.49 mM MgCl₂-6H₂O; RCA 120, Ricinus communis agglutinin (M, 120,000); HPLC, high performance liquid chromatography.
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; GNt, [1H]-N-acetylglucosaminitol; ± residues are present in 90% of chains.

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 (Man8GlcNAc2 to Man12GlcNAc3). 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 erythrophaghemagglutinin and Ricinus communis agglutinin (M, 120,000)) and on Bio-Gel-P4 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 sialyl-oligosaccharides (3.6%) structures common to rgp120 and gp120, oligosaccharides with bisecting N-acetylgalactosamine residues were detected in gp120. These included bi-, tri-, and tetraantennary complex-type structures with and without core region fucose residues and amounted to 16% of the total oligosaccharides from gp120. Just over 80% of the rgp120 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-N-acetylgalactosaminidase suggests the presence of other substituents on the N-acetylgalactosamine or galactose residues of their outer chains.

EXPERIMENTAL PROCEDURES AND RESULTS

DISCUSSION

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The detection of a higher proportion (80%) of high mannose-type oligosaccharides in a preparation of gp120 from H9 cells in an earlier study (6) 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 is that there is a great diversity of oligosaccharides present and that alternative structures are likely to occur on at least some of the glycosylation sites of gp120. Hence, many glycosylation variants are likely to exist in the glycoprotein even when produced in a single cell line. Viral glycosylation is the product of host cell glycosyltransferases. Therefore, in infected individuals, innumerable glycosylation variants 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 recongitive 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 (29) 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-acetylglucosamine, 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 by immunosorbent affinity chromatography. The protein was isolated from the cell culture medium by immunoprecipitation with a specific monoclonal antibody. The purified gp120 was further analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and stained with Coomassie blue or silver stain to determine the purity and integrity of the glycoprotein. The recombinant gp120 was produced in E. coli BL21 (DE3) cells and refolded in vitro using a refolding buffer containing 0.1 M sodium phosphate, 10% glycerol, and 1 mM dithiothreitol. The refolded gp120 was purified by nickel affinity chromatography and analyzed by size exclusion chromatography. The purified gp120 was further analyzed by mass spectrometry to determine its molecular weight and amino acid sequence. The purified gp120 was used as a source of glycosylation and for further experiments.

Methods

Paper chromatography (10), high voltage paper electrophoresis (10), Bio-Gel P-4 column chromatography (13), and lectin (15) affinity HPLC (26) were performed according to the cited references.

Preparation of the radioactive oligosaccharides from gp120

The radioiodination of the radioactive oligosaccharides from gp120 was performed by the chloramine T method (14). The radioactive oligosaccharides were isolated by high performance liquid chromatography (HPLC) and characterized by mass spectrometry.

Results

Preparation of the radioactive oligosaccharides from gp120

Four fractions, A, B, C, and D, were obtained and analyzed for the radioactive oligosaccharides. The fractions were separated by size exclusion chromatography and analyzed for the presence of radioactive material.

Electrophoretic analysis on the cation exchange chromatography 1 x 5.0 cm x 0.5 mm

The electrophoretic analysis of the radioactive oligosaccharides was performed using a cation exchange chromatography column (1 x 5.0 cm x 0.5 mm) and the results were compared with the reference materials.

Structural studies on oligosaccharides from fraction 1 x 0.5 mm

The structural studies of oligosaccharides from fraction 1 x 0.5 mm were performed using mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy. The results showed that the oligosaccharides were composed of N-acetylgalactosamine, galactose, and fucose.

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Discussion

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Conclusion

The preliminary results from our study indicate that the oligosaccharides from fraction 1 x 0.5 mm are composed of N-acetylgalactosamine, galactose, and fucose. Further studies are needed to determine the full structure of these oligosaccharides and their biological activities.
Fraction a: This fraction which was eluted from Bio-Gel P-4 column in the presence of 0.5 M NaCl (Fig. 4) accounted for 74% of the total radioactivity and was separated into oligosaccharides fractions by Bio-Beads S-X1 column of the elution volume of 0.5-1.5 ml. Two major fractions were obtained. One was a fast-migrating reductive, and the other a slower-moving non-reductive form of the same structural unit.

Fraction b: This fraction which was eluted from Bio-Gel P-4 column in the presence of 0.1 M NaCl (Fig. 4) accounted for 13% of the total radioactivity and was also separated into oligosaccharides fractions by Bio-Beads S-X1 column of the elution volume of 0.5-1.5 ml. Two major fractions were obtained. One was a fast-migrating reductive, and the other a slower-moving non-reductive form of the same structural unit.

Fraction c: This fraction which was eluted from Bio-Gel P-4 column in the presence of 0.05 M NaCl (Fig. 4) accounted for 6% of the total radioactivity and was also separated into oligosaccharides fractions by Bio-Beads S-X1 column of the elution volume of 0.5-1.5 ml. Two major fractions were obtained. One was a fast-migrating reductive, and the other a slower-moving non-reductive form of the same structural unit.

Fraction d: This fraction which was eluted from Bio-Gel P-4 column in the presence of 0.005 M NaCl (Fig. 4) accounted for 2% of the total radioactivity and was also separated into oligosaccharides fractions by Bio-Beads S-X1 column of the elution volume of 0.5-1.5 ml. Two major fractions were obtained. One was a fast-migrating reductive, and the other a slower-moving non-reductive form of the same structural unit.

Fraction e: This fraction which was eluted from Bio-Gel P-4 column in the presence of 0.001 M NaCl (Fig. 4) accounted for 1% of the total radioactivity and was also separated into oligosaccharides fractions by Bio-Beads S-X1 column of the elution volume of 0.5-1.5 ml. Two major fractions were obtained. One was a fast-migrating reductive, and the other a slower-moving non-reductive form of the same structural unit.

Fraction f: This fraction which was eluted from Bio-Gel P-4 column in the presence of 0.0001 M NaCl (Fig. 4) accounted for 0.1% of the total radioactivity and was also separated into oligosaccharides fractions by Bio-Beads S-X1 column of the elution volume of 0.5-1.5 ml. Two major fractions were obtained. One was a fast-migrating reductive, and the other a slower-moving non-reductive form of the same structural unit.

Summary of the fractionation of oligosaccharides: The data show that the fractionation of oligosaccharides is dependent on the concentration of NaCl used for the elution from the Bio-Gel P-4 column. At higher concentrations of NaCl, the fractionation is more efficient, resulting in a greater number of distinct fractions. At lower concentrations of NaCl, the fractionation is less efficient, resulting in fewer distinct fractions. This suggests that the oligosaccharides are more heterogeneous at lower concentrations of NaCl.

Figure 1: Separation of oligosaccharides on Bio-Gel P-4 column. The oligosaccharides were chromatographed on an AG 50W-X8 column, monitored by ultraviolet absorption at 280 nm. The fractions were collected and assayed for radioactivity.

Figure 2: Separation of the fast-migrating fraction from the Bio-Gel P-4 column on Bio-Beads S-X1 column. The fractions were collected and assayed for radioactivity.

Figure 3: Separation of the slow-migrating fraction from the Bio-Gel P-4 column on Bio-Beads S-X1 column. The fractions were collected and assayed for radioactivity.
Oligosaccharides of the Envelope Glycoprotein gp120 of HIV-1

Figure 1
Chromatography on a Mono-Q 5/4 column of radioactive oligosaccharides: fractions A (panel A), B (panel B), C (panel C), D (panel D), E (panel E), and F (panel F). Each of the 500 µl fractions A to F was eluted after chromatography on the HPLC column of the oligosaccharides obtained by the method described. Panel A: fractions A, I, K, and L were pooled as indicated by the horizontal bars. Fractions A, I, K, and L were not subfractionated at this stage. Arrows indicate the position of glucose oligosaccharides; values against the arrows indicate the number of glucose units.

Figure 2
(See detailed explanation in the text.)
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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