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-ACETYLGLOCAPSAMINE 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 (M₉ 20,000) and in 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 endo-oligosaccharides from virus-derived gp120 isolated from infected H9 cells after metabolic labeling with D-[2-¹³C]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 sialo-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-acetylglucosaminyl 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 characterizations by methylation analyses and enzymatic microsequencing of NaB³H⁴-labeled oligosaccharides after release by hydrazinolysis from recombinant gp120 produced in Chinese hamster ovary cells (rrg120) has been reported (6, 7). A diversity of structures were identified including high mannose-type (Man₉ 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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These are complex-type chains with bisecting N-acetylglucosamine residues. Both radioactive labels, high mannose-type oligosaccharides that study, the oligosaccharides had been enzymatically released by hydrazinolysis and radiolabeled with NaB\(^3\)H\(_4\). We identified as a series of high mannose-type oligosaccharides (Man\(_n\)GlcNAc\(_n\) to Man\(_n\)GlcNAc\(_3\)). 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 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 sia-loligosaccharides (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 gp120 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 \(\beta\)-galactosidase and \(\beta\)-N-acetylgalactosaminidase suggests the presence of other substituents on the N-acetylgalactosamine or galactose residues of their outer 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 NaB\(^3\)H\(_4\). These were identified as a mixture of high mannose-type oligosaccharides (Man\(_3\)GlcNAc\(_2\) to Man\(_6\)GlcNAc\(_3\)). 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 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 sia-loligosaccharides (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 gp120 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 \(\beta\)-galactosidase and \(\beta\)-N-acetylgalactosaminidase suggests the presence of other substituents on the N-acetylgalactosamine or galactose residues of their outer chains.

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**Fig. 7. Proposed structures for oligosaccharides contained in fractions a–k derived from cgpl20.** The molar percentages (determined on the basis of radioactivity) refer to the portions of total cgpl20 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 Gal1–4GlcNAc) were not confirmed but are the most probable. G, galactose; GN, N-acetylglucosamine; M, mannose; F, fucose; GNor, [\(\beta\)H]-N-acetylgalactosaminol; ± residues are present in 90% of chains.

| Fraction | Structure | Percentage |
|----------|-----------|------------|
| a–k      |           |            |
| h        |           | n.d. 3.0%  |
| k2       |           | n.d. 0.8%  |
| k3       |           | n.d. 0.5%  |
| k4       |           | n.d. 2.2%  |
| k5       |           | n.d. 0.4%  |
| k6       |           | n.d. 0.8%  |
| k7       |           | n.d. 0.8%  |
| k8       |           | n.d. 0.8%  |
| k9       |           | n.d. 0.8%  |
| k10      |           | n.d. 0.8%  |
| k11      |           | n.d. 0.8%  |
| k12      |           | n.d. 0.8%  |

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1 Portions of this paper (including "Experimental Procedures," "Results," and Figs. 1–6), are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.
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 some of the glycosylation sites of gp120 would be predicted to occur with 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 recogntive 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 mannose-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

Antigenic Material

The envelope glycoprotein, gp120, of HIV-1 was purified from the lymphoid cell line 293 chronically infected with MT-2/115 by immunoprecipitation on a micro-agarose. Briefly, the cells were collected in PBS (130 mm NaCl, 2.7 mm KCl, 10 mm Na2HPO4, 1.75 mm KH2PO4, 0.5 mm EDTA, pH 7.4). The glycoprotein was extracted with 100 mm NaOH and purified on micro-agarose. The purified gp120 was then treated with a mixture of D-tosylglycine phenylthiohydantoin (Gly), D-tosyl phenylalanine phenylthiohydantoin (Phe), and D-tosyl-3-carboxybenzyl-phenylthiohydantoin (Cys). The glycoprotein was then subjected to electrophoresis on a 5% polyacrylamide gel under reducing conditions. The gel was stained with Coomassie Blue R-250 and destained with destain solution. The stained bands were then excised and subjected to liquid chromatography. The eluates from the column were analyzed by amino acid analysis and mass spectrometry.

Experimental Procedures

Chemical and Physical Methods

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Figure 1

High voltage paper electrophoresis of radioactive oligosaccharides from cgp120. Total radioactive oligosaccharides released from cgp120 were electrophoresed at pH 5.6 (lane 1) and after 14 days (lane 2) treatment with sialidase. Lanes 3 and 4 show oligosaccharides treated with sialidase. Lanes 5 and 6 show oligosaccharides prepared from the sialidase-treated cgp120. Oligosaccharides were recovered and eluted with 0.1 M sodium acetate buffer containing 0.1% sodium dodecylsulfate.

Figure 2

Separation of radiolabelled sialidase-treated oligosaccharides (AB) from cgp120 on an E-PA affinity column. The oligosaccharides were eluted after sialidase treatment of fraction A and were chromatographed on an E-PA affinity column. The radioactivity in each tube was determined by liquid scintillation counting. The part through and the eluted fractions were pooled as indicated by the horizontal bars.
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Figure 3
Chromatography on a Bio-Gel P-4 column of radioactive oligosaccharides: fraction 9 (panel A), the retained fraction from the DEAE-cellulose (panel B), and the final fraction A to C obtained after chromatography on the Bio-Gel column of the oligosaccharides obtained by the final treatment. Panels 2 and 3 were pooled and further purified by the Bio-Gel column. Panels 2, 3, and 4 were not subjected to this stage. Arrows indicate elution positions of glucose oligosaccharides; numbers against the arrows indicate the number of glucose units.

Figure 4
Chromatography on the Bio-Gel P-4 column of the radioactive oligosaccharide fractions A to C, after dialysis in water (shown again in panels A, B, and C). The final fraction A to C obtained after chromatography on the Bio-Gel column of the oligosaccharides obtained by the final treatment. Panels 2, 3, and 4 were pooled and further purified by the Bio-Gel column. Panels 2, 3, and 4 were not subjected to this stage. Arrows indicate elution positions of glucose oligosaccharides; numbers against the arrows indicate the number of glucose units.
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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