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Comparative Glycoprofiling of HIV gp120 Immunogens by Capillary Electrophoresis and MALDI Mass Spectrometry

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Abstract

The Human Immunodeficiency Virus (HIV) envelope glycoprotein (Env) is the primary antigenic feature on the surface of the virus and is of key importance in HIV vaccinology. Vaccine trials with the gp120 subunit of Env are ongoing with the recent RV144 trial showing moderate efficacy. gp120 is densely covered with N-linked glycans that are thought to help evade the host's humoral immune response. To assess how the global glycosylation patterns vary between gp120 constructs, the glycan profiles of several gp120s were examined by capillary electrophoresis with laser induced fluorescence detection and MALDI-MS. The glycosylation profiles were found to be similar for chronic vs. transmitter/founder isolates and only varied moderately between gp120s from different clades. This study revealed that the addition of specific tags, such as the gD tag used in the RV144 trial, had significant effects on the overall glycosylation patterns. Such effects are likely to influence the immunogenicity of various Env immunogens and should be considered for future vaccine strategies, emphasizing the importance of the glycosylation analysis approach described in this paper.

Keywords

gp120; glycosylation; Env; HIV; immunogen; CE-LIF; gD tag

1 INTRODUCTION

The HIV Envelope glycoprotein (Env) is the sole target for neutralizing antibodies and is the focus in the field of rational immunogen design [1]. The surface subunit, gp120, contains most of the antigenic surface of the molecule, and the moderate efficacy of the recent RV144 trial has revitalized interest towards the use of soluble gp120 constructs as immunogens [2]. Env is a particularly challenging system from a glycan analysis perspective as nearly 50% of its mass is carbohydrate. It has 20 to 30 N-linked glycosylation sites per gp120 molecule and the glycosylation profile has been shown to influence both antigenicity and immunogenicity [3, 4]. Additionally, several key epitopes have glycan components as some of the most broadly neutralizing antibodies are glycan dependent [5-8]. Therefore, glycosylation profiles of gp120 immunogens represent a critical factor for the elicitation of an effective humoral response against HIV [8]. Such effects have already been observed due

to differences in the degree of sialylation of gp120 subunits from different expression systems [9].

Precise glycoprofiling of proteins with multiple glycosylation sites is challenging, not only because the sugar moieties in a given oligosaccharide can have several positional and linkage isomers, but each potential N-linked glycosylation (PLNG) site can bear many different glycan structures ("microheterogeneity"). High performance liquid chromatography (HPLC), capillary electrophoresis (CE), mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR) are some of the most frequently applied techniques for detailed glycosylation analysis [10]. Liquid separation based techniques (HPLC and CE) usually require tagging of the released oligosaccharides with a chromophore or fluorophore label. The fluorophore label enables very high sensitivity glycoprofiling especially with laser induced fluorescence (LIF) detection [11] and is well suited for monitoring glycosylation patterns on viral glycoproteins [12]. Capillary electrophoresis (CE) requires the use of a charged tag to ensure adequate electrophoretic mobilization of the sugar molecules, even the sialylated species. One of the most frequently used tags for CE-LIF is 1-aminopyrene-3,6,8-trisulfonic acid (APTS) that features excellent fluorescent characteristics and is highly negatively charged enabling rapid analysis times in CE. The migration behavior of many glycan structures with CE-LIF is now well documented enabling structural identification thanks to glycan databases [13]. Targeted exoglycosidase digestions with sialidase, fucosidase and other enzymes is a powerful additional tool to obtain precise structural information (sugar linkage and position) with highly detailed glycosylation patterns [14]. MALDI-MS has also become a useful orthogonal method for semi-quantitative glycan profiling of highly material limited samples [15, 16].

In this study, global N-glycosylation patterns are compared for gp120 constructs from various isolates, including the protein boost immunogen used in the RV144 trial [2]. Capillary electrophoresis with laser induced fluorescence detection combined with the use of exoglycosidase digestion steps was used to analyze the peak distribution in the glycosylation profiles and to elucidate the structures. MALDI-MS was also applied as a complementary method to verify the molecular masses of the components identified in the samples, helping to decipher the complex peak patterns. This comparative study revealed that while overall glycoform distributions are relatively invariant among different gp120s, the addition of the gD tag used in the RV144 trial substantially altered the glycosylation profile, therefore emphasized the importance of glycosylation analysis.

2 MATERIALS AND METHODS

2.1 Chemicals and reagents

The maltooligosaccharide ladder used for glucose unit calculation was from Grain Processing Corporation (Muscatine, IA, USA). High purity APTS was from Beckman Coulter (Brea, CA, USA). Sodium cyanoborohydride (1 M in THF), acetic acid, 2-aminoacridone (AMAC) and acetonitrile were from Sigma Aldrich (St. Louis, MO). PNGase F endoglycosidase and the exoglycosidases of *Arthrobacter ureafaciens* sialidase (ABS) (GK80040), Bovine kidney fucosidase (BKF) (GKX-5006), Almond meal alpha-fucosidase (AMF) (GKX-5019), Jack bean galactosidase (JBG) (GKX-5012), and Jack Bean

hexosaminidase (JBH) (GKX-5003) were from Prozyme (Hayward, CA). Recombinant gp120 samples CM244 (90TH_CM244) [17], gD tagged A244 [18], “Mother” (ML274.WOM.EnvF1) and “Infant” (BL274.W6M.EnvA3) [19] purified from 293 cell culture were purchased from Immune Technology Corp (New York, NY).

2.2 Glycan release and derivatization

HIV gp120 samples (approximately 20 µg protein/sample) were reduced with DTT and dissolved in 50 µL of 50 mM sodium-bicarbonate (pH 7.0). Release of the N-linked glycans was accomplished by the addition of 2 U of recombinant PNGase F (Prozyme) and incubation at 37°C overnight. SDS-PAGE was used to monitor the deglycosylation to ensure complete deglycosylation for all four gp120 samples. The released glycans were separated from the remaining polypeptide chains and the digestion enzyme by means of 10 kDa cut-off spin filters (Nanosep 10 kDa, Sigma-Aldrich) and dried in a centrifugal vacuum evaporator (Thermo Scientific, Asheville, USA).

The dried sugars were fluorescently labeled via reductive amination by the addition of 5.5 µL of 20 mM APTS in 15% v/v acetic acid and 1.5 µL of 1 M sodium cyanoborohydride in tetrahydrofuran at 37°C overnight. The reaction was stopped by the addition of 93 µL of water and the unreacted fluorescent dye was removed by normal phase microcolumns (PhyNexus, San Jose, CA, USA) with the binding step in 90% acetonitrile (100 µL of samples were diluted to 1000 µL with acetonitrile), following by removal of the unreacted fluorophore reagent with 95% acetonitrile and eluting the glycans with HPLC grade water.

2.3 Exoglycosidase digestion

Exoglycosidase digestions included *Arthrobacter ureafaciens* sialidase (ABS) to remove α2-3, 6 and 8 linked sialic acids; Bovine kidney fucosidase (BKF) to release α1-6 core-linked fucose; Almond meal alpha-fucosidase (AMF), to release α1-3 and 4 linked arm-fucose; Jack bean galactosidase (JBG) to remove β1-4 and 6 linked galactose; and Jack Bean hexosaminidase (JBH) to remove the β1-2, 4 and 6 linked N-acetyl-glucosamine. Briefly, APTS-labeled samples were first analyzed by CE-LIF, then sequentially digested using the above listed enzymes (0.5 U each) at 37°C overnight in 50 mM ammonium acetate buffer (pH 5.5). Samples were dried in a centrifugal vacuum evaporator after each digestion to remove the salt (ammonium acetate) content.

2.4 Capillary electrophoresis with laser induced fluorescent detection

Glycoprofiling of the APTS-labeled N-glycans was performed on a P/ACE MDQ automated capillary electrophoresis instrument (Beckman Coulter) equipped with an Ar-ion laser based fluorescent detector (ex: 488 nm, em: 520 nm). 50 cm effective length (60 cm total) NCHO coated 50 µm id capillary columns were used for all CE-LIF analyses (Beckman Coulter) filled with the N-CHO Carbohydrate Separation Gel Buffer (Beckman Coulter). In all electrophoretic separations, 500 V/cm electric field strength was applied in reversed polarity mode (cathode at the injection side and the anode at the detection side). Samples were pressure injected by 1 psi (6.89 kPa) for 5 sec. APTS labeled maltose (G2, lower bracketing standard) and 2-aminoacridone (AMAC) labeled glucuronic acid (upper bracketing standard) were co-injected with all samples for migration time normalization. The

normalized migration times were converted to glucose unit (GU) values by the application of a fifth order polynomial time based standardization against the maltooligosaccharide ladder [20]. Undigested samples were run in duplicate to get an estimate of the run to run variance in migration time and peak intensities. The Karat 32 version 7.0 software package (Beckman Coulter) was used for data acquisition and analysis. Highly overlapped peaks were deconvoluted by fitting to two Gaussian functions with custom Excel macros (Microsoft, Redmond WA). Identification of glycan structures corresponding to all major peaks were aided by the Glycobase ver 3.0 from NIBRT (Dublin, Ireland) [13] based on their GU values measured with identical CE-LIF conditions. N-glycan nomenclature and symbolic representations use the notation previously described by Harvey et al [21].

2.5 Quantification of branching degree and complex to high mannose glycan ratio analysis

Glycan types were quantified from the relative peak intensities within the series of electropherograms throughout exoglycosidase digests. Peaks disappearing upon ABS treatment were integrated providing a precise measure of the population of sialylated glycans present in the starting material. The overall high mannose content and distributions of the high mannose glycoforms were readily obtained from the peak intensities after JBH digestion, including the minor M7 D2; M7 D3; M8 D1, D2; and M8 D2, D3 structures [11]. Branching degrees of complex glycans were obtained from the relative peak intensities of the mono-, bi-, tri-, and tetra-antennary structures obtained after ABS, AMF, BKF and JBG treatment. Structures with residual core fucosylation from incomplete BKF treatment were also accounted for when calculating branching degrees. Lastly the overall percentage of complex type glycans was calculated from two metrics 1) the sum of all of the differently trimmed complex structures in the JBG trace, and 2) the sum of the M3 and F(6)M3 peaks in the JBH trace.

2.6 MALDI MS analysis

The released glycans (1 μ L after resuspending in 20 μ L ultra-pure water) were spotted with 1 μ L of saturated 2',4',6'-Trihydroxyacetophenone monohydrate (THAP) in 50/50 acetonitrile / water and analyzed on a Bruker Autoflex II mass spectrometer (Bruker Daltonics, GmbH, Bremen Germany) in linear positive mode. Spectra were calibrated externally with peptide standards with identical matrix. Mass spectra data were processed using Data Analysis 3.4 (Bruker Daltonics). After 1 round of 0.2 width (m/z) Gaussian smoothing, peak lists (2.5% threshold from base peak intensity) were exported into GlycoMod (<http://web.expasy.org/glycomod>) for glycan composition identification. Potential monosaccharide residues were restricted to Hex, HexNAc, DeoxyHex, and NeuAc. After initial searches for sodium adducts, a secondary was performed for multiply sodiated species, which was predominant for sialic acid containing glycans. Glycan structures matching within 1000 ppm were considered and potential hits were filtered to those present in the UniCarbKB database [22]. For the major species a weak signal was also observed for the potassium adducted species. At this level of mass accuracy the combination of potassium and deoxyhexose results in the same nominal mass as sodium and hexose, and such ambiguity for the major peaks is indicated in Table 2.

3 RESULTS AND DISCUSSION

A comparative glycoprofiling study was used to investigate glycosylation pattern changes for various gp120 constructs. The N-glycans of the gp120 samples were released by PNGase F and profiled by MALDI-MS as well as CE-LIF after fluorophore labeling with APTS. Application of these two orthogonal techniques provided the necessary information for comprehensive characterization of the glycoforms.

3.1 CE-LIF analysis of gp120 glycans

We first investigated the N-glycosylation profiles of gp120 from a primary isolate (CM244) and the re-engineered construct of this same isolate (“A244”), which was used as protein boost for the recent RV144 vaccine trial [2]. A244 gp120 differs by 7 point mutations from the original CM244 and an additional N-terminal herpes simplex virus gD protein-derived tag. The upper traces in Figure 1 show the electropherograms obtained from these two gp120s (CM244 and A244) along with the trace of the maltooligosaccharide ladder that was used for the downstream glucose unit calculation (upper trace). Both samples show the same major peaks (peaks 1-8), but with clear differences in their distribution.

A second comparative study assessed the glycosylation differences of gp120 between a transmitted/founder and chronic viral isolate. Mother to infant transmitted/founder variants are often used to identify key changes within Env that govern the infectivity and escape from the antibody response [23]. The “Mother” and “Infant” gp120s examined here were derived from the same isolate and shared 74% sequence identity with 7 changes in PNLGs [19]. In contrast to the A244/CM244 comparison, the glycoform distribution for the Mother and Infant gp120s appears remarkably similar with only minor changes in peak heights (Figure 1, lower traces).

3.2 Glycoform structural elucidation

Since the electropherograms of the global glycoform distributions were relatively complex, exoglycosidase enzyme digestion followed by CE-LIF analysis were used to identify and resolve all major species present within each sample. A series of the various exoglycosidase reactions are shown in Figure 2. The upper trace depicts the undigested trace of sample CM244. After sialidase treatment (ABS trace), some minor peaks disappeared (labeled with asterisks) at the lower GU unit range of 5-6 (migration time 10.5–11.5 min) and apparently altered the size of some of the peaks in the neutral glycan range of GU 6-15 (11.5–17.5 min). Next the desialylated glycan pool was subject to AMF digestion to remove possible arm fucosylated moieties (AMF trace). This trace revealed no changes in any of the glycan structures indicating no antennary fucosylation. The lack of antennary fucosylation was similarly observed with the other three gp120 samples.

Samples were subsequently treated with BKF enzyme to remove core fucose residues. In contrast to AMF treatment, this enzyme dramatically affected peak distribution (BKF trace). Such apparent changes decreased the areas of peaks 3, 8 and 9, with new peaks showing up at different locations (peaks 10, 11 and 12). These changes represented the shift of structures from the core fucosylated versions to afucosylated ones. The fact that the area of peak 3 only

decreased suggested co-migration of at least two structures, one of which shifted to peak 10. Peak 9 also shifted down to the new peak 12 but not fully. This latter phenomenon was most likely due to incomplete defucosylation, which was apparent especially for the larger glycan structures. Galactosidase (JBG) treatment trimmed all galactosylated complex-type glycans (JBG trace), consistent with the extensive shifts observed, with complex bi-, tri-, and tetra-antennary structures shifting to peaks 10, 13 and 14. Lastly, hexosaminidase (JBH) treatment removed all GlcNAc moieties and shifted all complex glycans to the common trimannosyl core structure (peak 15), as well as the trimannosyl core structure with residual core fucosylation due to incomplete BKF digestion (peak 16; JBH trace). The remaining peaks represent the high mannose structures and hybrid glycans that have been trimmed down to M5. An M4 structure is also present that was observable throughout all the digestion steps (peak 17).

The GUs obtained from the series of glycosidase treatments were compared to the Glycobase v3.0 database from NIBRT to elucidate each glycan structure (Table 1). All matched structures were within 0.22 GU of the reported migration times (by CE) in the database. MALDI-TOF was performed in parallel to analyze the predominant species (Figure 3). Although it provides no information on linkage or stereochemistry, the carbohydrate compositions of the various species can be extracted from the intact mass (Table 2). The predominant species identified by MS, were consistent with those obtained from CE-LIF analysis, corroborating the structures identified. Hybrid type glycans could not be identified as of yet, as GU units for hybrid structures have not been established in the NIBRT database. Masses corresponding to hybrid type glycans were present in the MS data, though the same masses could arise from potassium adducts of other predominant glycans structures (Table 2). Through the course of exoglycosidase digestions all hybrid glycans should be trimmed down to M5 structures after JBH treatment. The small difference observed in the relative intensity of the M5 peak in response to JBH treatment in all four samples suggests that the content of hybrid-type glycans is very low (<0.5%).

3.3 Relative distributions of gp120 N-linked glycans

The various glycoforms were quantified to assess the differences in their abundance among the gp120 samples. Although several peaks within the CE electropherograms of the untreated samples actually contained multiple species, from the exoglycosidase treated samples it was possible to resolve, and obtain quantitative measures of the different types of glycan structures (see methods). The relative degree of sialylation and complex-type glycans, as well as branching and distribution of high mannose glycans for each sample are shown in Table 3. Though the exoglycosidase digested samples were each only analyzed once, duplicate analysis of the undigested samples showed an average deviation in relative peak heights of less than 0.2%. Therefore quantitative analysis by this method is expected to have a high degree of precision. The overall percentage of complex type glycosylation was calculated from both the JBG and JBH traces. Quantitative integration of all peaks in the electropherograms was comprehensive and very likely included spurious peaks resulting from low levels of isomerization [24] (see Figure 2 legend), incomplete exoglycosidase digestion, the presence of LacdiNAc structures [25], and other possible sources of background noise. However, since all samples were handled and analyzed under identical

conditions, as a first approximation we consider each having similar background and therefore such artifacts should not compromise the comparative analysis between the gp120 samples.

In general, the Mother and Infant samples had very similar glycosylation profiles, as observed from the initial electropherogram comparison. The distributions among the high mannose glycoforms were remarkably similar within the two samples (Figure 4). The only notable differences were the higher degree of sialylation within the Infant sample and a higher portion of bi- and tri-antennary complex glycans in the Mother sample (Table 3, Figure 5). The overall similarity in the net glycoform distribution is a bit surprising considering the two constructs share only 74.0% sequence identity and the Infant sample contains 4 more PNLG sites. These additional PNLGs were located within variable loops V1, V2, and V4, which display mostly complex-type glycans based on previous examination of Env constructs [26-28]. From this one would expect a higher degree of complex type glycosylation in the Infant samples; however, the overall percentage of such glycosylation in the two samples was remarkably similar. Therefore, even the difference in number and location of several PNLG sites among similar gp120 constructs does not appear to drastically alter the net glycosylation profile.

Unlike the Mother and Infant samples, the A244 vs. CM244 showed very pronounced differences in their glycan structure distribution (Figures 4, 5). The CM244 sample had a similar ratio of high mannose to complex type glycans as both the Mother and Infant. However, this ratio was distinctively higher in the A244 sample. Furthermore, the distributions among the high-mannose type structures were very different for the A244/CM244 pair with A244 bearing much less M9 glycoforms as depicted in Table 3 and shown in Figure 4. The large differences in the glycosylation profiles of A244 and CM244 are surprising considering that the only differences between the two are the gD tag and 7 point mutations that only affect 2 of the 25 PNLG sites.

The overall glycosylation trends for the three non-gD-tagged gp120s (CM244, Mother and Infant), were relatively similar, implicating the gD tag as the primary cause for the unique glycosylation profile for the A244 sample. The presence of this tag has been shown to alter the antigenicity of gp120, which is attributed to the removal of the native 11 N-terminal residues of gp120 [29]. Regardless of the cause, from the work reported here we consider that some of the resulting changes may be associated with altered glycosylation profiles, which are known to affect the antigenicity of gp120 [3, 4]. The leader sequence, expression vector and purification techniques were constant for all samples therefore the remaining possible sources of glycosylation differences are altered trafficking through the ER and Golgi, or differences in protein conformation that alter susceptibility of various sites to glycan processing enzymes. Both of these affects may result in the observed changes in mannose trimming in A244, and therefore both remain a possible source for the phenotypic difference. Though the original RV144 gp120 immunogen was expressed in CHO cells, which have a different glycosylation profile [3], the comparative antigenic studies on the effects of the gD tag in A244 utilized the same 293 cell expression system as the material analyzed here [29]. Site-specific glycoprofiling, which was beyond the scope of this study,

should provide further information in regard to whether the effects are global or localized to a specific region of gp120 that is critically influenced by gD tag modification.

From a vaccinology standpoint, the presence of dense high mannose glycans on gp120 was found to limit its immunogenicity through enhanced interaction with mannose C-type lectin receptor(s) on dendritic cells [30]. For this reason, demannosylated variants were suggested as a possible strategy for improving immunogenicity. Based on the current results, the A244 vaccine boost indeed bears both smaller as well as a lower degree of high mannose type glycans relative to CM244, and even the other two isolates examined. A better understanding of how modifications to gp120 alters the resulting glycosylation patterns will greatly aid the development of more effective HIV vaccine immunogens with optimal antigenicity and immunogenicity.

4. Concluding remarks

CE-LIF in conjunction with exoglycosidase digestion and MALDI-MS provided a robust and reliable method for comprehensive structural elucidation and precise quantitation of very complex glycoform distributions of gp120 N-glycans with excellent detection sensitivity. From the current comparisons, there appears to be only minor variations in glycoform distributions in gp120s from different HIV isolates, given identical expression systems and conditions. However, modifications such as the removal of the native 11 N-terminal residues and the addition of the gD tag used for the RV144 gp120 immunogen, resulted in a significant glycosylation changes. The way in which gp120 modifications alter the overall glycosylation profile should be a key consideration in the development of new HIV immunogens.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

APTS	1-aminopyrene-3,6,8-trisulfonic acid
PLNG	potential N-linked glycosylation
Env	HIV envelope glycoprotein
NMR	nuclear magnetic resonance
PNGaseF	peptide N-glycosidase F
GU	glucose unit
gD	herpes simplex virus glycoprotein D

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Figure 1.

Comparative N-glycosylation profiling of gp120 glycan samples by CE-LIF. The upper trace shows the maltooligosaccharide ladder with the GU units indicated above each peak for precise migration shift determination. Electropherograms are aligned and shown for CM244, A244, Mother and Infant. Major peaks are numbered with corresponding peak and glycan structure information presented in Table 1.

Figure 2.

Electropherograms of the consecutive exoglycosidase digestion reaction products of CM244 N-glycans. The abbreviated names of the individual exoglycosidase enzymes label the respective traces corresponding to the removal of sialic acids (ABS), arm fucose (AMF), core fucose (BKF), galactose (JBG) and N-acetylglucosamine residues (JBH). Gray arrows indicate the major shifts occurring upon exoglycosidase treatment. Major peaks are numbered and the structures are shown in Table 1. * Sialylated glycans that disappear with ABS treatment. ° The peak preceding peak 15 (at GU 10.5 in the JBH trace) is likely an isomer of the M3 peak, similar to that observed previously [24].

Figure 3. MALDI-MS analysis of glycans released from gp120 by PNGaseF digestion (sample CM244). Major peaks are numbered and compositions are listed in Table 2.

Figure 4. Electropherograms of the four gp120 glycan samples after full exoglycosidase treatment (ABS, AMF, BKF, JBG and JBH) with the structures of the major peaks labeled. Quantitative analysis of the ratios of complex and high mannose glycans along the distribution of high mannose glycans is shown in Table 3.

Figure 5.

Electropherograms of the four gp120 glycan samples after ABS, AMF, BKF, and JBG treatment, with the structures of the major peaks labeled. Degree of branching within complex glycans is evident from the distribution of the A2, A3 and A4 glycans along with these same structures with residual core fucosylation (F(6)A2, F(6)A3, and F(6)A4). Quantitative analysis of the distributions of branching patterns is shown in Table 3.

Table 1

Structure abbreviated names, CE migration times and glucose unit values (GU) of the N-glycans of this study. Numbering correspond to the peaks in Figures 1 and 2. Diagrams of each structure are shown in Supporting Information Figure S1.

Peak #	Structure ^{a)}	Migration time (min)	GU measured	GU database
1	F(6)A2[6]G(4)2S(6)1	11.94	6.70	6.67
2	M5	12.00	6.80	6.75
3 ^{b)}	M6	12.61	7.66	7.60
3 ^{b)}	F(6)A2	12.61	7.66	7.63
4 ^{b)}	M7 D1	13.34	8.65	8.68
4 ^{b)}	F(6)A2[6]G(4)1	13.34	8.65	8.72
5	F(6)A2[3]G(4)1	13.55	9.03	9.06
6	M8 D1, D3	13.88	9.50	9.54
7 ^{b)}	M9	14.26	10.10	10.13
7 ^{b)}	F(6)A2G(4)2	14.26	10.10	10.10
8	F(6)A(6)3G3 ^{c)}	15.57	12.09	-
9	F(6)A4G(4)4	17.06	14.39	14.36
10	A2	11.94	6.69	6.67
11	A(6)3G3	14.99	11.12	11.25
12	A4G(4)4	16.52	13.33	13.55
13	A3	12.69	7.79	7.81
14	A4	13.42	8.85	8.89
15	M3	10.53	4.85	4.83
16	F(6)M3	11.17	5.60	5.58
17	M4 ^{c)}	11.31	5.80	-

^{a)} Glycan structure nomenclature as described by Harvey et al [21].

^{b)} Peaks contained two major glycan components with nearly identical migration times.

^{c)} Glycan structures were not available in the Glycobase v3.0 database and the structures were inferred from shifts during exoglycosidase digestions and masses observed by MADLI-MS.

Table 2

Suggested compositions of gp120 derived glycans based on MALDI-MS analysis. Average masses and glycan compositions for peaks in Figure 3 are listed. Structures that correspond to a major peak observed by CE-LIF are shown in bold with the corresponding CE peak ID (Figure 1, Table 1).

Peak ID	m/z obs.	m/z calc.	Error (ppm)	Composition	CE peak ID
1	934.4	933.8	602.9	Hex ₃ HexNAc ₂ Na ₁	
2	1096.9	1096.0	814.7	Hex ₄ HexNAc ₂ Na ₁	
3	1137.4	1137.0	337.2	Hex ₃ HexNAc ₃ Na ₁	
4	1258.1	1258.1	19.4	Hex₅HexNAc₂Na₁	2
5	1282.9	1283.2	-205.4	Hex ₃ HexNAc ₃ DeoxyHex ₁ Na ₁	
6	1420.4	1420.3	108.8	Hex₆HexNAc₂Na₁	3
7	1445.2	1445.3	-92.3	Hex ₄ HexNAc ₃ DeoxyHex ₁ Na ₁	
8	1461.2	1461.3	-106.2	Hex ₅ HexNAc ₃ Na ₁ or Hex ₄ HexNAc ₃ DeoxyHex ₁ K ₁ ^a	
9	1486.4	1486.4	37.8	Hex₃HexNAc₄DeoxyHex₁Na₁	3
10	1582.6	1582.4	116.7	Hex₇HexNAc₂Na₁	4
11	1623.3	1623.4	-77.0	Hex ₆ HexNAc ₃ Na ₁ or Hex ₇ HexNAc ₂ K ₁ ^a	
12	1648.5	1648.5	-8.2	Hex₄HexNAc₄DeoxyHex₁Na₁	4 & 5
13	1689.7	1689.6	104.2	Hex ₃ HexNAc ₅ DeoxyHex ₁ Na ₁	
14	1744.6	1744.5	8.7	Hex₈HexNAc₂Na₁	6
15	1810.8	1810.6	64.4	Hex₅HexNAc₄DeoxyHex₁Na₁	7
16	1851.9	1851.7	111.4	Hex ₄ HexNAc ₅ DeoxyHex ₁ Na ₁	
17	1906.5	1906.7	-80.9	Hex₉HexNAc₂Na₁	7
18	2013.9	2013.8	18.3	Hex ₅ HexNAc ₅ DeoxyHex ₁ Na ₁	
19	2123.8	2123.9	40.3	Hex₅HexNAc₄DeoxyHex₁NeuAc₁Na₂	1
20	2175.9	2176.0	-15.1	Hex₆HexNAc₅DeoxyHex₁Na₁	8
21	2217.0	2217.0	-19.4	Hex ₅ HexNAc ₆ DeoxyHex ₁ Na ₁	
22	2378.8	2379.2	-173.2	Hex ₆ HexNAc ₆ DeoxyHex ₁ Na ₁	
23	2541.2	2541.3	-32.4	Hex₇HexNAc₆DeoxyHex₁Na₁	9

^aMass corresponds to either hybrid type glycans or potassium adducts.

Table 3

Percent distributions of glycan types in gp120s. Relative amounts of each glycan type are reported as percentage of total glycan content.

	CM244	A244	Mother	Infant
Degree of sialylation	9.91	10.05	9.61	13.65
Total complex type ^{a)}	54.11	69.85	49.26	51.60
Total high mannose (M5–M9) & hybrid type ^{b)}	34.04	18.81	27.24	28.61
M5 ^{b)}	6.15	4.10	8.11	8.41
M6	3.59	2.62	3.15	2.42
M7	7.54	4.65	5.21	4.61
M8	10.25	5.68	6.79	8.86
M9	6.51	1.76	3.98	4.32
Degree of branching				
Mono	5.69	7.26	6.18	8.88
Bi	19.34	32.52	29.12	25.08
Tri	14.66	15.54	10.24	8.12
Tetra	4.32	6.73	6.00	6.82
Total complex type ^{c)}	44.00	62.05	51.55	48.91

^{a)} Obtained from the relative intensity of M3 and F(6)M3 after JBH digestion (Figure 4).

^{b)} The M5 peak represents both M5 in the undigested starting material and hybrid type glycans that are trimmed to M5 during exoglycosidase digestion.

^{c)} Obtained from the sum of complex type glycans after JBG digestion (Figure 5).