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HIV-1 Variants with Reduced Sensitivity to Sulfated Oligosaccharide Muparfostat Contain Mutations in the Envelope Glycoproteins gp120 and gp41

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Abstract

Attachment of human immunodeficiency virus type 1 (HIV-1) to host cells is primarily mediated by cell surface molecules CD4 and either of the chemokine co-receptors CCR5 or CXCR4, and is facilitated by cellular heparan sulfate chains of syndecans. Although mimetics of heparan sulfate exhibit potent anti-HIV-1 activity in cultured cells, these compounds failed to prevent infection in humans when used in clinical trials as microbicides. We have previously shown that the low molecular weight and extensively sulfated oligosaccharide muparfostat coupled to cholestanol exhibited virucidal activity while the non-conjugated muparfostat (formerly known as PI-88) inhibited HIV-1 infection of cultured cells in a reversible manner only. To initiate clarification of distinct anti-HIV-1 potencies of muparfostat and muparfostat-cholestanol conjugate, in this work we sought to select for viral resistance using the less potent muparfostat. The laboratory strain HIV-1^{IIIB} was successively propagated in H9 cells in the presence of the compound. The virus selected for after 21-24 passages appeared to be approximately 3-4 times less sensitive to muparfostat than the original HIV-1^{IIIB} strain or control virus passaged in parallel in the absence of muparfostat. Comparative analysis of nucleotide sequences of these viruses revealed presence of the I152V substitution in V2, the K276R change in V3, the deletion of five amino acid repeat 366FNSTW370 in V4 of gp120, and the L33S and A101T alterations in transmembrane gp41 component of the muparfostat passaged virus. Selection for viral variants with mutations in gp41 was an unexpected observation as this protein of HIV-1 is seldom targeted by sulfated polysaccharides.

Keywords: HIV-1; Muparfostat; Resistance; Escape variants; gp120; gp41

Introduction

Initial interaction of human immunodeficiency virus type 1 (HIV-1) with susceptible host cells is binding of viral envelope glycoprotein subunit gp120 to cell surface CD4 [1]. This binding is known to induce changes in the conformation of gp120 that exposes binding sites required for the virus interaction with chemokine receptors CCR5 (R5) or CXCR4 (X4) depending on the amino acid sequence of gp120 [2-5]. Interaction of trimeric gp120 with both CD4 and chemokine co-receptor triggers fusogenic activity of the transmembrane gp41 subunit of envelope glycoprotein. These include uncovering and insertion of fusion peptide into plasma membrane of targeted cells, exposure of two separate heptad-repeat regions at positions close to viral and cellular membranes, and strong interaction between these domains that opposes viral and cellular membranes provoking their fusion, pore formation, and insertion of the viral core structure into host cells [6,7]. This canonical route of HIV-1 entry into cells is strongly influenced by the level of expression of required receptors. Thus, in some cells targeted by HIV-1 such as macrophages, where expression of CD4 is low, this interaction is accompanied or strengthened by the virus binding to heparan sulfate (HS) glycosaminoglycan (GAG) chains of cell surface proteoglycan syndecan [8]. Furthermore, in cells of genital epithelium, that do not express CD4, HS chains of syndecan were found to provide binding sites for the virus attachment to cells and its transmission to susceptible CD4 expressing cells [9-12]. In addition, in two separate studies, heparinase treated T cells showed reduced sensitivity to HIV-1 [13,14] suggesting that the cell surface HS chains may facilitate infectivity even in cells with abundant expression of CD4. The viral gp120 contains four HS binding domains that in part overlap with the chemokine receptor binding sites [2] and their activity is most likely based on electrostatic interactions between the basic, positively charged residues of V3 loop of HIV-gp120 and/or the co-receptor binding sites on gp120 and the negatively charged sulfate/carboxylate groups of HS [13]. Attachment of X4-using viruses and dual-tropic viruses using both chemokine receptors R5 and X4 to HS is stronger than that of R5-using viruses, probably because of the higher net positive charge of their V3 loop [14-16].

The HS mimetics, such as sulfated polysaccharide cellulose sulfate (CS) [17,18] or naphthalene sulfonate polymer PRO2000 [18,19] are known inhibitors of adherence of HIV-1 particles to cells and their anti-HIV-1 potency was exploited in clinical trials. However, intravaginal application of CS not only failed to prevent against HIV but in fact increased the risk of acquiring this infection [20,21]. One possible explanation to the CS failure in human trials, regardless of the convincing antiviral activity in cell culture, is that HS chains of cell surface syndecans were reported to capture, protect and transmit HIV-1 particles to susceptible cells. The protective activity of HS chains was manifested among others by the fact that the syndecan-bound virions remained viable for a week while unbound virus particles lost infectivity after one day [11]. The same seems to be true for HS mimetics,

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which are hydrophilic and negatively charged polymeric molecules. Consequently their mode of antiviral activity relies on multiple but weak electrostatic associations with viral components which are reversible and non-virucidal [22], and as such can be exploited by a variety of viruses for their masking, controlled release, and spread. To verify this assumption, we have previously conjugated a cholestanol group to the sulfated oligosaccharide muparfostat (formerly known as PI-88), and found that although both compounds inhibited infectivity of HIV-1, herpes simplex virus (HSV), and respiratory syncytial virus (RSV) [23-25] only the cholestanol-muparfostat conjugate exhibited virucidal activity i.e., it irreversibly blocked infectivity of these viruses. Muparfostat is a mixture of extensively sulfated di- to hexasaccharides showing broad antiviral potency against enveloped viruses that use GAGs for attachment to and entry into target cells [23-26]. However, as already mentioned its antiviral activity, like that of the majority of HS mimetics [22], is reversible and a simple dilution of the virus-HS mimetic complexes, such as that employed in virucidal assays, can release infectious virus.

To investigate the mechanism of antiviral activity of muparfostat and the related virucidal compounds we attempted to select for resistant viral variants, using the weaker inhibitor muparfostat, by passaging HIV-1 in H9 cells. This very approach was used in our previous studies with HSV that was passaged in the presence of muparfostat in monkey kidney AH1 cells. Sequence analysis of the muparfostat-resistant HSV-1 variants revealed mutations in the mucin-like region of glycoprotein gC [27], which is thought to interact with cell surface HS prior to the virus entry into the cell [28,29]. Likewise, mutations in the mucin-like region of glycoprotein gG were found in muparfostat-resistant variants of HSV-2. The procedure of passaging HIV-1 in H9 cells has been verified in our laboratory in a previous antiretroviral study [30]. Our present work demonstrated that variants of HIV-1 with decreased sensitivity to muparfostat could indeed be selected for after 24 passages in cells, and that these viral mutants had amino acid alterations in the HS-binding domain and in the V2 and V4 loops of gp120, including a pentamer deletion in the latter. Amino acid substitutions were also found in close proximity to the pocket-forming sequence and the N-terminal heptad repeat region of gp41.

Materials and Methods

Muparfostat

Muparfostat was prepared by hydrolysis of the extracellular phosphomannan polysaccharide of the yeast *Pichia holstii*, to yield a phosphorylated oligosaccharide fraction, which was subsequently chemically sulfonated [31-33]. The compound was obtained from Progen Pharmaceuticals Ltd. (Australia).

Cells and viruses

H9-cells were cultured in RPMI-1640 media (Gibco, Paisley, UK) supplemented with 10% heat-inactivated fetal calf serum (FCS), penicillin (60 µg/mL), streptomycin (100 µg/mL), and polybrene at 2.24 µg/mL. The cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2. The HIV-1^{IIIB} laboratory strain used was kindly provided by Dr. R.C. Gallo and Dr. M. Popovic (at the time at the National Cancer Institute, NIH, Bethesda, MD).

Selection of resistant viral variants

H9-cells (5×10^5 cells/mL) were infected with 100 cell culture infective doses ($CCID_{50}$) of HIV-1^{IIIB} pre-treated with 12.5, 25, 50, and 100 µg/mL of muparfostat and cultured in 24-well plates for 7-8 days. Viral replication was monitored by observation of the cytopathic effect (CPE), i.e. syncytium formation, and cultures showing extensive CPE, normally after 7 days of virus inoculation, were used for further passage, where upon 75 µL of supernatants from these wells were mixed with the same concentrations of muparfostat as mentioned above and added to fresh H9 cells. The virus was also passaged in H9 cells in the absence of muparfostat to serve as controls. After 19 passages, the dominant virus population was isolated by the limiting dilution method. To this end, the supernatants were diluted 10-fold in media and cultured in fresh H9 cells supplemented with 50 µg/mL of muparfostat. The highest dilution of viral inoculum that induced syncytium formation in H9 cells served as a source of material for next round of virus isolation. This virus was subjected to three additional passages in the presence of 100 µg/ml of muparfostat resulting in a total of 24 passages. The IC_{50} determinations for the virus passaged 21 and 24 times respectively in the presence of muparfostat (P21 and P24), the control virus passage (C21) and the original wild type virus HIV-1^{IIIB} (wt) were performed in H9 cells. Cells (5×10^5 cells/mL) in triplicate wells were inoculated with a mixture of 100 $CCID_{50}$ of each virus and serial 2-fold increasing concentrations of muparfostat (10-160 µg/mL) and incubated at 37°C. Media were changed on day 6, and supernatants were collected on days 6-9 and analysed for p24 antigen content by enzyme-linked immunosorbent assay (ELISA) using *in house* produced antibodies as previously described [34]. The final dilution of the virus stocks used in this experiment was approximately 200-fold thus making it unlikely that any residual muparfostat present in the diluted P21 and P24 virus (0.5 µg/ml) would affect the IC50 value. The efficacy of replication of viral variants P21 and C21 as well as wt virus HIV-1^{IIIB} was compared by inoculation of H9 cells with 100 $CCID_{50}$ of respective strain and monitoring the p24 production in the supernatant fluid daily at days 6 through 10 after infection.

Gene sequencing

The RNA of the passaged viral variants and original wt HIV-1^{IIIB} strain was extracted from infectious supernatants using QiaAmp Viral RNA Mini Kit (Qiagen GmbH, Hilden, Germany). The envelope (env) gene fragments coding for gp120 and gp41 were amplified by reverse transcriptase PCR using forward and reverse primer pairs specified in table 1. The PCR products were purified with Qiaquick PCR purification kit (Qiagen GmbH, Hilden, Germany) and the PCR sequence reactions were performed using Big Dye Terminator v1.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and forward and reverse primer pairs (Table 1). Samples were then precipitated with NaAc and 99.5% ethanol, solubilized in formamide, and sequenced by a 16-channel Applied Biosystems 3130/3130*xl*Genetic Analyzer (Hitachi). Results were analysed using Sequencher version 4.9.

Results

Muparfostat selects for moderately resistant variants of the HIV-1^{IIIB} strain

To investigate whether muparfostat could select for resistant variants of the HIV-1^{IIIB} strain, the compound was mixed with the virus just prior to inoculation of H9 cells, and then the virus-compound mixture was incubated with these cells for 7-8 days. Passages of HIV-1^{IIIB} were performed 24 and 21 times respectively in the presence (P24 and P21) or 21 times in the absence (C21) of muparfostat. Sensitivities of P24, P21, C21, and wt HIV-1^{IIIB} strain to muparfostat, determined by monitoring of the production of viral p24, are shown in Figure 1A. The IC50 values were 60 µg/ml for P24 and P21, 20 µg/mL for C21, and 15 µg/ml for the original wt virus (Figure 1A). These data indicate

that muparfostat selected for virus variants that were ~3-4 times less sensitive to this compound than the C21 or the original wt virus (Figure 1A). Note that no further increment in IC50 occurred between passage 21 and 24. No difference in viral replication efficacy was seen between the P21 and C21 variants (Figure 1B).

HIV-1 virus escape mutants contain amino acid changes in gp120 and gp41

The env gene fragments coding for gp120 and gp41 of the P24, C24, P21, C21, P16, C16 and HIV-1^{IIIB} viruses were sequenced using three and two pairs of forward and reverse primer for gp120 and gp41 respectively (Table 1). Three point mutations and one deletion of 15 nucleotides at positions 6956 through 6970 were found in the gp120 coding sequence of P24 and P21 but not in C24, C21 or wt HIV-1^{IIIB}. Two of these point mutations resulted in amino acid substitutions I152V and K276R while the loss of 15 nucleotides gave rise to the deletion of amino acid residues 366-FNSTW-370 (Table 2). The I152V amino acid substitution (Figure 2A) was located right at the distal part of the hypervariable V2 loop (Supplementary Figure 1A), the K276R change (Figure 2B) occurred at the interior of the HS-binding domain of the V3 loop of gp120. The deletion of the 366FNSTW370 amino acid stretch comprising a glycosylation site for an *N*-linked glycan concerned the repeat region of the V4 loop (Supplementary Figure 1A). In addition, two mutations in the gp41 transmembrane protein were found. The L33S amino acid substitution (Figure 2C) was located just prior to the beginning of the N-terminal heptad repeat region (Supplementary Figure 1B) [35] while another amino acid change, the A101T (Figure 2D), occurred at the intervening domain between the N-terminal and C-terminal heptad repeat regions (Supplementary Figure 1B). The virus passaged alongside P24 and P21 in the absence of muparfostat, C24 and C21, also exhibited amino acid changes in two positions in the gp41 region. The I69V alteration occurred in the N-terminal heptad repeat region while the A300T amino acid substitution was found in the cytoplasmic tail of this protein (Supplementary Figure 1B). These two mutations were not found in P24/P21 or in the wt HIV-1^{IIIB} strain. Note that while single amino acid substitutions in gp120 and gp41 were associated with 3-4-fold resistance to muparfostat as determined at passage 21 (Figure 1A), the 333-FNSTW-370 deletion that occurred after passage 21 (Table 2) did not increase the virus resistance (Figure 1A).

Discussion

Development and analysis of drug resistant virus variants, although an unwelcome feature in clinical settings, is a helpful tool in deciphering both the modes of antiviral activity of a drug in question and biological activities of targeted viral proteins or its domains. Analysis of HIV-1 escape variants may be especially warranted for compounds aimed for development of microbicides, given the recent negative outcome of human prophylactic trials. In this study we prepared and analysed variants of the HIV-1^{IIIB} strain resistant to HS mimetic muparfostat, the carbohydrate part of the microbicide candidate compound designated as P4 or PG545 [23,25]. Muparfostat [23] and other HS mimetics/

Gene region	Outer primer, RT-PCR[a]	
	Forward	Reverse
gp120	5′TGTGTGGTCCATAGTAATCATAGAATA′3	5′CTCAATAGCCCTCAGCAAATT′3
gp41	5′CAATTGGAGAAGTGAATTATAT′3	5′CTGGCTCAGCTCGTCTCATTC′3
	Inner primer, Sequencing-PCR	
	Forward	Reverse
gp120	5′GAAAGAGCAGAAGACAGTGGCAATGA′3	5′ATTGGCTCAAAGGATACCTTTGGACA′3
	5′CAATATCAGCACAAGCATAAGAGGTAA′3	5′TCCTGAGGATTGCTTAAAGATTATTGT′3
	5′GGAAATATGAGACAAGCACATTGTAAC′3	5′TACCAGACAATAATTGTCTGGCCTGTA′3
gp41	5′AAGAGAAGAGTGGTGCAGAGAG′3	5′GCCTAACTCTATTCACTATAGA′3
	5′TATTGGAATTAGATAAATGGGC′3	5′GCCATCCAATCACACTACTT′3

[a]Reverse transcriptase PCR reaction for generation of cDNA and elongation.

Table 1: PCR primers used for sequencing of env gene of HIV-1.

		HIV-1 passaged with muparfostat			HIV-1 passaged without muparfostat	
Passage no.	Day	Muparfostat concentration (μg/mL)	Amino acid change in gp120	Amino acid change in gp41	Amino acid change in gp120	Amino acid change in gp41
0	0	12.5	wt[a]	wt	wt	wt
1	7	12.5				
12	85	12.5	I152V[b]	wt	K276R[b]	wt
14	99	12.5				
15	106	25				
16	113	25	I152V[b]	wt	K276R[b]	wt
19	134	25				
20	141	50				
21	148	50	I152V K276R	L33S A101T	wt	I69V A300T
22	155	100				
24	162	100	I152V K276R Δ366FNSTW370	L33S A101T	wt	I69V A300T

[a]Amino acid sequence of the original wild type (wt) virus HIV-1^{IIIB}
[b]Confers to partial amino acid substitution with the wild type background

Table 2: Amino acid alterations in HIV-1 variants selected by virus passaging in H9 cells in presence of muparfostat.

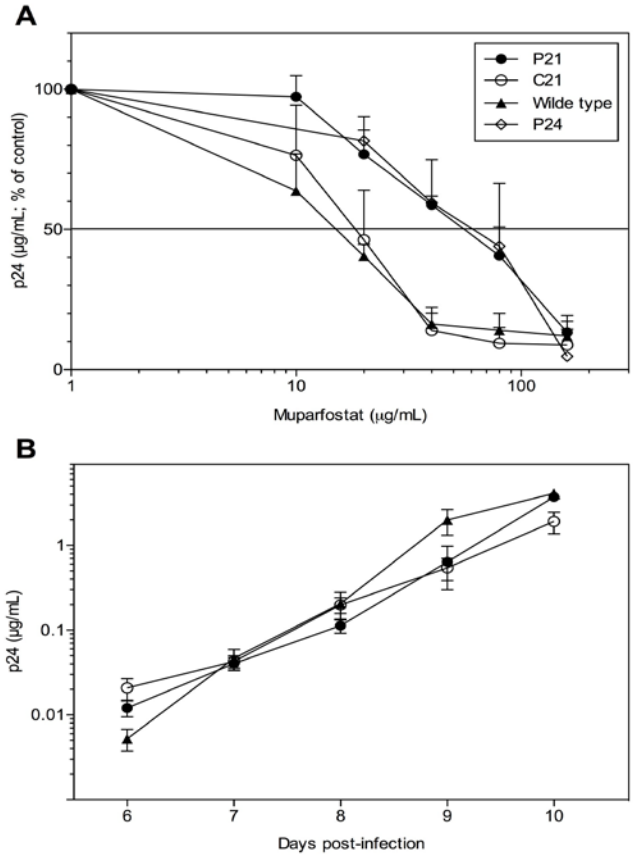

Figure 1: Sensitivity of HIV-1 variants to muparfostat and efficacy of their replication in cells. (A) The viral variants selected for after 24 and 21 passages of HIV-1^{IIIB}strain in H9 cells in the presence of muparfostat (P24, P21) or in the absence of this compound (C21) as well as original non-passaged (Wild type) virus were tested for sensitivity to muparfostat in H9 cells. (B) The H9 cells were infected with 100 CCID50 of P21, C21, and wild type HIV-1, and the amount of viral p24 released into the culture supernatant medium determined after 6-10 days of incubation of cells. The results are expressed as a percentage relative to mock treated viral control in the absence of muparfostat (Figure 1A) and as µg/mL (Figure 1B) of the amount of viral p24 produced. Results are shown as mean values of three separate experiments.

polyanionic compounds such as dextran sulfate [36] or PRO2000 [37] are known to interfere with HIV-1 infectivity at the stages of virus attachment to and/or entry into the cells. Following 24 passages of HIV-1^{IIIB} in the presence of muparfostat, the selected virus was ~3-4 times less sensitive to this compound than the original strain or the virus passaged in parallel in the absence of muparfostat. In spite of this relatively low resistance, comparative analysis of sequence data revealed presence of five specific alterations, i.e., deletion of the 366-FNSTW-370 amino acid stretch, the I152V and K276R amino acid substitutions in gp120, and the L33S and A101T changes in gp41. All four amino acid substitutions were seen as sole peaks after 24 and 21 passages (Figure 2A-D) as illustrated in the chromatograms from the sequencing reactions. The viral variant carrying the I152V mutation was selected already at passage 12, and this phenotype coexisted with wild type virus until passage 16 as deduced from the presence of a double peak at the site of the nucleotide alteration (not shown). The fact that a smaller guanine peak was observed in the wild type virus aside the dominating adenine suggests that the selected variant upon the lead drug pressure indeed corresponds to a selection of specific variants in the mixed virus population of the HIV-1^{IIIB} strain. Interestingly some of mutations found in the muparfostat resistant virus were different from those conferring resistance of HIV-1 NL4-3 strain to dextran sulfate. In particular the latter virus comprised numerous amino acid substitutions and these were the S114N in V1, the S134N in V2, the K269E, Q278H and N293D in V3, and the N323S in the C3 region. In addition, the reported deletion of five amino acid residues at positions 364-368 [38] is identical to the 366-FNSTW-370 deletion found in muparfostat resistant HIV-1^{IIIB} strain in our study. This deletion was also observed in HIV-1 strains resistant to both lipophile-modified cyclodextrinsulfate [39] and bicyclam, a macrocyclic polyamine targeting the CXCR4 chemokine receptor [40,41]. These data indicate that the 366-FNSTW-370 deletion in the V4 loop of gp120 is a frequent alteration in viral variants resistant to polyanionic compounds that target the HS- or chemokine receptor-binding domain(s) of gp120, and in variants resistant to specific compounds of polycationic nature that block HIV infectivity by interaction with negatively charged sites on cell surface HS chains or chemokine CXCR4 receptor. Interestingly, in the present study, this deletion occurred as a late event during selection with muparfostat and did not result in increased resistance to the compound.

It is not surprising that many mutations that confer resistance of HIV-1 to polyanions and polycations occur in the positively charged V3 loop region known to be involved in the interaction of HIV-1 with HS chains and chemokine co-receptors [42]. A tendency is that, the virus resistance to polyanions such as dextran sulfate is conferred by amino acid alterations that decrease an overall net positive charge in the V3 loop region [38] thus reducing the likelihood of interaction of virus particles with negatively charged inhibitor. In contrast, resistance to polycations such as bicyclams is associated with an increase in a net positive charge of V3 [40] which provide the virus with selective advantage to displace this inhibitor from its binding to HS chains or chemokine receptor. Different to other polyanions [38,39] we found the K276R mutation as a sole change in the V3 loop region of the muparfostat resistant HIV-1. The significance of this alteration is obscure since, in contrast to V3 loop mutations conferring resistance to dextran sulfate, it does not change an overall net positive charge in this region. In addition, it should be emphasized that there was a partial selection of the K276R variant at passage 12 and passage 16 (indicated in Table 2) in the virus passaged without muparfostat, which indicates that fluctuations in the dominating viral sequence may occur during passages. Two structural features of sulfated oligosaccharides, i.e. the degree of sulfation and the chain length are known to be important for antiviral activity. In this respect it is noteworthy that muparfostat possesses on average on each monosaccharide one sulfate group more than dextran sulfate (MW 5000) while its chain length, i.e., di-hexsasaccharide is significantly shorter than that of dextran sulfate. Thus, it is likely that selection for the viral variants comprising distinct resistance mutations could be attributed to differences in structure of these sulfated oligosaccharides. Analysis of published sequences of HIV-1 gp120 revealed that lysine at position 276 is not a well-conserved amino acid residue as serine or arginine residues can be found at this position.

In addition to interference with the binding of gp120 to HS, some sulfated polymers such as PRO2000 are capable of inhibiting the interaction of this protein with the CD4 receptor [37]. The I152V mutation of the muparfostat resistant P24 or P21 virus variants occurs at a site proximal to the CD4 binding domain. Interestingly, a mutation at the neighbouring amino acid, i.e. the I153K alteration, was found in an HIV-1 variant resistant to feglymycin, a peptide-

like antibiotic targeting the gp120-CD4 interaction [43]. Although the I152V mutation occurs in the hypervariable region of gp120, and valine at position 152 was found in some published sequences of this glycoprotein [44,45], clonal analysis of HTLV-IIIB viruses passaged 50 and 5 times in H9 cells revealed no I152V alteration suggesting that this mutation is unlikely to result from extensive virus passaging in cells.

Surprisingly, muparfostat also selected for HIV-1 variants with L33S and A101T mutations in the gp41. These alterations were seen at nucleotide level as sole peaks at passage 21 (Figure 2C-2D) and 24, and no indication of their emergence was found at passage 16 (not shown). The major amino acid changes conferring resistance of HIV-1 to the entry inhibitor enfuvirtide occur within the so called GIV motif at positions 36-38, and other sites of the heptad repeat region 1 of gp41 (reviewed in [46]) thus making it uncertain whether muparfostat has any effect on fusion events mediated by gp41. Serine in position 33 of gp41 is also found in isolates from patients who have failed treatment with enfuvirtide, however different hydrophobic amino acids can be found in the same position, including alanine, proline, methionine, valine and glutamine.

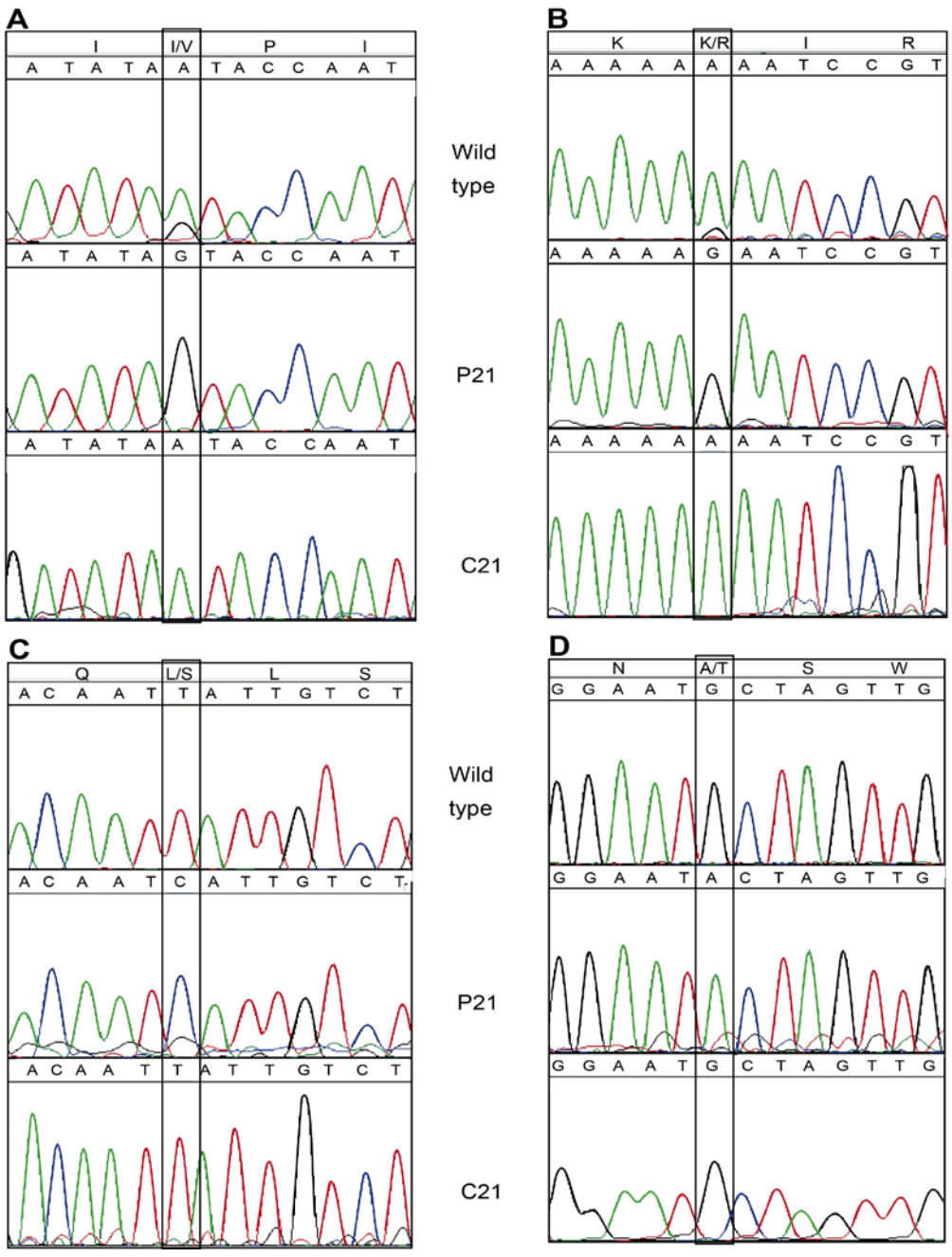

Figure 2: Chromatograms from gene sequencing of the HIV-1 envelope glycoproteins gp120 and gp41. Partial chromatograms of original wild type strain as well as viral variants selected for in 21 passages in the presence of muparfostat (P21) or in the absence of this drug (C21) showing nucleotide as well as predicted amino acid substitutions (boxed) at positions 152 (A) or 276 (B) in gp120, and at positions 33 (C) and 101 (D) in the gp41.

In HSV and RSV muparfostat targeted the viral envelope glycoproteins that comprise the mucin-like domains, and are involved in the virus attachment to cells and/or in modulation of this activity. The resistance to muparfostat was conferred by deletion of entire mucin-like domain in glycoprotein gC of HSV-1 [27], loss of entire mucin-like glycoprotein mgG of HSV-2 [47], or the N191T mutation within the HS-binding domain of the G glycoprotein of RSV [25]. The HIV-1 attachment protein gp120 possesses no typical mucin-like domain but the deletion of one of the repeats (366-FNSTW-370) of V4 loop region is associated with a loss of single *N*-linked glycan that could shield and modulate activity of gp120. Anyhow, some important issues of how exactly the I152V or K276R mutations in gp120 affect its interaction with HS chain and whether there is any similarity as regards to functional impact of these mutations and the resistance alterations found in HSV or RSV need to be further addressed.

Taken together, the decreased sensitivity of HIV-1^{IIIB} after passage in the presence of muparfostat and the selection of viral variants with amino acid alterations in regions of envelope glycoproteins gp120 and gp41 important for attachment and fusion further support that this compound, and possibly its glycoconjugates, inhibits infection at an early stage. We intend to prepare HIV-1 variants resistant to the muparfostat-cholestanol conjugate, and comparative analysis of all these variants would help to clarify molecular basis for virucidal activity found in the cholestanol-coupled muparfostat and its absence in unconjugated muparfostat. These studies are of interest for the further development of microbicidal compounds active against HIV-1.

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